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The effect of sleep restriction on coagulation and fibrinolysis after heavy exercise

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The Effect of Sleep Restriction on Coagulation and Fibrinolysis After Heavy Exercise

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JAMES MADISON UNIVERSITY

in partial fulfillment of the requirements for the degree of

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Abstract

Introduction: Research has shown exercise elicits a hemostatic response affecting coagulation and fibrinolysis. Furthermore, prior research has determined circadian fluctuations exist where clotting potential increases in the morning as a result of increased PAI-1 and decreased tPA which is further exacerbated by exercise. These circadian fluctuations and exercise responses have the potential to be accentuated by poor sleep. The purpose of this study is to determine the effects of sleep restriction on tPA activity, PAI-1 activity, and Factor VIII antigen activity (FVIII) while resting and after exercise.

Methods: 7 Subjects underwent two similar exercises sessions (EX). EX1 occurred in the evening and involved a resting blood draw, peak leg extension torque, 20 min warm-up (10 min-50% Wmax; 10 min-60% Wmax), post-exercise blood draw, 3-km time trial, 60 min of intervals (2 min-50% Wmax; 2 min-95% Wmax), and 3x10 leg press (80% 1RM). The following morning, EX2 mimicked EX1 except no intervals or leg press were performed. Sleep prescription was assigned between EX1 and EX2. Subjects received both full and disturbed sleep.

Results: Two-way ANOVA found no significant main effect for sleep or any interactions between sleep and any of the other factors. There was a main effect for exercise for all variables, as well as for time of day for tPA and FVIII. Interactions for exercise and time of day were significant for tPA (Evening [pre-exercise = 0.32±0.14, post-exercise = 1.89±0.60 AU/mL] vs. Morning [pre-exercise = 0.27±0.13, post-exercise = 0.69±0.18 AU/mL]). and PAI-1 (Evening [pre-exercise = 0.78±0.26, post-exercise = 0.69±0.29 AU/mL] vs. Morning [pre-exercise = 7.06±2.66, post-exercise = 5.40±2.31 AU/mL]).

Conclusion: Sleep restriction does not appear to play a role in hemostasis. Diurnal rhythms increase coagulation potential in the morning shown by increases in PAI-1 and FVIII and decreases in tPA while resting and after exercise.
Keywords: hemostasis, sleep, exercise, coagulation, fibrinolysis
Chapter 1

Introduction

In adults, one in every four deaths can be attributed to cardiovascular disease (CVD) (CDC, 2015). CVD is the leading cause of death in the world representing 17.3 million deaths per year and is expected to grow to 23.6 million by 2030 (AHA, 2014). A major contributing factor to ischemic events that occur due to CVD are alterations in hemostasis favoring a pro-coagulant state. Most ischemic events, and an even greater portion of exertion-related ischemic events, occur as a result of a blood clot (Giri, 1999).

Coagulation involves two pathways: intrinsic and extrinsic. In the intrinsic pathway, platelet aggregation occurs following vessel damage, which activates coagulation proteins starting with Factor XII. Once Factor XII is activated the intrinsic pathway cascade begins, which leads to activation of Factor X. Factor X will convert Prothrombin to Thrombin which converts Fibrinogen to Fibrin. Fibrin forms a tight mesh over aggregated platelets and blood cells. The extrinsic pathway is initiated by trauma and activates free floating Factor VII. Factor VII activates Factor X as well, resulting in thrombin formation (Smith, 2011). It has been shown in cardiovascular disease patients that the potential to form a clot is enhanced, characterized by Fibrinogen, Factor VIII, Thrombin Antithrombin Complex, and other coagulation factors (Folsom, 1993; Rice, 1998; Mustonen, 1998); as well as, impaired fibrinolysis assessed by Tissue Plasminogen Activator (tPA), Plasminogen Activator Inhibitor-1 (PAI-1), and Fibrin Dimer Proteins (Danesh, 2001; Kannel, 1987; Lowe, 2001).

Dissolution of excessive clot, fibrinolysis, counterbalances increased coagulation potential. Free-floating Plasminogen is converted to Plasmin by tissue plasminogen activator (tPA). Plasmin then degrades Fibrin into Fibrin Dimer Proteins. Plasminogen Activator Inhibitor (PAI-1) is the main circulating inhibitor of tPA. Angleton et al. conducted a study comparing tPA and PAI-1 in CVD patients against healthy controls and found that PAI-1 was significantly elevated without a complementing increase in tPA (Angleton, 1989). Similarly, Francis et al.
observed mean PAI-1 activity and plasma fibrinogen levels were elevated in subjects with coronary artery disease (CAD) compared to healthy controls. Researchers also noted a positive correlation between PAI-1 activity and fibrinogen levels. (Francis, 1988).

There is evidence to suggest that abdominal adipose tissue, will express PAI-1 (Alessi, 1997; Cigolini 1999). An experiment conducted by Ericksson, et al. revealed a two-fold increase in adipocyte PAI-1 mRNA levels, six-fold increase in adipose tissue PAI-1 secretion, and seven-fold increase in plasma PAI-1 activity compared to healthy controls (Eriksson, 1998). It is clear that as individuals become severely affected by CVD, an environment of increased coagulation potential and decreased fibrinolytic potential is created and enhanced by disease progression which can lead to adverse health reactions such as stroke or myocardial infarction.

It is recommended by the National Sleep Foundation that individuals sleep between seven and nine hours per night (Hirshkowitz, 2015). However, it appears that greater than 30% of adults receive an average of less than 6 hours of sleep per night (Schoenborn, 2010). Sleep disruptions could impact markers of coagulation and fibrinolysis. Weil, et al investigated fibrinolysis in habitual short sleepers and determined that tPA release following bradykinin infusion was approximately 25% lower in habitual short versus normal duration sleepers. There was an inverse relationship between average nightly sleep duration and tPA release (Weil, 2013). In conjunction, it has been shown that there is a circadian effect on PAI-1 with the highest activity in the morning and decreasing throughout the day (Angleton, 1989).

Liu, et al. determined that after complete sleep deprivation, prothrombin time, thrombin time, and activated partial thromboplastin time significantly decreased with trends for increased fibrinogen (Liu, 2009). Even with sleep restriction, intermittent or shortened sleep, there is an increase in Von Willibrand Factor (vWF) and PAI-1 in subjects with subclinical sleep apnea (von Kanel, 2007). Furthermore, it has been observed that healthy population who received less than six hours of sleep have higher Von Willebrand Factor (vWF) than those who received seven hours of sleep (Miller, 2010). Similarly, increased levels of Fibrin Dimer Proteins have been
observed in Alzheimer caregivers, whose sleep patterns tend to be erratic (von Kanel, 2010). It is important note however that these studies primarily investigated the effects of chronic average sleep duration and not intermittent or acute sleep restriction or deprivation.

Exercise can contribute to a reduction in CVD progression (Blair, 1995; Kodama, 2009). However there is inherent risk when during acute exercise given the physiological stress (Willich, 1993; Vuori, 1986). During physical exertion there is an increase in coagulation potential (Bounameaux, 1992; Herren, 1992; Prisco 1993) and fibrinolytic potential; the latter evidenced by an increase in tPA and decrease in PAI-1. (Gough, 1992; Rankinen, 1995; Szymanski, 1994). After cessation of exercise however, markers of coagulation remained elevated after physical exertion for at least 1 hour, whereas tPA returns to baseline within the same post exercise period (Paton, 2004; Hedge, 2001). Likewise, it has been shown this increased coagulation potential persists for 6-10 hours after ceasing exercise (Hansen, 1990; Lin, 1999). This pro-coagulant environment after physical activity could prove hazardous, if not fatal, for diseased individuals or even those unaccustomed to exercise.

Thus, both exercise and sleep can elicit a pro-coagulant state. To the researchers knowledge there is no study that has determined the effects of exercise in conjunction with sleep restriction on the hemostatic environment. Therefore, the purpose of this study is to determine the effect of sleep restriction on coagulation and fibrinolysis following intense exercise.
Chapter 2
Methodology

Subjects
Approximately 10-15 male cyclists, age 18-25, will be recruited from James Madison University (JMU) and the surrounding area. Recruitment efforts will focus on the JMU Triathlon and Cycling Club teams and local cycling shops. Additionally, subjects will have no reported injuries or sleeping disorders according to the Pittsburgh Sleep Quality Index (PSQI). Subjects will be provided verbal and written information regarding procedures, and a subsequent written informed consent will be obtained. All procedures were approved by the JMU Institutional Review Board prior to data collection.

Experimental Design
The protocol is designed to examine the influence of sleep restriction on recovery from heavy exercise. Subjects will complete a preliminary trial, a familiarization phase, and two experimental phases, each separated by approximately seven days. The familiarization phase and two experimental phases will consist of three exercise sessions performed on consecutive days (EX1, EX2, EX3). EX1 will include baseline performance testing followed by a heavy exercise protocol modeled to elicit fatigue. EX2 will be performed the following morning and will be used to assess recovery from EX1. EX3 will be performed 24 hrs thereafter to further evaluate recovery. EX1 and EX2 performed during the experimental phases will be will be separated by a full night of sleep (+) or a night of sleep restriction (-). Accordingly, EX1 and EX2 sessions separated by a full night of sleep will be referred to as EX1+ and EX2+, whereas EX1 and EX2 sessions separated a night of sleep restriction will be referred to as EX1- and EX2-. Order of subject assignment to sleep+ and sleep- will be determined using a randomized crossover design.

Experimental Trials:

Preliminary Trial
Following height (cm) and body weight (kg) measurements, maximal oxygen consumption (VO$_{2\text{max}}$) and peak power (W$_{\text{max}}$) will be obtained on an electronically braked cycle ergometer (Velotron, RacerMate, Inc., Seattle, WA, USA). Subjects will perform a self-selected warm-up for five minutes and begin the test at an intensity corresponding to what the subject perceives as comfortable for a 60-min ride. Intensity will then be increased by 25 W every 2 minutes until voluntary termination or until cadence drops below 50 RPM. W$_{\text{max}}$ will be used to determine workloads during the EX trials. Expired air will be continuously analyzed for oxygen uptake (VO$_2$), ventilation (VE), respiratory exchange ratio (RER) using indirect calorimetry (Moxus; Pittsburgh, PA, USA); heart rate will be measured using a Polar heart rate monitor (Lake Success, NY, USA). Subjects will indicate rate of perceived exertion by pointing to a Borg (6-20) RPE scale at the end of each stage.

Following maximal testing, subjects will be assessed for one repetition maximum (1RM) for Leg Press. Subjects will self-select a comfortable weight and perform one repetition. Weight lifted will increase 20 lbs. until 1RM is achieved. Subjects will be given two minutes of rest between sets (maximum of five). Weight lifted will increase until 1RM is achieved. 1RM will be used to determine load during experimental trials.

**Familiarization Phase**

The familiarization phase will be identical to the EX phases (detailed below), and will be used to ensure that subjects can complete the EX protocols at the pre-determined intensity, and to minimize learning associated error variance during EX trials. Sleep will be monitored, but not controlled, during this phase.

**Physical Activity & Dietary Control**

Subjects will record all food and beverage intake for 24 hrs preceding EX1. Subjects will report to EX1 after a ≥2 hr fast. Subjects will consume Ensure Active High Protein Shake
according to body weight within 1 hour of completing EX1. Additionally, subjects will be instructed to refrain from consuming any other macronutrients two hrs following EX1. Subjects will be instructed to consume a pre-determined meal two hours prior to EX2. Subjects will arrive to EX3 after an overnight fast. After the initial EX phase, subjects will be provided with copies of their dietary records and instructed to replicate their dietary habits for the second EX phase.

Subjects will be instructed to record all physical activity 72 hrs prior to EX1 in both phases. Subjects will also be instructed to avoid physical activity between EX1 and EX3 and to keep physical activity habits consistent between EX phases.

Exercise Trial 1 (EX1)

Subjects will arrive at the human performance lab between 3-5pm, not having consumed alcohol, tobacco, or caffeine within 24 hrs of testing. Following 15 min of supine rest, venous blood samples (~15 ml) will be obtained from an antecubital vein. Subjects will then warm up on a treadmill for five min at 3.5 mph and 0% grade. Afterwards, subjects will perform a peak isokinetic dynamometer test at 60 and 120 deg/sec achieved by completing two sets of two repetitions at each velocity (BioDex, Shirley, NY, USA). Following a 5-min rest, subjects will perform a 20-min warm-up (10 min at 50% $W_{max}$ followed by 10 min at 60% $W_{max}$) on the Velotron cycle ergometer. During each 10 min stage HR, RPE, glucose, lactate, RER, VE, and $VO_2$ later discussed will be assessed. Immediately Following the 20 min warm-up, venous blood samples (~15 ml) will be obtained within two minutes according to Cooper, et al (Cooper, 2004). Subjects will then begin a three km self-paced time trial. Subjects will be verbally encouraged to give maximal effort prior to start but not during the time trial. After five min of rest, subjects will perform a 60-min sprint interval session as detailed by Goh, et al (Goh, 2012). Sprint intervals will last two min at 95% $W_{max}$, followed by two min at 50% $W_{max}$, each at a cadence of >50 rpm. Should subjects fail to maintain cadence at “sprinting” $W_{max}$, the intensity will be reduced by 10%
in subsequent sprints. Following a five min rest period, subjects will perform three sets of 10 repetitions on a Leg Press exercise machine at 80% 1RM.

Experimental Trial 2 (EX2)

Subjects will arrive at the human performance lab between 7-9am, not having consumed alcohol, tobacco, or caffeine 24 hrs prior to testing. Following 15 min of supine rest, venous blood samples (~15 ml) will be obtained from an antecubital vein. Subjects will then warm up on a treadmill for five min at 3.5 mph and 0% grade. Afterwards, Subjects will perform a peak isokinetic dynamometer test as they did previously. Following a five min rest, subjects will perform a 20-min warm-up (10 min at 50% \( W_{\text{max}} \) followed by 10 min at 60% \( W_{\text{max}} \)) on the Velotron cycle ergometer, and physiological variables will be evaluated accordingly. Immediately following the 20 min warm-up, venous blood samples (~15 ml) will be obtained within two minutes according to Cooper, et al (Cooper, 2004). Subjects will then begin a three km self-paced time trial. Subjects will be verbally encouraged to give maximal effort prior to start but not during the time trial.

Experimental Trial 3 (EX3)

Subjects will arrive at the human performance lab 24 hours after the start of EX2 not having consumed alcohol, tobacco, or caffeine 24 hrs prior to testing. Following 15 min of supine rest, venous blood samples (~5 ml) will be obtained from an antecubital vein. Subjects will then warm up on a treadmill for five min at 3.5 mph and 0% grade. Afterwards, Subjects will perform a peak isokinetic dynamometer test as they did previously.

Heart Rate & Rate of Perceived Exertion (HR & RPE)

HR will be recorded at the end of the 15 min supine rest to achieve a resting HR. During EX trials, HR and RPE will be measured at min 10 and min 20 of the 20-min warm-up preceding
the 3km time trial. HR will be observed during the 3km time trial at approximately 2km. HR and RPE will also be taken during the 60 min every two min.

*Glucose & Lactate (GLU & LAC)*

Whole blood GLU and LAC will be obtained via finger-stick samples at min 10 and min 20 of the warm-up preceding the 3km time trial. Both variables will be assessed using an automated YSI 2300 Stat Plus analyzer (Yellow Springs, OH, USA).

*Oxygen Consumption (VO₂), Ventilation (VE), & Respiratory Exchange Ratio (RER)*

VO₂, VE, and RER will be assessed continuously during EX phases using a Moxus metabolic cart (Pittsburgh, PA, USA). Breath samples will be obtained during the 20 min warm-up preceding the 3km time trial. Minutes 7-10 and 17-20 will be averaged and recorded.

*Muscle Function*

Peak isokinetic torque will be determined using the BioDex dynamometer mentioned above (60 deg/sec, 120 deg/sec), at the aforementioned times during EX phases.

*Muscle Soreness*

Muscle soreness ratings will be obtained using a visual analog scale from 0-100 mm, with 0 mm indicating no muscle soreness and 100 mm indicating maximal impaired movement due to muscle soreness, as detailed by Saunders, et al (Saunders, 2009). Soreness ratings will be obtained upon arrival for all trials by walking up a flight of stairs, down a flight of stairs, and standing.

*3km Time Trial Performance*
3km cycling time trials will be performed on the Velotron ergometer mentioned above. 3km time trials will be performed on EX1 and EX2, but not EX3. 3km finishing times and average power output will be used as the performance criterion.

**Sleep Restriction**

Subjects will undergo the first phase of EX trials separated by either sleep+ or sleep-. Subjects will then be assigned the alternate sleep condition seven days later for the following phase. Subjects will attempt onset of sleep as normal to their sleep schedule as possible. Onset of sleep will be measured using an Actigraph accelerometer, Model- WGT3X-BT Monitor (Actigraph, LLC; Pensacola, FL, USA), a smartphone application, “Sleep Cycle”, which uses motion detection to determine actual time of sleep onset, and a modified PSQI. Subjects will be instructed on a sleep- night to attempt to get three hrs of sleep, while a sleep+ night attempting to get eight hrs of sleep. A sleep- night is acceptable if the subject achieves 1-4 hrs of sleep, while a sleep+ night is will be acceptable if 6-9 hrs of sleep is achieved.

**Processing Blood Samples:**

Following 15 min of supine rest, venous blood samples (~5 ml) will be obtained from an antecubital vein. Blood samples will be centrifuged at 3500 RPM for 20 min. Plasma samples will be stored at -80°C until analysis.

*Plasma Creatine Kinase (CK)*

Plasma CK will be measured using a ChemWell analyzer (Palm City, FL, USA).

*Hematocrit and Hemoglobin*

Capillary tubes lined with heparin will be used to assess hematocrit and hemoglobin changes. Once blood is collected, tubes will be placed in Readacrit Centrifuge (Clay Adams,
Parsippney, New Jersey) and spun at 7000 RPM for five min. Measurement of red blood cell volume and plasma volume will be done using a micrometer.

*Coagulation and Fibrinolysis*

*Plasma Tissue Plaminogen Activator (tPA), Plaminogen Activator Inhibitor-1 (PAI-1), and Factor VIII*

Plasma samples will be measured for Factor VIII antigen, tPA activity, Pai-1 activity, and tPA antigen using ELISA kits. Samples will be corrected for plasma changes in accordance with Dill and Costill (Dill, 1974).

*Statistics*

Repeated measures ANOVA utilizing a within-subject design for condition (Sleep+, Sleep-), Trial (EX1, EX2), and Time (Pre, Post) will be used to determine changes in tPA, PAI-1, Factor VIII, and tPA antigen. Post-hoc testing will be done using paired samples t-tests with a Bonferroni correction. A priori significance will be set to an alpha of p-value < 0.05.
Chapter 3

Manuscript
The Effect of Sleep Restriction on Coagulation and Fibrinolysis

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Abstract

Introduction: Research has shown exercise elicits a hemostatic response affecting coagulation and fibrinolysis. Furthermore, prior research has determined circadian fluctuations exist where clotting potential increases in the morning as a result of increased PAI-1 and decreased tPA which is further exacerbated by exercise. These circadian fluctuations and exercise responses have the potential to be accentuated by poor sleep. The purpose of this study is to determine the effects of sleep restriction on tPA activity, PAI-1 activity, and Factor VIII antigen activity (FVIII) while resting and after exercise.

Methods: 7 subjects underwent two similar exercises sessions (EX). EX1 occurred in the evening and involved a resting blood draw, peak leg extension torque, 20 min warm-up (10 min-50% Wmax; 10 min-60% Wmax), post-exercise blood draw, 3-km time trial, 60 min of intervals (2 min-50% Wmax; 2 min-95% Wmax), and 3x10 leg press (80% 1RM). The following morning, EX2 mimicked EX1 except no intervals or leg press were performed. Sleep prescription was assigned between EX1 and EX2. Subjects received both full and disturbed sleep.

Results: Two-way ANOVA found no significant main effect for sleep or any interactions between sleep and any of the other factors. There was a main effect for exercise for all variables, as well as for time of day for tPA and FVIII. Interactions for exercise and time of day were significant for tPA (Evening [pre-exercise = 0.32±0.14, post-exercise = 1.89±0.60 AU/mL] vs. Morning [pre-exercise = 0.27±0.13, post-exercise = 0.69±0.18 AU/mL]) and PAI-1 (Evening [pre-exercise = 0.78±0.26, post-exercise = 0.69±0.29 AU/mL] vs. Morning [pre-exercise = 7.06±2.66, post-exercise = 5.40±2.31 AU/mL]).

Conclusion: Sleep restriction does not appear to play a role in hemostasis. Diurnal rhythms increase coagulation potential in the morning shown by increases in PAI-1 and FVIII and decreases in tPA while resting and after exercise.
Introduction

Most ischemic events, and an even greater portion of exertion-related ischemic events, occur as a result of a blood clot (Giri, 1999). Dissolution of excessive clot, fibrinolysis, counterbalances increased coagulation potential that occurs during physical exertion. Free-floating Plasminogen is converted to Plasmin by Tissue Plasminogen Activator (tPA). Plasmin then degrades Fibrin in to Fibrin Dimer Proteins. Plasminogen Activator Inhibitor (PAI-1) is the main circulating inhibitor of tPA. During physical exertion there is an increase in coagulation potential (Bounameaux, 1992; Herren, 1992; Prisco 1993) and fibrinolytic potential; the latter evidenced by an increase in tPA and decrease in PAI-1. (Gough, 1992; Rankinen, 1995; Szymanski, 1994). However, after cessation of exercise, markers of coagulation remain elevated hours after physical exertion whereas tPA returns to baseline rapidly. (Cooper, 2004; Hansen, 1990; Lin, 1999; Paton, 2004; Hedge, 2001).

Greater than 30% of adults receive an average of less than 6 hours of sleep per night (Schoenborn, 2010). Sleep disruptions could impact markers of coagulation and fibrinolysis. Liu, et al. determined that after complete sleep deprivation, prothrombin time, thrombin time, and activated partial thromboplastin time significantly decreased with a trend for increased fibrinogen (Liu, 2009). It has also been observed that a healthy population who received less than six hours of sleep have higher Von Willebrand Factor (vWF) than those who received seven hours of sleep (Miller, 2010). Similarly, increased levels of Fibrin Dimer Proteins have been observed in Alzheimer caregivers, whose sleep patterns tend to be erratic (von Kanel, 2010). It is important to note, these studies primarily investigated the effects of chronic sleep duration and not intermittent or acute sleep restriction or deprivation. Furthermore, there is a circadian effect on PAI-1 and tPA with the highest PAI-1 and lowest tPA activity in the morning (Angleton, 1989; Szymanski, 1994).

Thus, both exercise and sleep deprivation can elicit a pro-coagulant state. To our knowledge there is no study that has determined the effects of exercise in conjunction with sleep
restriction on the hemostatic response to exercise. Therefore, the purpose of this study is to determine the effect of sleep restriction on coagulation and fibrinolysis following intense exercise.
Methods

Subjects

17 cyclists were recruited although only 5 male cyclists and 2 female cyclists, aged 18-40, were recruited from James Madison University (JMU) and the surrounding area. Subjects had no reported injuries or sleeping disorders according to the Pittsburgh Sleep Quality Index (PSQI). Subjects were provided verbal and written information regarding procedures, and a subsequent written informed consent was obtained. All procedures were approved by the JMU Institutional Review Board.

Experimental Design

Subjects completed a preliminary trial, a familiarization phase, and two experimental phases, each separated by approximately seven days. The familiarization phase and two experimental phases consisted of two exercise sessions performed on consecutive days (EX1, EX2). EX1 included baseline performance testing followed by a heavy exercise protocol modeled to elicit fatigue. EX2 was performed the following morning and was used to assess recovery from EX1. EX1 and EX2 performed during the experimental phases were separated by a full night of sleep (+) or a night of sleep restriction (-).

Experimental Trials:

Preliminary Trial

Following height (cm) and body weight (kg) measurements, maximal oxygen consumption (VO$_{2\text{max}}$) and peak power (W$_{\text{max}}$) were obtained on an electronically braked cycle ergometer (Velotron, RacerMate, Inc., Seattle, WA, USA). Subjects performed a self-selected warm-up for five minutes and began the test at an intensity corresponding to what the subject perceived as comfortable for a 60 min ride. Intensity was increased by 25 W every 2 min until voluntary termination or until cadence dropped below 50 RPM. W$_{\text{max}}$ was used to determine
workloads during the EX trials. Expired air was continuously analyzed for oxygen uptake (VO₂), ventilation (VE), respiratory exchange ratio (RER) using indirect calorimetry (Moxus; Pittsburgh, PA, USA); heart rate was measured using a Polar heart rate monitor (Lake Success, NY, USA). Subjects indicated rate of perceived exertion by pointing to a Borg (6-20) RPE scale at the end of each stage.

Following maximal testing, subjects were assessed for one repetition maximum (1RM) for Leg Press. Subjects self-selected a comfortable weight and performed one repetition. Weight lifted increased 20 lbs. until 1RM was achieved. Subjects were given two minutes of rest between sets (max of five sets). Weight lifted increased until 1RM was achieved. 1RM was used to determine load during experimental trials.

**Familiarization Phase**

The familiarization phase was identical to the EX phases (detailed below), and was used to ensure that subjects could complete the EX protocols at the pre-determined intensity, and to minimize learning associated error variance during EX trials.

**Physical Activity & Dietary Control**

Subjects recorded all food and beverage intake for 24 hrs preceding EX1 until the end of EX2. Subjects reported to EX1 after a ≥4 hr fast. Subjects consumed Ensure Protein Shake according to body weight within 15 min of completing EX1. Additionally, subjects were instructed to refrain from consuming any other food two hrs following EX1. After consuming dinner, subjects fasted until they consumed a pre-determined meal two hours prior to EX2. After the initial EX phase, subjects were provided with copies of their dietary records and instructed to replicate their dietary habits for the second EX phase.
Subjects were instructed to record all physical activity 72 hrs prior to EX1 in both phases. Subjects were instructed to avoid physical activity within EX phases and to keep physical activity habits consistent between EX phases.

**Exercise Trial 1 (EX1)**

Subjects arrived at the human performance lab between 3-5pm, not having consumed alcohol, tobacco, or caffeine within 24 hrs of testing. Following 15 min of supine rest, venous blood samples (~15 ml) were obtained from an antecubital vein. Subjects then warmed-up on a treadmill for five min at 3.5 mph and 0% grade. Afterwards, subjects performed a peak leg extension isokinetic dynamometer test on the right quadricep at 60 and 120 deg/sec achieved by completing two sets of two repetitions at each velocity (BioDex, Shirley, NY, USA). Following a 5-min rest, subjects performed a 20-min warm-up (10 min at 50% \( W_{\text{max}} \) followed by 10 min at 60% \( W_{\text{max}} \)) on the Velotron cycle ergometer. During each 10 min stage HR, RPE, RER, VE, and \( VO_2 \) were assessed. Immediately following the 20 min warm-up, venous blood samples (~15 ml) were obtained within two minutes as described by Cooper, et al (Cooper, 2004). Subjects then began a three km self-paced time trial. Subjects were verbally encouraged to give maximal effort prior to start but did not receive encouragement during the time trail. After five min of rest, subjects performed a 60-min sprint interval session. Sprint intervals lasted two min at 95% \( W_{\text{max}} \), followed by two min at 50% \( W_{\text{max}} \), each at a cadence of >50 rpm. Subjects who failed to maintain cadence at “sprinting” \( W_{\text{max}} \), intensity was reduced by 10% in subsequent sprints. Following a five min rest period, subjects performed three sets of 10 repetitions on a Leg Press exercise machine at 80% 1RM.

**Experimental Trial 2 (EX2)**

Subjects arrived at the human performance lab between 6-10am, not having consumed alcohol, tobacco, or caffeine 24 hrs prior to testing. Following 15 min of supine rest, venous
blood samples (~15 ml) were obtained from an antecubital vein. Subjects warmed up on a treadmill for five min at 3.5 mph and 0% grade. Afterwards, Subjects performed a peak isokinetic dynamometer test as they did previously. Following a five min rest, subjects performed a 20-min warm-up (10 min at 50% $W_{max}$ followed by 10 min at 60% $W_{max}$) on the Velotron cycle ergometer, and physiological variables were evaluated accordingly. Immediately following the 20 min warm-up, venous blood samples (~15 ml) were obtained within two minutes according to Cooper, et al (Cooper, 2004). Subjects then began a three km self-paced time trial as before.

_Heart Rate & Rate of Perceived Exertion (HR & RPE)_

HR was recorded at the end of the 15 min supine rest for resting HR. During EX trials, HR and RPE were measured at min 10 and min 20 of the 20-min warm-up preceding the 3km time trial. HR and RPE were also taken during the 60 min sprint intervals every two min.

_Oxygen Consumption ($VO_2$), Ventilation (VE), & Respiratory Exchange Ratio (RER)_

$VO_2$, VE, and RER were assessed continuously during EX phases using a Moxus metabolic cart (Pittsburgh, PA, USA). Expired air samples were obtained during the 20 min warm-up. Minutes 7-10 and 17-20 were averaged and recorded.

_Sleep Restriction_

Subjects underwent the first phase of EX trials separated by either full or restricted sleep. Subjects were then assigned the alternate sleep condition seven days later for the following phase. Subjects attempted onset of sleep as normal to their sleep schedule as possible. Onset of sleep was measured using an Actigraph accelerometer, Model- WGT3X-BT (Actigraph, LLC; Pensacola, FL, USA), a smartphone application, “Sleep Cycle”, and a modified PSQI. Subjects were instructed on a restricted night to attempt to get three hrs of sleep, while a full night to
attempt to get eight hrs of sleep. A restricted night was acceptable if the subject achieved 1-4 hrs of sleep, while a full night was acceptable if 6-9 hrs of sleep was achieved. Five subjects started their first experimental phase with a full night of sleep while two subjects started with a night of sleep restriction. The two female subjects were counterbalanced for sleep prescription to control for menstrual cycle hormonal variations. One subject was excluded from analysis due to incomplete data.

**Processing Blood Samples:**

Following 15 min of supine rest, venous blood samples (~15 mL) were obtained from an antecubital vein. Plasma samples were collected in both 3.2% Sodium Citrate and Acidified Citrate vacutainers. Blood samples were centrifuged at 3500 RPM for 20 min. Plasma samples were stored at -80°C until analysis.

*Coagulation and Fibrinolysis*

Plasma samples from appropriate vacutainers were measured for Factor VIII antigen, tPA activity, PAI-1 activity, and tPA antigen using commercial ELISA kits.

**Statistics**

Repeated measures two-way ANOVA utilizing a within-subject design for condition (Sleep+, Sleep-), Trial (EX1, EX2), and Time (Pre, Post) was used to determine changes in tPA activity, PAI-1 activity, and Factor VIII antigen. Post-hoc testing was done using paired samples t-tests. A priori significance was set at P-value < 0.05.
Results

Average tPA activity for all trials is illustrated in Figure 1. There was a main effect for exercise with post-hoc testing indicating a significant (P<0.05) increase between pre and post-exercise. There was also a main effect for time of day with significantly (P<0.05) higher values in the evening compared to the morning. An interaction between exercise and time of day demonstrated the magnitude of the exercise response was significantly (P<0.05) higher in the evening (pre-exercise 0.32±0.14, post-exercise 1.89±0.60 AU/mL) vs. morning (pre-exercise = 0.27±0.13, post-exercise = 0.69±0.18 AU/mL). There was no main effect for sleep nor was there any significant sleep x exercise, sleep x time of day, or sleep x exercise x time of day interaction.

Average PAI-1 activity for all trials is illustrated in Figure 2. There was a main effect for exercise with post-hoc testing showing a significant (P<0.05) decrease between pre and post-exercise only in the morning. There was a trend for a main effect for time of day (P = 0.051) with higher values in the morning compared to the evening. An interaction between exercise and time of day demonstrated the magnitude of the exercise response was significantly (P<0.05) larger in the morning (pre-exercise = 7.06±2.66, post-exercise = 5.40±2.31 AU/mL) vs. evening (pre-exercise = 0.78±0.26, post-exercise = 0.69±0.29 AU/mL). There was no main effect for sleep nor was there any significant sleep x exercise, sleep x time of day, or sleep x exercise x time of day interaction.

Average Factor VIII activity for all trials is illustrated in Figure 3. There was a main effect for exercise with post-hoc testing denoting a significant (P<0.05) increase between pre and post-exercise. Also, a main effect for time of day was shown with significantly (P<0.05) higher values in the morning compared to the evening. There was no interaction between exercise and time of day. There was no main effect for sleep nor was there any significant sleep x exercise, sleep x time of day, or sleep x exercise x time of day interaction.
Discussion

The current study demonstrates sleep restriction does not alter resting or post-exercise hemostasis as it did not significantly change Tissue Plasminogen Activator (tPA), Plasminogen Activator Inhibitor-1 (PAI-1), or Factor VIII or their respective responses to exercise. Prior studies have investigated the effects of sleep apnea on hemostasis (von Kanel, 2003; Yaggi, 2005; Leung, 2001), but few have explored sleep restriction itself. Liu, et al. determined activated partial thromboplastin time (APTT), Prothrombin time (PT), and Thrombin time (TT) significantly decreased with sleep deprivation, suggesting increased coagulation potential (Liu, 2009). In contrast, Pinotti, et al. found decreases in Factor VII activity and thrombin generation from the extrinsic pathway with sleep deprivation in mice (Pinotti, 2010). It is important to note both studies utilized sleep deprivation while the present study used sleep restriction. Thus it appears sleep deprivation can influence coagulation potential while sleep restriction does not have an impact on coagulation or fibrinolysis based on the current study.

Markers of fibrinolysis and coagulation potential are subject to diurnal variation reflected by an increase in coagulation potential and decreased fibrinolytic potential in the morning versus the evening (Manfredini, 2005). Findings from the present study support this conclusion as tPA was lower and PAI-1 and Factor VIII were higher in the morning versus the evening. As such, morning appears to be associated with a pro-thrombotic state. Interestingly, a time of day x exercise interaction was observed for tPA and PAI-1 but not Factor VIII. The magnitude of the exercise response was lower in the morning for tPA and higher for PAI-1. These results are similar to those reported by Szymanski, et al. who observed higher tPA responses in the evening independent of intensity. PAI-1 values pre-exercise were significantly higher in the morning compared to the evening, but following exercise, time of day differences subsided (Szymanski, 1994). It should be noted that in the current study and Szymanski, et al. (Szymanski, 1994) that low resting levels of PAI-1 in the evening could have prevented any large decrease to occur
during exercise. Similar time of day effects are seen in sedentary and trained individuals where PAI-1 is higher and tPA is lower in the morning (Siahkoujian, 2013; Khodadadi, 2013). Not only is fibrinolysis impaired but coagulation is upregulated in the morning (Manfredini, 2005; Pinotti, 2005), promoting a prothrombotic environment.

The Factor VIII response to exercise seen in both the morning and evening would be more significant in the morning as it would be an additive to the higher morning values. Similarly, PAI-1 is significantly elevated in the morning. Furthermore, tPA activity and response to exercise is lower in the morning further contributing to a pro-coagulant state. Thus, the morning appears to promote a pro-thrombotic environment which is accentuated by physical activity. Although these circadian fluctuations exist, they do not seem to be altered by sleep restriction while resting or post-exertion. The majority of hemostasis studies involve collecting samples after an overnight fast but often ignore the quality of sleep of participants. The present study suggests sleep restriction does not influence resting hemostatic markers or their response to exercise.

Our findings suggest that exercise in the morning results in greater potential for thrombosis. Therefore, it would be logical to conclude that resultant clinical outcomes such as myocardial infarction and stroke would be higher during this time period. However, multiple studies have not observed greater risk for ischemic related events in the morning (Murry, 1993; Atkinson 2006; Jimenez, 1994). However, researchers have suggested that physical activity in the evening may offer protective effects not involving hemostasis that are not evident during exercise in the morning (Zhao, 2014; Zhao, 2016). Future research should elucidate why the altered hemostasis does not pose a more significant clinical threat.

A key limitation within the prevailing study was the low number of participants. However, it should be pointed out that none of the null findings approached statistical
significance. Also, it is unreasonable to say these results pertain to sleep deprivation or sleep restriction in the form of constant waking. In order to gain a full scope of the hemostatic environment, future research should include more coagulation pathway variables. Although there was a significant time of day by exercise interaction it is important to note the exercise that took place following the post-exercise blood draw. Although the exercise workload was similar between sleep conditions it was not feasible to maintain complete duplication. Studies have shown effects of exercise on coagulation to persist for hours (Hansen, 1990; Lin, 1999) but given the time between EX1 and EX2 these effects should be minimal. Also our findings confirm prior research which have shown similar circadian changes without any prior exercise (Angleton, 1989).

In conclusion, the current study demonstrates that sleep restriction does not influence hemostasis or the hemostatic response to exercise. Circadian fluctuations in tPA, PAI-1, and Factor VIII exhibit a pro-thrombotic environment in the morning not only while resting but in response to exercise.
Manuscript References


Table Legend

**Table 1.** Participant Demographics (Mean±SD)

**Table 2.** Participant Sleep Data (Mean±SD)
Figure Legend

**Figure 1.** tPA activity between full (black) and restricted (white) sleep per exercise trial (Mean±SD).

**Figure 2.** PAI-1 activity between full (black) and restricted (white) sleep per exercise trial (Mean±SD).

**Figure 3.** Factor VIII activity between full (black) and restricted (white) sleep per exercise trial (Mean±SD).
Table 1. Participant Demographics (Mean±SD)

<table>
<thead>
<tr>
<th>Sex - n</th>
<th>Male - 5</th>
<th>Female - 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.86 ± 6.91</td>
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</tr>
<tr>
<td>Height (cm)</td>
<td>170.24 ± 9.82</td>
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</tr>
<tr>
<td>Weight (kg)</td>
<td>68.46 ± 12.22</td>
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<tr>
<td>VO(_2) Max (mL/kg/min)</td>
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</tr>
<tr>
<td>VO(_2) 10min (mL/kg/min)</td>
<td>32.75 ± 3.25</td>
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<tr>
<td>VO(_2) 20min (mL/kg/min)</td>
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</tr>
<tr>
<td>Wmax (W)</td>
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<tr>
<td>W10min (W)</td>
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</tr>
<tr>
<td>W20min (W)</td>
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**Table 2. Participate Sleep Data (Mean±SD)**

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<tr>
<td>Sleep (min)</td>
<td>408.57 ± 32.64</td>
<td>151.50 ± 19.09</td>
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<td>In-bed (min)</td>
<td>446.29 ± 22.30</td>
<td>165.00 ± 24.36</td>
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<td>Efficiency (%)</td>
<td>0.92 ± 0.07</td>
<td>0.92 ± 0.06</td>
</tr>
<tr>
<td>Awakenings</td>
<td>16.00 ± 13.08</td>
<td>4.67 ± 4.23</td>
</tr>
</tbody>
</table>
Figure 1. tPA activity between full (black) and restricted (white) sleep prescription per exercise trial (Mean±SD).

Significant main effect for exercise (P<0.05)

(*) Significantly higher than resting values (P<0.05)

(†) Significantly lower in the morning trial (P<0.05)

(‡) Interaction for exercise x time of day (P<0.05)
Figure 2. PAI-1 activity between full (black) and restricted (white) sleep prescription per exercise trial (Mean±SD).

Significant main effect for exercise (P<0.05)

(*) Significantly lower than resting values (P<0.05)

(‡) Interaction for exercise x time of day (P<0.05)
Figure 3. Factor VIII activity between full (black) and restricted (white) sleep prescription per exercise trial (Mean±SD).
Significant main effect for exercise (P<0.05)
(*) Significantly higher than resting values (P<0.05)
(†) Significantly higher in the morning trial (P<0.05)
Appendices

James Madison University
Department of Kinesiology

Consent for Investigative Procedure

I, ______________________, hereby agree on _____________ (date) to participate in the research project conducted by Christopher J. Womack, Ph.D., Nicholas D. Luden, Ph.D., John Chase, and Paul Roberson from James Madison University titled The Effects of Sleep Restriction on Recovery from Heavy Exercise.

The purpose of this study is to determine the effects that sleep restriction has on subsequent performance and recovery from heavy exercise. Additionally, this study aims to determine the effects that sleep restriction has on performance and muscle recovery following heavy exercise, compared to a full night of rest.

Subject Responsibility

I understand that I will undergo the following testing:

This study consists of nine separate visits performed, nine of which will involve exercise on a resistance exercise device and seven of which will involve exercise on a stationary bike (cardiovascular fitness test, two familiarization trials, and six exercise trials). All testing will occur in Godwin Hall, room 209, on the campus of James Madison University. You will also be asked about lifestyle behaviors such as smoking and physical activity and complete dietary and physical activity records. The total time commitment is estimated to be less than 11 hours over the course of 4-6 weeks.

Preliminary Trial (n=1 visit; 60 min):

After completing this consent form and the health history screening, if you meet the inclusion criteria for the study, researchers will measure your height and body weight. You will then be asked to perform a maximal cardiovascular fitness test to determine your maximum oxygen consumption (VO$_{2\text{max}}$). You will be asked to ride a stationary bike at an initial workload that is ‘fairly easy’. The workload will then be increased every two minutes until fatigue is reached, determined by either: 1) your request to stop due to fatigue, or 2) inability to maintain a cadence of $\geq$50 revolutions per minute. You will be verbally encouraged to continue to obtain an accurate measurement of VO$_{2\text{max}}$. To access
oxygen consumption, you will need to breathe through a mouthpiece/breathing apparatus which collects expired air throughout the test (10-15 minutes).

You will then be asked to complete 1 repetition of leg press multiple times to determine max strength.

**Familiarization Phase (n=2 visits; total time of 180 min):**

The familiarization phase will consist of three consecutive days of exercise trials. Procedures will be the same as the experimental trials detailed below. Exceptions include that no blood samples will be obtained and you will not be assigned to a sleep condition.

**Experimental Phase**

**Exercise Trial 1 (n=2, 120 min each)**

You will be asked to arrive at the human performance lab between 3-5pm, not having consumed alcohol, tobacco or caffeine 24 hrs prior to testing.

**Blood Draw** - You will rest in a seated position for 5 minutes, after which a blood sample will be sampled for measurement of a blood marker of muscle damage (creatine kinase). Approximately 3 ml of whole blood will be obtained. The blood will be stored in the freezer for later analysis. Immediately following the blood draw, you will be asked to rate your level of muscle soreness using a scale ranging from 0-100 (taken walking up stairs, walking down stairs and standing) and subjective ratings of energy and fatigue will be assessed using a short questionnaire. Plasma samples will be stored in our laboratory freezer for at least 3 years to allow us to conduct follow-up studies related to emerging blood markers that may be impacted by sleep disruption.

**Skeletal Muscle Function** – Immediately following the blood draw, you will warm up for 5 minutes on a treadmill (3.5 mph) after which you will perform peak muscle function testing using a muscle function device. You will be prompted to push as hard as possible against a shin pad that is connected to an electronic device that controls speed of movement through the leg-extension.

**3-km Cycling Time Trial** – Immediately following skeletal muscle function testing, you will be prompted to perform a 20-min warm-up on a stationary cycle ergometer. During the warm-up, several measurements will be taken (detailed below). Following the warm-up, you will be prompted to transition into a 3-km computer-simulated time trial on the cycle ergometer. This will last approximately 4-7 minutes and you will be reminded to treat this like a competition.
60-minute Sprint Interval – 10 min following completion of the 3-km time trial, you will complete 60 min of sprint intervals. **You will only perform these intervals on Exercise Trial 1.** You will be asked to cycle at high intensities for 2-minute intervals, separated by 2 min of moderate intensity cycling. Intensity will be progressively decreased throughout to permit you to finish the 60-minute session.

Resistance Exercise – 10 minutes following the 60-min sprint interval, you will perform 3 sets of 10 at 75% of your peak strength on a traditional leg press device.

**Exercise Trial 2 (n=2, 60 min each)**

You will be asked to arrive at the human performance lab between 7-9 am, not having consumed alcohol, tobacco, or caffeine 24 hrs prior to testing.

Blood Draw - The same blood draws, muscle soreness, and energy/fatigue ratings described above will be used.

Skeletal Muscle Function – Immediately following the blood draw, you will again perform a warm-up for 5 minutes on a treadmill (3.5 mph) after which they will perform peak muscle function testing as described above.

3-km Cycling Time Trial – Immediately following skeletal muscle function testing, subjects will perform a 20-min warm-up on a stationary cycle ergometer and then perform a 3-km computer-simulated time trial 5 minutes after. This will last approximately 4-7 minutes.

**Measurements During 20-Minute Warm-Up**

*Heart Rate & Rate of Perceived Exertion (HR & RPE)*

You will be fitted for a heart rate monitor so that we can measure your heart rate throughout the exercise trial. Additionally, you will be asked to rate your level of exertion on a scale from 6 to 20 at various points throughout your warm up.

*Glucose & Lactate (GLU & LAC)*

Finger-stick blood samples (1-2 drops of blood) will be obtained at min 10 and min 20 of the warm-up preceding the 3km time trial for measurement of blood sugar and lactic acid.

*Oxygen Consumption, Ventilation, & Respiratory Exchange Ratio (VO₂, VE & RER)*

You will also be hooked up to a mouth-piece (and nose clip) in 2 x 5-minute increments during the warm-up for measurement of oxygen consumption and other measures of cardiorespiratory function.
Coagulation Factors

At the end of the 20-min warm-up and preceding the 3-km time trial, another 3 ml blood sample will be obtained to allow us to assess markers of blood clotting.

Exercise Trial 3 (n=2, 20 min each)

You will not be assigned to a sleep condition the night between EX 2 and EX 3.

You will be asked to arrive at the human performance lab between 7-9 am, not having consumed alcohol, tobacco 24 hrs prior to testing or caffeine 12 hours prior to testing.

Blood Draw - The same blood draw, muscle soreness, and energy/fatigue ratings described in EX1 will be used.

Skeletal Muscle Function – Immediately following the blood draw, you will warm-up for 5 minutes on a treadmill (3.5 mph) after which you will be asked to perform peak muscle function testing as described above.

Sleep Protocol:

Sleep will be measured, but not controlled, during the familiarization trials. During the experimental phases, you will be randomly assigned to a sleep condition: a full night of sleep or a night of sleep restriction. You will not know your assigned sleep condition until completion of the first exercise trial. The sleep condition will be randomly selected for each exercise phase such that you may be assigned each sleep condition, or two of the same sleep conditions. Following the first night of sleep assignment, you will be asked to record what you wore to sleep and replicate that on the second night of sleep assignment. You will also be asked to download a smartphone application that will detect movement during sleep, and wake you up either 3.5 or 7.5 hrs following sleep onset, dependent upon sleep assignment for the night. Should you not comply with sleep protocols, you will be given another chance to complete the study seven days later. Failure to comply with sleep protocol a second time will result in removal from the study.

Dietary and Exercise Controls:

You will be asked to record all food and beverage intake for 24 hrs preceding the first exercise trial and throughout the day of EX 2. After the initial exercise trial, you will be provided with copies of your dietary records and instructed to replicate their dietary habits for the second EX phase. You will be asked to report to all testing after a >2 hr fast. You will be asked to consume Ensure Active High Protein Shake within 1 hour of completing the first exercise trial. Additionally, you will be instructed to refrain from consuming anything other than water during the 2 hours following EX1. You will be asked to record dietary intake following EX1, and replicate that seven days later after the second
EX1 trial. You will also be instructed to record all physical activity 72 hrs prior to the first exercise trial in both phases and the day of EX 2. Additionally, you will be instructed to avoid physical activity between the first and third exercise trials, and to keep physical activity habits consistent between exercise phases.

**Risks/Benefits:**

**Skeletal Muscle Function**

The risks of muscle function testing include soreness from exertion 24-48 hours post and potential lightheadedness or loss of consciousness if correct form is not utilized. You will be instructed in correct form and breathing techniques prior to testing.

**Sleep Disruption**

The consequences of a single night of sleep restriction comparable to this investigation have not been well documented but include impaired insulin sensitivity, increased sleepiness and fatigue, and reduced alertness and constant attentiveness. The latter have the potential to impact short-term academic performance, decision-making and tasks such as driving ability but these have not been documented.

**Cardiovascular Exercise (3-km Time Trial, 60-min sprint interval session, and VO2max test)**

According to the American College of Sports Medicine’s Guidelines for Exercise Testing and Prescription, the risk associated with heavy exercise for individuals categorized as “low risk” is very minimal, and physician supervision is not necessary. The conditions that the exercise sessions are to take place are likely safer than the typical exercise environments of the subjects. If you do not meet ACSM criteria for “low risk”, you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, at least one of the listed investigators will be present during the exercise sessions, and all are CPR certified.

**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 OSHA protocols, including: investigators will wear latex gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a
separate waste disposal unit lined with a biohazard bag. All investigators who will be involved in blood draws (and handling of blood) have been trained in these phlebotomy techniques, and completed JMU blood-borne pathogen training. A total of ~35 milliliters of blood will be obtained throughout the course of the study, which is roughly 8% of the amount of blood typically obtained during blood donation (1 pint or 473 milliliters).

Performance incentive:

The top 5 3-km time trial performers will be entered into a drawing to win $150. Individuals with the top 6-10 times will be entered into a drawing to win $75. Times from both EX2 trials will be averaged to determine the top performers.

Confidentiality:

The results of this research will be presented at conferences and published in exercise science journals. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. However, you can ask that your data be removed from the study at any point prior to presentation and publication. 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 researcher. Final aggregate results will be made available to you upon request.

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. Your right to withdraw includes the right to request that your blood samples be discarded at any time. To dispose of your samples, your samples will be rinsed down a chemical drain in our laboratory or will be disposed of in a biohazard container. Again, your sample will not be identifiable without the coding document that will be locked away in a filing cabinet.

Questions:

You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact Nicholas D. Luden, Ph.D. at ludennd@jmu.edu or by phone at 540-568-4068.
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 Researcher(s) (Printed)

Name of Participant (Signed)  Name of Researcher(s) (Signed)

Date  Date

For questions about your rights as a research subject, you may contact the chair of JMU’s Institutional Review Board (IRB). Dr. David Cockley, (540) 568-2834, coxjule@jmu.edu.
AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire
Assess your health status by marking all true statements

History
You have had:
- [ ] a heart attack
- [ ] heart surgery
- [ ] cardiac catheterization
- [ ] coronary angioplasty (PTCA)
- [ ] pacemaker/implantable cardiac defibrillator/rhythm disturbance
- [ ] heart valve disease
- [ ] heart failure
- [ ] heart transplantation
- [ ] congenital heart disease

Symptoms
- [ ] You experience chest discomfort with exertion
- [ ] You experience unreasonable breathlessness
- [ ] You experience dizziness, fainting, or blackouts
- [ ] You take heart medications

Other Health Issues
- [ ] You have diabetes
- [ ] You have asthma or other lung disease
- [ ] You have burning or cramping sensation in your lower legs when walking short distances
- [ ] You have musculoskeletal problems that limit your physical activity
- [ ] You have concerns about the safety of exercise
- [ ] You take prescription medication(s)

Cardiovascular risk factors
- [ ] You are a man older than 45 years
- [ ] You smoke, or quit smoking within the previous 6 months
- [ ] Your blood pressure is > 140/90 mmHg
- [ ] You do not know your blood pressure
- [ ] You take blood pressure medication
- [ ] Your blood cholesterol level is > 200 mg/dl
- [ ] You do not know your cholesterol level
- [ ] You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
- [ ] You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)
- [ ] You are > 20 pounds overweight

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.
None of the above

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.
The Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions. During the past month,

1. When have you usually gone to bed? ______________
2. How long (in minutes) has it taken you to fall asleep each night? ______________
3. When have you usually gotten up in the morning? ______________
4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) ____________

5. During the past month, how often have you had trouble sleeping because you…
   a. Cannot get to sleep within 30 minutes
   b. Wake up in the middle of the night or early morning
   c. Have to get up to use the bathroom
   d. Cannot breathe comfortably
   e. Cough or snore loudly
   f. Feel too cold
   g. Feel too hot
   h. Have bad dreams
   i. Have pain
   j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):

6. During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?

7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

9. During the past month, how would you rate your sleep quality overall?

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<tr>
<th>Component 1</th>
<th>#9 Score</th>
<th>Component 2</th>
<th>#2 Score</th>
<th>Component 3</th>
<th>#4 Score</th>
<th>Component 4</th>
<th>(total # of hours asleep)/(total # of hours in bed) x 100</th>
<th>Component 5</th>
<th>Sum of Scores #5b to #5j</th>
<th>Component 6</th>
<th>#6 Score</th>
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<td>&gt;85%=0, 75%-84%=1, 65%-74%=2, &lt;65%=3</td>
<td>#5a Score</td>
<td>(if sum is equal) 0=0; 1-2=1; 3-4=2; 5-6=3</td>
<td></td>
<td>#6 Score</td>
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</table>
Component 7  #7 Score + #8 Score (0=0; 1-2=1; 3-4=2; 5-6=3)........................................... C7_____

Add the seven component scores together ________ Global PSQI Score ________


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### Daily Activity Records

**Subject #** __________  **Date:** ______________

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#### Intensity Scale

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<th>Intensity</th>
<th>Description</th>
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<tbody>
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<td>Very, very light</td>
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<tr>
<td>7</td>
<td>Very light</td>
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<tr>
<td>8</td>
<td>Fairly light</td>
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<tr>
<td>9</td>
<td>Somewhat hard</td>
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<tr>
<td>10</td>
<td>Hard</td>
</tr>
<tr>
<td>11</td>
<td>Very hard</td>
</tr>
<tr>
<td>12</td>
<td>Very, very hard</td>
</tr>
<tr>
<td>Time</td>
<td>Food and/or Drink</td>
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References


Center for Disease Control Web Site [Internet]. Atlanta (GA): Center for Disease Control; [Cited 2016 Jan]. Available from http://www.cdc.gov/heartdisease/facts.htm


