PROTOCOL TITLE:

Aerobic Exercise: A Potential Rescue from the Negative Repercussions of Poor Sleep

PRINCIPAL INVESTIGATOR:

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VERSION NUMBER/DATE:

Include the version number and date of this protocol.

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	13 May 2025	Title change, participant compensation added	Yes

Table of Contents

1.0 Study Summary

Study Title	Aerobic Exercise: A Potential Rescue from the Negative Repercussions of Poor Sleep
Study Design	Randomized, crossover
Primary Objective	To differentiate the effects of a multi-day partial sleep deprivation intervention on central hemodynamics in individuals with high vs low cardiorespiratory fitness.
Secondary Objective(s)	To differentiate the effects of a multi-day partial sleep deprivation intervention on performance in individuals with high vs low cardiorespiratory fitness. To differentiate the effects of a multi-day partial sleep deprivation intervention on overall health in individuals with high vs low cardiorespiratory fitness.
Research Intervention(s)/ Investigational Agent(s)	1) 30% restriction in total sleep time for 4 days 2) 0% restriction in total sleep time for 4 days
IND/IDE #	N/A
Study Population	Men and women (age 18-39)
Sample Size	30
Study Duration for individual participants	~1.5 months
Study Specific Abbreviations/ Definitions	Heart rate (HR): the frequency of heart beats over a given time (usually one minute) Resting heart rate (RHR): the frequency of heart beats over a given time (usually one minute) measured at rest Heart rate variability (HRV): term encompassing the time variation between successive heart beats over a given time (usually 5-10 minutes) Interleukin (IL): specialized group of cytokines (i.e., protein); in this paper, the interleukins referenced will be those that are produced and released in response to inflammatory stimuli Rating of Perceived Exertion (RPE): self-perceived measure of effort required from a task

2.0 Objectives

Purpose

The purpose of the proposed study is to differentiate the effects of a multi-day partial sleep deprivation intervention on markers of central hemodynamics, cardio-autonomic function, physical and cognitive performance, as well as overall health in individuals with high vs low cardiorespiratory fitness (CRF). The proposed study would further elucidate the role that CRF plays in the repercussions of poor/insufficient sleep – a homeostatic disruption that is becoming more and more common place in today's age. Delineating this role would ultimately allow for a better understanding of potential factors than can be used to mitigate the deleterious effects of poor/insufficient sleep.

Aims/Hypotheses

Aim 1: To differentiate the effects of a multi-day partial sleep deprivation intervention on central hemodynamics (i.e., central blood pressure and arterial stiffness) in individuals with high vs low CRF.

Hypothesis: Individuals with higher CRF will exhibit a blunted response to the restricted sleep intervention (i.e., lesser increase in both blood pressure and arterial stiffness).

To accomplish Aim 1, we will assess central hemodynamics in individuals with high (i.e., $\geq 75^{th}$ percentile) and low ($\leq 25^{th}$ percentile) CRF (i.e., maximum oxygen consumption capacity [VO_2max]; determined via maximal treadmill testing) following 4 days of normal sleep and 4 days of partially deprived sleep.

Aim 2: To differentiate the effects of a multi-day partial sleep deprivation intervention on overall health (i.e., cardio-autonomic function and serum cytokine levels) and well-being (i.e., psychological mood states) in individuals with high vs low CRF.

Hypothesis: Individuals with higher CRF will exhibit a blunted response to the restricted sleep intervention (i.e., lesser augmentation of cardio-autonomic function, smaller increase in pro-inflammatory cytokines and decrease in anti-inflammatory cytokines, and lesser deviation in mood states).

To accomplish Aim 2, we will assess cardio-autonomic function (i.e., heartrate variability [HRV], resting heart rate [RHR], and recovery score), serum pro- (C-reactive protein [CRP], interleukin-6 [IL-6], IL-1β, and tumor necrosis factor-α [TNF-α]) and anti-inflammatory (IL-10) cytokines, and psychological mood states in individuals with high and low CRF following 4 days of normal sleep and 4 days of partially deprived sleep.

Aim 3: To differentiate the effects of a multi-day partial sleep deprivation intervention on physical (i.e., reactive strength index [RSI]) and cognitive performance (i.e., psychomotor vigilance testing [PVT]) in individuals with high vs low CRF.

Hypothesis: Individuals with higher CRF will exhibit a blunted response to the restricted sleep intervention (i.e., lesser decrease in both RSI and psychomotor vigilance).

To accomplish Aim 3, we will assess RSI, performed during countermovement jump testing as well as PVT tasks in individuals with high and low CRF following 4 days of normal sleep and 4 days of partially deprived sleep.

3.0 Background

Though not fully understood, sleep is accepted to be a paramount mediator of homeostasis (1). Indeed, sleep allows the human body to both recover from as well as restore the metabolic, hormonal, neural, immunological, cognitive, and even emotional processes that it carries out during the waking hours of the day (2–6). This ultimately serves to prime the body for functional optimization the following day, assuming enough sleep is achieved (6–8). Echoing sleep's importance, though from the opposite perspective, is the effect that inadequate sleep has on the body. Disordered or disturbed sleep (i.e., sleep habits/conditions resulting in insufficient sleep quantity and/or quality), for example, has been shown to be associated with impaired cognitive function, hypertension, increased arterial stiffness (i.e., impaired vascular function), increased sympathetic activity, reduced fine-motor skills, as well as undesirable mood states (9–13). In conjunction, many of these symptoms have been shown to increase an individual's risk for cardiovascular disease (CVD) (14). This ominous relationship between poor sleep and CVD risk is particularly noteworthy given that approximately one-third of the American population do not achieve the amount of sleep recommended by the American Academy of Sleep Medicine (15,16). Moreover, this chronic sleep insufficiency represents a state of partial sleep deprivation (PSD) (5) and poses a legitimate threat of cardiovascular events (17–19). This is particularly noteworthy, as acute bouts of PSD, defined as two or more consecutive nights of insufficient sleep (20), are common in young adults, especially college students (21,22), putting them at an increased risk of an event.

Of the CVD risk factors, much emphasis is placed on those that are modifiable given their value in intervention-based approaches for reducing risk. CRF is the focus of many of these approaches due to its robust, inverse relationships with CVD risk as well as all-cause mortality (23–25). Multiple mechanisms exist that aid in explaining the beneficial effect of fitness on CVD risk, with several being anti-atherosclerotic in nature (26). Pertinently, high CRF has been shown to be associated with improved endothelial function (27,28) as well as reduced pro-inflammatory biomarkers (i.e., CRP, IL-6, IL-1β, and TNF-α) (29,30) and increased anti-inflammatory markers (i.e., IL-10) (31) in the plasma that are relevant to CVD manifestation (32,33). Additionally, the relation between CRF and heart rate variability (HRV; a marker of autonomic nervous system function/balance) have been observed (34), concomitant with HRV's relation with vascular function (34,35). Thus, the notion exists that the link between CRF and CVD risk is multifactoral. Indeed, factors such as blood glucose and lipid profiles, dietary habits, and body composition have all been shown to be related to both CRF and CVD risk (26).

Due to the numerous, positive effects stemming from being cardiovascularly fit, many of which pertain to the outcomes that link insufficient sleep to increased CVD risk (e.g., hypertension, increased arterial stiffness, autonomic imbalance, and a pro-inflammatory cytokinetic environment) (27,28,34,36), it stands to reason that high CRF could play a cardio-protective role in blunting the body's response to disordered and/or disturbed sleep. Therefore, **the overall objective of this study** is to differentiate the effects

of an acute partial sleep deprivation intervention on markers of cardio-autonomic function, physical and cognitive performance, as well as overall health in individuals with high vs low CRF.

3.1 While the current literature is clear on the deleterious effects of disordered or disturbed sleep (i.e., sleep habits/conditions resulting in insufficient sleep quantity and/or quality) on an individual's CVD risk, a paucity exists in characterizing the potentially protective role that high CRF, an accepted CVD risk mitigator, may play in that relationship.

3.2 A small handful of preliminary data exist showcasing the effectiveness of high CRF in dampening negative responses to sleep deprivation on cognitive health and performance *(37–40)*, though these studies were either conducted in rat models and/or did not deal with physiological markers directly associated with CVD risk. Thus, these data indicate and bolster the necessity for further research to aid in the understanding of the impact of CRF in protecting against the negative ramifications of insufficient sleep.

3.3 The rationale and significance of the research based on the existing literature lies in the potential for identifying a viable health-promoting and performance-enhancing practice. This research will add to existing knowledge by being the first to distinguish the effects of acute partial sleep deprivation in individuals with high vs low CRF on metrics of central hemodynamics, overall health and well-being, and physical and cognitive performance in humans, thereby exemplifying novelty and thus, warranting research.

4.0 Study Endpoints

4.1 The primary and secondary study endpoints are as follows:

- Do individuals with higher CRF exhibit differing central hemodynamic responses to acute partial sleep deprivation compared to individuals with lower CRF.
- Do individuals with higher CRF exhibit differing central cardio-autonomic function responses to acute partial sleep deprivation compared to individuals with lower CRF.
- Do individuals with higher CRF exhibit differing central serum cytokine responses to acute partial sleep deprivation compared to individuals with lower CRF.
- Do individuals with higher CRF exhibit differing central psychological mood state responses to acute partial sleep deprivation compared to individuals with lower CRF.

- Do individuals with higher CRF exhibit differing central RSI responses to acute partial sleep deprivation compared to individuals with lower CRF.
- Do individuals with higher CRF exhibit differing central PVT responses to acute partial sleep deprivation compared to individuals with lower CRF.

4.2 Describe any primary or secondary safety endpoints.

5.0 Investigational Test Articles or Products

5.1 Device Description:
The WHOOP band (Boston, MA) is a wrist-worn multi-sensory biometric and cardio-autonomic function monitor. The device primarily collects continuous heart rate data and determines various metrics of well-being and cardio-autonomic (e.g., sleep, heart rate variability, resting heart rate, respiration rate, and recovery) via use of proprietary algorithms established by the company.

5.2 Device Handling/Usage:
The WHOOP band will be worn on the participant's non-dominant wrist throughout the entirety of the project timeline (~1.5 months), excluding anytime that the device might become submerged in water for extended periods of time (e.g., swimming or bathing). In order to use the device, the participant will need to and be instructed on how to download and use the smartphone application that accompanies the device. Upon completion of the study, the participant will return the device, and all accompanying equipment (i.e., device charger) to the primary investigator.
The Bod Pod (Cosmed, Chicago, IL) will be used to assess the participant's body composition during the initial visit. In order to perform the test, the participant will sit within in the device for a short period of time (~2 minutes) while the device measures the amount of air that is displaced by the participant (i.e., amount of air in the device while empty minus the amount of air in the device with the participant in the device). The air displacement value will then be used to estimate body composition via an automated program as part of the Bod Pod software.
The metabolic cart (Parvomedics; Salt Lake City, UT). will used to assess oxygen consumption and carbon dioxide production (i.e., metabolic testing) during the VO_{2max} protocol, described in detail below. In order to use the device, the participant will don a face mask with a Hans Rudolph valve attached to it. The mask and valve allow for the inhalation of room air (i.e., normal breathing) while measuring expired air, via the cart, to establish metabolic values. Further, the mask will be worn for the duration of the test (~8-12 minutes).
The SphygmoCor Xcel device (AtCor Medical, New South Wales, Australia) will be used to assess central hemodynamics (i.e., central blood pressure) and arterial stiffness following the protocols set forth in the attached operations manual. In short, the participant will lie in a supine position while wearing

two cuffs, one on their right arm and one on their right upper thigh. The arm cuff will be used for central blood pressure measurement following standard blood pressure testing. The thigh cuff will be used to measure arterial stiffness via applanation tonometry, a process involving the use of a tonometer to palpate for the participant's carotid pulse and assessing the time difference between the carotid pulse and femoral pulse (measured with the thigh cuff) for each heartbeat, resulting in the estimation of pulse transit time, a proxy for arterial stiffness.

The treadmill (4Front; Woodway USA, Waukesha, WI) will be used during the VO_{2max} test following the protocol outlined in the *VO_{2max} Testing Protocol* below.

The heart rate monitor (H10; Polar, Kempele, Finland) will used to assess the participants heart rate during the VO_{2max} test. The participant will wear the device on the surface of the skin, superficial to the xyphoid process of the sternum (i.e., middle of the chest).

The Sway Medical application (Tulsa, OK) will be used to assess cognitive performance via two short (~1 minute) tests, reaction time and impulse control. In order to use the application, the participant will need to and be instructed on how to download and use the application. In short, both tests begin with a blank screen. For the reaction time test, participants will be tasked with tapping the blank screen as quickly as possible once the screen changes colors. For the impulse control test, the blank screen will display either a check mark or an X. Participants will be tasked with tapping the blank screen as quickly as possible if the check mark is displayed and avoiding tapping the screen if the X is displayed.

5.3 N/A

5.4 N/A

6.0 Procedures Involved

6.1 This will be a randomized, crossover study designed to differentiate the effects of an acute partial sleep deprivation intervention on markers of central hemodynamics, cardio-autonomic function, physical and cognitive performance, as well as overall health in individuals with high vs low CRF. The study, outlined in Figure 1 and described in detail below, will consist of 5 total visits to the Institute of Sports Sciences and Medicine (ISSM) located on the main campus of Florida State University. On the first visit, participants will report to the ISSM to be informed of the details of the study before giving their oral and written informed consent. Thereafter, participants will be assessed for exercise training status (i.e., trained vs sedentary), body composition, and maximal oxygen consumption capacity (i.e., VO_{2max}). Participants will be dichotomized into one of two test groups – fit or unfit – based on both self-reported training status and VO_{2max} values. The utilization of both training status and VO_{2max} will better allow the selection of individuals with high CRF values not due

primarily to genotype – a known confounder *(41)*. The American College of Sports Medicine's classifications of training status (i.e., <150 minutes of moderate-intensity and/or <75 minutes of vigorous aerobic exercise per week for the last 3 months) as well as normative VO_{2max} values will be used to dichotomize the groups with the fit (and chronically-trained) and unfit (and sedentary) group having values in the upper and lower quartiles (i.e., $\geq 75^{th}$ percentile and $\leq 25^{th}$ percentile) for their age and sex, respectively *(42)*. This equates to a VO_{2max} value of ≥55.2 and 44.7 ml/kg/min for fit males and females, respectively, and ≤40.1 and 30.5 ml/kg/min for unfit males and females, respectively. Participants will then be provided with a wearable, wrist-worn sleep and cardio-autonomic function monitor, to be worn on the non-dominant wrist throughout the entirety of the project timeline, including a two-week initialization period to establish baseline sleep cardio-autonomic data. This initialization period will take place following consent and prior to the first data collection visit. Next, participants will then be familiarized with daily sleep, physical activity, and 3-day nutrition logs as well as a PVT smart phone application, all of which will be performed throughout the study. Lastly, participants will be assessed for sleep chronotype via a morningness-eveningness questionnaire to inform any potential differences in sleep intervention response post hoc. Following the initialization period, participants will be randomly assigned to either a normal or partial sleep deprivation intervention, both of which will last 4 days. The partial sleep deprivation intervention will consist of prescribed bed and wake times eliciting a 30% reduction in habitual time in bed, identical to a protocol utilized by Roberts, et al. *(43)*. Following the first sleep intervention period, participants will again report to the ISSM for the first of four data collection visits (i.e., Post-Test 1). Data collection will consist of assessment of central hemodynamics, cardio-autonomic function, physical and cognitive performance, as well as overall health. Participants will then undergo a 7-day washout/ post-intervention observation period, in which they will continue to track and log their sleep and perform daily PVT to allow for recovery evaluation in those metrics, prior to crossing over to the other sleep intervention. During the washout period, participants will also return to the lab to repeat the blood work and CMJ testing to allow for recovery assessment in those values (i.e., immediate post-intervention vs 3-day post-intervention values; Recovery 1). Following completion of the washout/observation period, participants will crossover to the other sleep intervention and repeat the testing protocols (i.e., Post-Test and Recovery 2). Data collection visits will occur at the same approximate time of day for each individual to account for diurnal patterns in the data collected. In total, the protocol will last approximately 5 weeks and consist of 5 visits.

6.2 Research Procedures

Initial Visit: All data collection time points are outlined in Figure 1 below. Prior to participation, voluntary consent to participate will be obtained. Eligible participants (i.e., those deemed fit to participate via the Physical Activity Readiness Questionnaire [PAR-Q+] in conjunction with the American College of Sports Medicine guidelines for physical activity) will then complete a questionnaire to establish typical training characteristics (i.e., frequency, intensity, time, type, and history). Specifically, the PAR-Q+ screens individuals for signs or symptoms of cardiovascular, metabolic, and or renal disease which would make it inadvisable for them to participate in physical activity. Individuals who are not deemed fit to participate, according to the PAR-Q+, will not be allowed to continue with participation. They will then be assessed for body composition (Bod Pod; Cosmed, Chicago, IL) prior to performing a maximal treadmill test (protocol described below) to determine maximal oxygen consumption capacity (VO_{2max}). In the case that an individual does not qualify for the study (i.e., those meeting requirements for training status and VO_{2max}), all data collected on that individual will be destroyed. Individuals who do qualify will be provided with a WHOOP (Boston, MA) wearable, wrist-worn multi-sensory biometric and cardio-autonomic function monitor. The WHOOP band will be worn on the non-dominant wrist throughout the entirety of the project timeline (~1.5 months), including a two-week initialization period prior to the first data collection visit. Participants will then be familiarized with daily sleep, physical activity, and 3-day nutrition logs as well as a PVT smart phone application, all of which will be performed throughout the study. Specifically, participants will complete the sleep and physical activity logs and PVT task daily throughout the enrollment period, while they will complete the 3-day nutrition log in the 3 days immediately prior to Post Test 1 and 2 visits (described in detail below). Lastly, participants will be assessed for sleep chronotype via a morningness-eveningness questionnaire to inform any potential differences in sleep intervention response post hoc.

VO_{2max} Testing Protocol: Testing will take place on a motorized treadmill (Woodway USA, Waukesha, WI). Oxygen consumption will be measured via a Parvomedics TrueOne 2400 metabolic cart (Salt Lake City, UT). Each testing stage will last for two minutes, and heart rate (HR) and rating of perceived exertion (RPE) will be recorded in the last 15-20" of each stage. The participant will begin at a speed of 3 miles per hour for the warm-up stage. Upon completion of the warm-up the speed will be increased to 5 miles per hour. The speed will be increased by 1.5 miles per hour for each successive stage until an RPE of 13 or greater is achieved. Following this, the speed will remain constant for the remainder of the test. For the following stage, the grade of the treadmill will increase to 2%, and will be increased by an additional 2% for each subsequent stage until volitional exhaustion. Attainment of maximal oxygen consumption will be ensured by satisfying two of three criteria: plateau in oxygen consumption with increasing workload, respiratory exchange rate (RER) >1.1, or RPE >17. Standardized verbal encouragement will be provided throughout the test. Immediately after the test, participants will be given a three-minute cooldown period in which they will be asked to walk at 3 mph.

Sleep Intervention: Following the two-week initialization period, participants will be randomly assigned to either a normal or partial sleep deprivation intervention, both of which will last 4 days. The last week of the two-week initialization period will be used to

characterize typical sleep duration (i.e., total sleep time, total time in bed, and typical bed/wake times [values not dependent on initialization of WHOOP device]). During the normal sleep intervention, participants will be prescribed bed and wake times consistent with those observed to ensure the maintenance of typical sleep. The partial sleep deprivation intervention, conversely, will consist of prescribed bed and wake times eliciting a 30% reduction in habitual time in bed, identical to a protocol utilized by Roberts, et al. (43). Specifically, wake times will be individualized to the participant's typical sleep habits determined previously, while delayed bed times will be prescribed to achieve the 30%-time reduction. Administration of the partial sleep deprivation intervention in this manner will allow for consistent time intervals between waking and data collection between both sleep interventions, thus mitigating the effects of diurnal patterns altered by intervention. Intervention compliance will be monitored via use of the sleep data collected with the WHOOP bands as well as the daily sleep logs. Those who deviate from the prescribed in-bed and out-of-bed times by more than 30 mins will be asked to restart the intervention. During both interventions, participants will be asked to maintain typical physical activity, including the timing of said activity, and eating habits as well as complete the physical activity and nutrition logs to characterize those lifestyle habits.

Experimental Visits: Following the first sleep intervention period, participants will come in for the first of four data collection visits (i.e., Post-Test 1). Data collection visits will begin of the completion of the Pittsburgh Sleep Quality Index (PSQI) and Profile of Mood States (POMS) questionnaire to characterize sleep quality and psychological mood states, respectively. Next, central arterial pulse wave form analysis and pulse transit time (carotid-femoral PWV [cf-PWV]; via applanation tonometry) will be assessed using a SphygmoCor XCEL device (AtCor Medical, New South Wales, Australia), followed by a standard venipuncture procedure to allow for inflammatory marker characterization, total blood cell counting, as well as blood lipid and glucose profiling to account for known hemodynamic confounders (26). The venipuncture procedure will be performed by the primary investigator (certified phlebotomist trained; 5+ years of experience) following the attached standard operating procedure for venipuncture and will entail the collection of 10-30 mL of blood, as is typical of a standard blood venipuncture procedure. If the primary investigator is unavailable to perform the procedure or is unable to do so, the Clinical Research and Trials Unit will be utilized. Lastly, participants will perform a short countermovement jump (CMJ) test to quantify their reactive strength index (RSI).

Participants will then undergo a 7-day washout/ post-intervention observation period, in which they will continue to track and log their sleep and perform daily PVT to allow for recovery evaluation in those metrics, prior to crossing over to the other sleep intervention. During the washout period, participants will also return to the lab to repeat the blood work and CMJ testing to allow for recovery assessment in those values (i.e., immediate post-intervention vs 3-day post-intervention values; Recovery 1). Following completion of the washout/observation period, participants will be assigned to the other sleep intervention in a crossover fashion and complete the second 4-day intervention before returning for another data collection visit (i.e., Post Test 2) identical to Post Test 1. They will then enter another 7-day post-intervention observation period and performing a second recovery data collection visit (i.e., Recovery 2). Data collection visits will occur at the same

approximate time of day for each individual to account for diurnal patterns in the data collected. In total, the protocol will last approximately 5 weeks and consist of 5 visits.

Central Hemodynamic Assessment: Participants will be assessed for central arterial pulse wave form and cf-PWV following both sleep interventions with re-analysis occurring 3 days later to quantify the response to, and recovery from, the two sleep interventions between the two groups. The central hemodynamic (i.e., central blood pressure, augmentation index [absolute and relative to a heart rate of 75 beats per minute], mean arterial pressure, and pulse pressure) and arterial stiffness (i.e., cf-PWV) variables of interest will be collected and analyzed following methods identical to those utilized by Grosicki, et al. (34).

Health and Well-Being Assessment: Both metrics of sleep quality and cardio-autonomic function (i.e., resting heart rate, heart rate variability, and a proprietary, WHOOP-generated recovery score) will be collected via the WHOOP devices. The wearing of the device throughout the entirety of the project timeline will allow for the quantification of these measures in response to, and recovery from, the two sleep interventions, which will be compared between the two groups. Further, data from the devices will be analyzed following protocols set forth by Greenwalt, et al. (44). Using blood samples collected during the venipuncture protocols performed at the four data collection visits, sleep intervention response and recovery levels of circulating plasma concentrations of pro- (CRP, IL-6, IL-1β, and TNF-α) and anti-inflammatory (IL-10) cytokines will be assessed using protocols similar to that outlined by Smith, et al. (45). Additionally, portions of the blood samples will undergo hematologic analysis to characterize shifts in the composition of immune cells through methods similar to those of Schluter et al. (46). Finally, POMS data will be used to evaluate intervention-associated shifts in psychological mood states between the two groups following the methodology of Shacham (47).

Physical and Cognitive Performance Assessment: Group comparisons of intervention response and recovery in physical performance will be evaluated via RSI testing performed during repeated CMJ testing using the guidelines set forth by Jarvis, et al (48). RSI, a measure of an individual's ability to transition from an eccentric muscular contraction to a concentric, serves as a more sensitive marker of power production (i.e., a proxy for overall muscular fitness) (48) than CMJ height alone. Cognitive performance, on the other hand, will be quantified using a daily PVT task and used to track and compare changes in response time following both interventions between the two groups through methods similar to those of Sauvet, et al. (37).

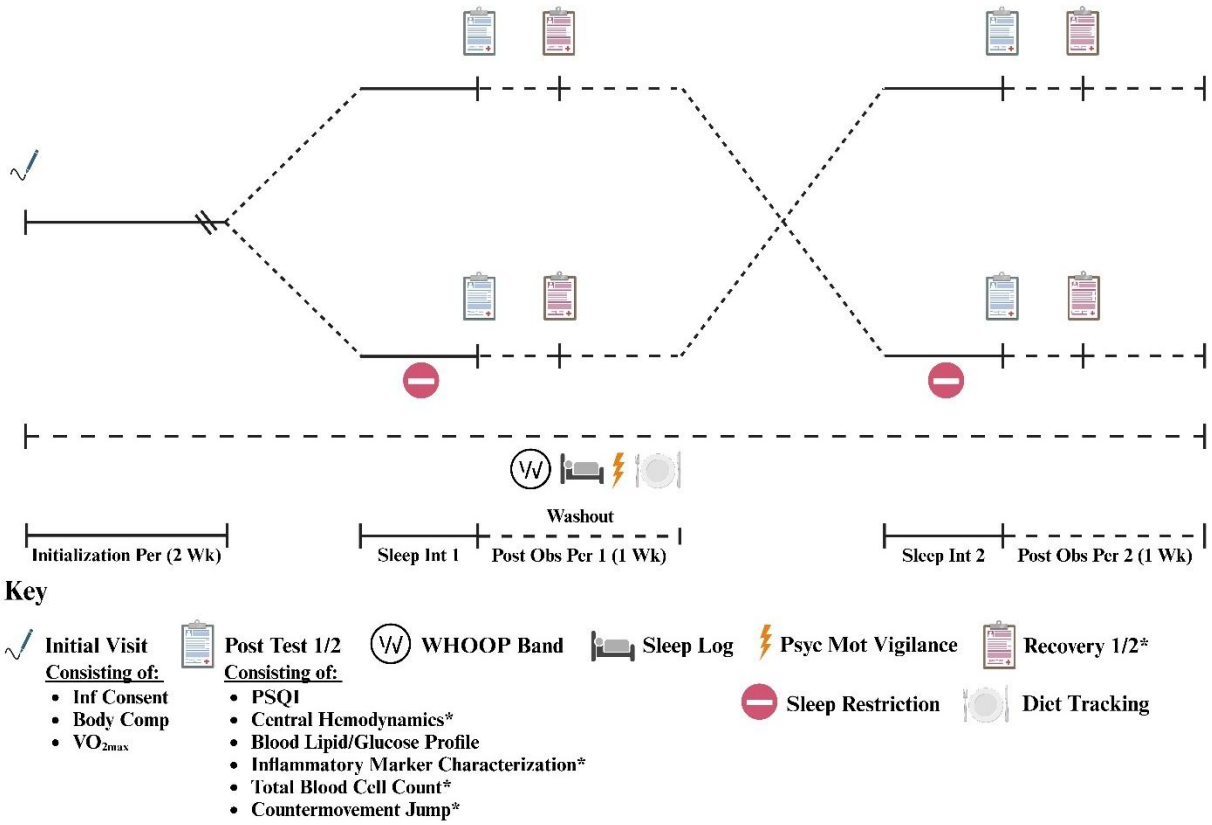

Figure 1. Data Collection Overview

6.3 Describe:

- N/A
- N/A

6.4 Biochemical and metabolic data will be collected via antecubital venous blood draw during the study. Further, surveys, questionnaires, and physical outputs will be collected throughout experimental trials.

6.5 N/A

6.6 N/A

7.0 Data and Specimen Banking

7.1 Blood specimens will be kept in closed containers within the ISSM freezer (-80° C) behind two locked doors for batch analysis. After the analysis of the data, specimens will be maintained for 3 years. Data will be kept on password protected FSU computers or in physical copies behind locked drawers and doors in the PI's office. The verified research team are the only people who will have access to the data or specimens.

7.2 The information will be included in the data or associated with the specimens will be coded and unidentifiable without the code. The

code will be kept in physical copy behind two locked doors and a third locked drawer, or in electronic copies in a password protected file.

7.3 The PI will be responsible for receipt or transmission of the data or specimens until graduation. After such event, the co-investigator, Dr. Ormsbee, will be responsible for receipt or transmission of data or specimens.

7.4 N/A

8.0 Sharing of Results with Subjects

8.1 Individual subject results for anthropometric data will be provided upon the completion of the study protocol. The results will be shared exclusively with the individual via print or word of mouth.

9.0 Study Timelines

9.1 Describe:

- The duration of an individual subject's participation in the study is ~1.5 months (~35 days).
- The duration anticipated to enroll all study subjects is 18 weeks.
- The estimated date for the investigators to complete this study (complete primary analyses) is December 2025.

10.0 Inclusion and Exclusion Criteria

10.1 Eligibility.

Inclusion Criteria:

Men and women will be recruited to participate in this study. Inclusion criteria will be as follows:

a) Age: 18-39 yrs

b) Active (>150 minutes of aerobic exercise per week)

c) Sedentary (<150 minutes of aerobic exercise per week)

Exclusion Criteria:

Participants will be excluded form participation in the study for the following reasons:

a) Participants known to have chronic, uncontrolled metabolic, cardiovascular, or hepatic disease including:

   a. Diagnosed cardiovascular diseases or previous myocardial infarction

   b. Uncontrolled hypertension

   c. Diabetes (Type 1 or 2)

d. Uncontrolled thyroid conditions

b) Participants known to have injuries limiting exercise capacity
c) Participants classified as grade III obese (BMI: ≥40 kg/m^2)
d) Participants known to be pregnant

10.2 N/A

10.3 None of the aforementioned special populations will be included in this research.

11.0 Vulnerable Populations

N/A

12.0 Local Number of Subjects

12.1 30

12.2 The PI anticipates <20% attrition in this population, therefore the PI plan to recruit no more than 36 participants to ensure a final number of 30 successfully complete the protocol.

13.0 Recruitment Methods

13.1 Participants will be recruited from the general population of Tallahassee, FL and surrounding rural areas within reasonable commuting distances. Participants will be recruited via public service announcements, social media, and flyers posted on campus, in health clinics and fitness centers, and in employment development centers.

13.2 The source of subjects will be from active local members of the community who meet the inclusion criteria.

13.3 The PI will, in the recruitment materials, include general inclusion and exclusion criteria, as well as the investigators' contact information. Individuals interested in participating in the study will contact an investigator (via telephone or email). Volunteers interested in participating will then be scheduled to meet with the investigator to have the study and what is expected of them explained in detail and have their questions answered. Prior to the initiation of any tests or measurements, an informed consent to participate in the study will be obtained in writing by the investigator.

13.4 Participants will be recruited via public service announcements, social media, and flyers posted on campus, in health clinics and fitness centers, and in employment development centers. (Copy attached)

13.5 Upon completion of the study, participants will be compensated for their participation will a $100 VISA gift card, an amount comparable to past studies similar in nature and extent. Complete participation is required to earn the gift card.

14.0 Withdrawal of Subjects

14.1 Subjects will be withdrawn from the research without their consent if they do not comply with prescribed bed and wake times for the entirety of either intervention.

14.2 Upon discovering the adherences problem, participants will be contacted via telephone or face-to-face and politely told they are disqualified from continuing the study due to adherence.

14.3 If participants withdraw of their own volition or are withdrawn by the PI, the research team will notify them via phone call or word of mouth in person. All data collected up until the point of withdrawal may be used at the PI's discretion for the results.

15.0 Risks to Subjects

15.1 The reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research include:

- Physical discomfort from intravenous blood draws: Yet, blood will be taken by researchers previously trained and experienced in phlebotomy, following the biological safety procedures in place for a level two laboratory.
- Possible inconvenience of missing work if they adhere to a traditional work schedule: Yet we can schedule experimental visits early in the morning before traditional hours to accommodate these needs.
- Possible risk of adverse cardiac event or injury during maximal treadmill testing: Yet, the established risk of event is 6 in 10,000 and the tests will be performed by researchers previously trained and extensively experienced (10+ years) in administering the test.

15.2 Unforeseeable risks may include the expression of a genetic condition that was not "caught" by the screening process. Yet, efforts will be made to ask the subject if there is any reason why they think it is to their detriment if they participate in this protocol.

15.3 N/A. Pregnant women will not be included in this study

15.4 Blood will be collected following biological safety procedures in place for a level 2 laboratory. However, accidental needle sticks or exposure to potential pathogens via blood is always a possibility. In such a case, wounds will be cleaned and bandaged appropriately and incidents will be reported.

16.0 Potential Benefits to Subjects

16.1 The potential benefits that individual subjects may experience from taking part in the research include gathering important metrics for

their health and fitness. Depending on the outcome of the study, participants may also discover a viable health-promoting practice.

16.2 N/A

17.0 Data Management and Confidentiality

17.1

Sample Size Estimation

Sample size requirements were determined via a priori power analyses using G*Power software (Ver. 3.1.9.4; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to ensure sufficient statistical power. To inform the expected effect size, we referenced Sunbul, et al. *(49)* who reported significantly higher wave velocity (PWV) values (5.33±0.46 m/s) following one night of total sleep deprivation compared to one night of normal sleep (5.15±0.26 m/s; p=0.001) in young, healthy males and females. Acknowledging that this will consist of an acute partial sleep deprivation intervention, a Cohen's f value of 0.24 was used in conjunction with an α level of 0.05, power of 0.80, with 2 groups (crossover design; 4 measures each), and a conservative correlation among repeated measured of 0.5. This resulted in a sample size estimate of 24 participants. The planned sample size was then increased based on the differences between the current study, the previous work, and the assessment of several secondary outcomes, as well as to account for expected attrition (~20%). Therefore, 30 participants will be recruited to maximize statistical power and account for expected attrition.

Statistics

Data will be presented as means ± SD if normally distributed and assessed for normality via a Shapiro-Wilk Test. Assuming normality, all variables will undergo null-hypothesis testing with the α level set to 0.05. In the case that normality is violated, the appropriate non-parametric alternatives will be utilized. Analyses will be conducted using SPSS version 29.0 (SPSS, Inc., Chicago, IL).

Analysis of Variance: Central hemodynamic and arterial stiffness variables will be interpreted using a mixed-model (i.e., within-between) repeated measures analysis of variance (RMANOVA) to identify interactions between multiple groups and conditions. Specifically, to test the hypothesis that individuals with higher CRF would demonstrate a blunted arterial stiffness response (i.e., maintained pulse transit times) to the acute partial sleep deprivation intervention, cf-PWV will be assessed following 4 consecutive nights of both normal sleep and restricted sleep and compared between the two groups. Likewise, all secondary variables will be assessed at pre-specified time points using a within-between interaction RMANOVA to test the hypotheses that individuals with higher CRF would demonstrate a blunted effect of partial sleep deprivation on markers of physical and cognitive performance (i.e., maintained RSI and PVT, respectively) as well as on markers of overall health (i.e., maintained cardio-autonomic function parameters, pro- and anti-inflammatory serum cytokine levels, and mood states).

In the case that sphericity is violated, a Greenhouse-Geisser correction will be performed. In the case of significance, a post hoc one-way ANOVA with Bonferroni tests will be used to identify significant differences. Effect sizes (ES) will be calculated by standardizing mean differences to the standard deviations (SD) of the placebo condition. Effect sizes will be qualified as follows: small $d = 0.2$, moderate $d = 0.5$, and large $d \geq 0.8$ (50).

17.2 The data will be kept secure through several routes. First, only the CITI certified research team will be allowed to be involved in the data collection process. Further, after acute bouts of data collection occur, all data will be collected together by the PI. If the data is physical, it will be kept in a locked drawer, behind a locked laboratory door. If the data is electronic, it will be kept on a password secured computer that only the PI may gain access. All human data will be coded, such that without the code it becomes unidentifiable.

17.3 Quality control of collected data will be ensured by controlling as many external variables as is reasonable. Further, biological measures (primary outcomes) will be measured in duplicate with the average of each measure used in the final assessment.

17.4 Describe how data or specimens will be handled study-wide:

- The information will be included in the data or associated with the specimens will be coded and unidentifiable without the code. The code will be kept in physical copy behind two locked doors and a third locked drawer, or in electronic copies in a password protected file.
- Specimens will be kept in closed containers within the ISSM freezer (-80° C) behind two locked doors for batch analysis. After the analysis of the data, specimens will be maintained for 3 years. Data will be kept on password protected FSU computers or in physical copies behind locked drawers and doors in the PI's office.
- Specimens will be stored for 3 years after initial analysis.
- The verified research team are the only people who will have access to the data or specimens.
- The PI will be responsible for receipt or transmission of the data or specimens until graduation. After such event, the co-investigator, Dr. Ormsbee, will be responsible for receipt or transmission of data or specimens.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

18.1 Describe*:*

- The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe will occur throughout each experimental visit.

- Any unanticipated adverse events reported will be recorded.
- The safety information will be collected at every study visits.
- The frequency of data collection, including when safety data collection will occur at every study visit.
- The PI and the research team will review the data. If further review is necessary, the research team will recruit the associate dean of research for the College of Education, Health, and Human Sciences.
- N/A
- N/A
- N/A

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Only the research team will have access to personal information. Furthermore, it will be coded to protect participant privacy.

19.2 The research team will be kind, gentle, and explain every step of the research protocol to the participant prior to conducting each test of the experiment. Together, these conversations and familiarizations, paired with a friendly demeanor, will promote the participants of the study feeling at ease.

19.3 The research team may only access personal information about participant's by going through the PI. The PI is the only person who has access to the password protected computer and key to the locked drawer. The password and key will not be given out, ensuring the bottleneck of information going through the CITI trained PI.

Further, the data collected via the WHOOP and Sway Medical app will be provided, by the participant, to the PI for analysis of the data needed for study participation.

20.0 Compensation for Research-Related Injury

20.1 If the participant needs medical care because of taking part in this research study, contact the investigator and medical care will be made available. Generally, this care will be billed to the participant's insurance, or other third party. Florida State University has no program to pay for medical care for research-related injury.

21.0 Economic Burden to Subjects

21.1 There are no anticipated costs that subjects may be responsible for due to participation in the research.

22.0 Consent Process

22.1 Indicate whether you will you be obtaining consent, and if so describe*:*

- Consent will take place within the ISSM research laboratory.
- The prospective participant may take as long as they would care to between being informed and signing the informed consent. This is completely up to their discretion.
- Ongoing consent will be ensured via weekly check-ins with regard to their adherence to the supplementation protocol.
- Yes, we will follow "SOP: Informed Consent Process for Research (HRP-090)."
    - Only the trained PI or a co-investigator will be permitted to lead the informed consent process with the potential participant.
    - The discussion of informed consent will take as long as is needed for the potential participant to feel informed and comfortable about their voluntary participation in this research.
    - We will emphasize that this is completely voluntary and that the participant is able to refuse or withdraw from the study at any time.
    - To ensure the subjects' understanding, we will ask the participants to repeat back to us the perceived risks and benefits.
    - The informed consent document will be at a 10th grade reading level, however as previously mentioned all topics will be read to the participants and explained.

Non-English Speaking Subjects

- There are no current indications that our subjects will not speak English. If a potential participant would like to take part in the study and meets the inclusion criteria appropriate accommodations will be made to ensure their consent is informed in their language. Accommodations will include a non-English consent form.
- N/A
- N/A

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure you have provided sufficient information for the IRB to make these determinations.*

Subjects who are not yet adults (infants, children, teenagers)

- N/A

Cognitively Impaired Adults

- N/A

Adults Unable to Consent

- N/A

Adults Unable to Consent

- N/A

23.0 Process to Document Consent in Writing

23.1 Yes, the "SOP: Written Documentation of Consent (HRP-091)" will be followed.

23.2 N/A

23.3 Please see the attached Informed Consent Document

24.0 Setting

24.1 Describe the sites or locations where your research team will conduct the research.

- Participants will be recruited from the general population of Tallahassee, FL and surrounding rural areas within reasonable commuting distances. Participants will be recruited via public service announcements, social media, and flyers posted on campus, in health clinics and fitness centers, and in employment development centers.
- Research will be conducted with the Institute of Sport Sciences and Medicine (ISSM) on FSU's main Tallahassee campus.
- N/A
- *For research conducted outside of the organization and its affiliates describe:*
    - N/A

25.0 Resources Available

- Between various recruitment locations mentioned, we anticipate an ample amount (>50) of eligible subjects. As the power analysis revealed a required sample size of 30, the PI does not anticipate any issues regarding recruitment.
- Th PI aims to complete data collection no later than Spring 2026 and complete data analysis and writing no later than Summer 2026.
- The ISSM is a well-funded, fully stocked human performance laboratory. It has all the equipment and space needed to fully support this research. The facilities are more than satisfactory for the completion of this study.
- First Aid, automated external defibrillator (AED), fire extinguishers, and showers are available at each location. All data collection visits will be attended by CPR-trained staff. Although unlikely, any medical needs beyond the scope of first aid will consist of a researcher escorting the participant to the FSU medical center on campus.
- The PI will have several meetings with each person in the research team going over each individual role. This will help ensure that each member of the research team is adequately informed about the protocol, the research procedures, and their duties and functions.

26.0 Multi-Site Research

- N/A

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