# Sleep and Circadian Mechanisms of Non-dipping Blood Pressure (protocol and statistical analysis plan)

ClinicalTrials.gov ID: NCT03558893

Oregon Health & Science University

IRB Protocol: 16803

OREGON HEALTH & SCIENCE UNIVERSITY

IRB MEMO

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APPROVAL OF SUBMISSION

September 24, 2025

Dear Investigator:

On 9/24/2025, the IRB reviewed the following submission:

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|---|---|---|---|
| IRB ID: | STUDY00016803 | MOD or CR ID: | MODCR00032985 |
| Type of Review: | Modification and Continuing Review | | |
| Title of Study: | Sleep and Circadian Mechanisms Contributing to Disparity in Prevalence of Hypertension Between Black and White Americans | | |
| Title of modification | Continuing Review 2025 | | |
| Principal Investigator: | Steven Shea | | |
| Funding: | Name: DHHS NIH Natl Inst on Minority Hlth & Hlth Disp, PPQ #: 1012376; Name: DHHS NIH Natl Heart, Lung, and Blood Inst, PPQ #: 1018256, Funding Source: 1K01HL151745-01A1; Name: DHHS NIH Natl Ctr for Advancing Translational Sciences, PPQ #: 1010312; Name: DHHS NIH Natl Heart, Lung, and Blood Inst, PPQ #: 1017894; Name: Medical Research Foundation of Oregon, PPQ #: 1017672; Name: OHSU Foundation, PPQ #: 1022159; Name: DHHS NIH Natl Heart, Lung, and Blood Inst, PPQ #: 1012376, Funding Source: 3R01HL142064-02S1 | | |
| IND, IDE, or HDE: | None | | |
| Documents Reviewed: | • DIP_screening-consent_blooddraw_2020-12-15 (3).pdf<br>• Cohen's social support scale<br>• Research Match Ad for Remote Focus Groups<br>• HIPAA WoA<br>• Test - PVT<br>• General Self-Efficacy Scale<br>• resilience scale.docx<br>• Cohen's perceived stress<br>• Focus Group Interview Guide<br>• Questionnaire - PROMIS Sleep Disturbance<br>• Memo9102019.docx | | |

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|---|---|
| | • Questionnaire - POMS<br>• Instructions - 24-h Urine<br>• Focus Flyer<br>• Information Release Form<br>• Sleep Diary<br>• Questionnaire - PROMIS Sleep Related Impairment<br>• Online Ad - Mealtracking.pdf<br>• Final Portland Observer Ad (non-stamp)<br>• MRF application<br>• Shea R35 grant.pdf<br>• Memo<br>• Website Adv_Leaderboard_DIP.pdf<br>• DSMP<br>• PSRS<br>• K01_Jan21toDec25.pdf<br>• Questionnaire - Exit<br>• DIP_bike_questions.pdf<br>• Interview Demographics<br>• Package Insert - Heparin<br>• Information Sheet - DIP Supplement<br>• Consent and Authorization Form - Mealtrack<br>• Questionnaire - RPE<br>• Questionnaire - BDI<br>• DIP_COVID-screening_questions<br>• Meal Preference Form<br>• HTN_Use-of-Ionizing-Radiation-in-Humans-Form_2017-011-17.docx<br>• RedCap screening document<br>• Appointment Reminder and Instructions<br>• The COVID Stress Scales.pdf<br>• Questionnaire - New VAS<br>• Instructions - MealLogger<br>• Questionnaire - PANAS<br>• Questionnaire - Owl Lark<br>• Questionnaire - SDQ<br>• Study demographics<br>• Please update IRB approval stamp on Main DIP Flyer<br>• 16803_MEMO_2018-01-08.docx<br>• Evaluation of Dance Routine at McCoy Park 8-2-2019.docx<br>• Final Portland Observer ad.pdf<br>• Ordaz_Post-Baccalaureate_Research_Supplement<br>• Questionnaire - Appetite<br>• David Williams Perceived Stress Scale<br>• Questionnaire - Stanford Sleepiness Scale<br>• Fecal collection |

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|---|---|
| | • ResearchMatch Ad - Mealtracking.pdf<br>• Questionnaire - Old VAS<br>• Test - Math<br>• Bowles_KL2Application.pdf<br>• HTN Disparity Grant Application<br>• 2024_OHSU_Facebook_OccHealth_BPStudy.pdf<br>• Consent and Authorization - Main Study<br>• Consent and Authorization - Screening<br>• DIP Flyer Handout-Black Female tabs<br>• DIP Flyer Handout-Males tabs v2<br>• DIP Flyer Handout-Males v1<br>• DIP_Ad_Flyer-BP_tabs_2024-03-19 Black females.pdf<br>• DIP_Radio_Script_(Revised).pdf<br>• DIP_Reason for Non-Participation_Survey-Consent_2024-12-17.pdf<br>• DIP_Reason_for_Non-participation_Email_template_2024-12-4<br>• DIP_website<br>• Faculty Initiative Award Letter.Bowles.2022.pdf<br>• NPB_facultyinitiativepool10222021.pdf<br>• OCTRI 6092 Reason for Non-Participation Survey v2.pdf<br>• OCTRI 6092 Reason for Non-Participation_ Sleep and Circadian Mechanisms Contributing to Disparity in Prevalence of Hypertension between Black and White Americans _ RE.pdf<br>• Online Ad<br>• Phone Script<br>• Please update IRB approval of DIP pamphlet<br>• Protocol<br>• Reason for Non-Participation Survey_2024-11-06.pdf<br>• Redesigned DIP Flyer Blue 1 .pdf.pdf<br>• Redesigned DIP Flyer Blue 2 .pdf.pdf<br>• ResearchMatch Ad |

The IRB granted final approval on 9/24/2025. The study is approved until 9/23/2026.

Review Category: Expedited Category # 8c

Copies of all approved documents are available in the study's **Final** Documents (far right column under the documents tab) list in the eIRB. Any additional documents that require an IRB signature (e.g., IIAs and IAAs) will be posted when signed. If this applies to your study, you will receive a notification when these additional signed documents are available.

**Ongoing PI Responsibilities:**

- Six to ten weeks before the expiration date, submit a continuing review to request continuing approval.
- Submit changes to the project for IRB approval prior to implementation.
- Submit Reportable New Information per OHSU policy.
- Submit a continuing review to close the study when the research is completed.

**Guidelines for Study Conduct**

In conducting this study, you are required to follow the guidelines in the document entitled, "Roles and Responsibilities in the Conduct of Research," as well as all other applicable OHSU IRB Policies and Procedures.

**Requirements under HIPAA**

If your study involves the collection, use, or disclosure of Protected Health Information (PHI), you must comply with all applicable requirements under HIPAA. See the HIPAA and Research website and the Information Privacy and Security website for more information.

**IRB Compliance**

The OHSU IRB (FWA00000161; IRB00000471) complies with 45 CFR Part 46, 21 CFR Parts 50 and 56, and other federal and Oregon laws and regulations, as applicable, as well as ICH-GCP codes 3.1-3.4, which outline Responsibilities, Composition, Functions, and Operations, Procedures, and Records of the IRB.

Sincerely,

The OHSU IRB Office

**Sleep and Circadian Mechanisms Contributing to Disparity in Prevalence of Hypertension between Black and White Americans**

## 1. OBJECTIVES

In US adults, there are large, unexplained racial disparities in hypertension (HTN), a leading cause of morbidity and mortality. We propose to distinguish the relative contribution of sleep and circadian mechanisms to the increased risk for HTN in African American (Black) adults compared to European American (White) adults. The scientific premise for the proposed research, based partly on our recent work, is that both sleep and circadian mechanisms contribute to blood pressure (BP) regulation and HTN, and both sleep and circadian regulation differ between Black and White races. The innovation is to understand to what degree these sleep and circadian factors mechanistically explain this racial disparity in HTN. Recent work shows that Blacks have poorer sleep and a shorter circadian period, resulting in a tendency towards earlier sleep relative to circadian phase. BP usually decreases during sleep and this appears protective as there is an association between a <10% drop in the nocturnal vs. daytime BP (the so-called non-dipping BP) and more adverse cardiovascular (CV) events and mortality. Blacks have increased prevalence of this dangerous non-dipping 24-h BP fluctuation. Recently, we discovered a robust endogenous circadian rhythm in BP, unrelated to activity or sleep, with a circadian drop in BP across the biological night that likely contributes to nocturnal BP dipping. The diurnal variation in BP is the result of a summation of the effects on BP of varied behaviors across the day and night (e.g., sleep, waking up, exercise, other stresses) and the effects on BP of the circadian system. We plan to examine these interacting factors in 26 Black and 26 White adults, aged 30-60 years, to determine sleep and circadian mechanisms that may contribute to higher overall BP and reduced nocturnal drop in BP in Blacks. This will be achieved by using an intensive multi-day protocol where sleep-wake cycles are adjusted to 18-h so all behaviors occur evenly across all circadian phases while in a constant dim light (to avoid light resetting circadian phase). Sleep will be assessed with polysomnography and circadian phases by core body temperature. By studying standardized behaviors and regulators of BP during sleep and behavioral stresses across all circadian phases, this protocol will allow us specifically to:

**1. To determine if poor sleep, while controlling for circadian phase, contributes to the higher overall BP and reduced nocturnal drop in BP in Blacks compared to Whites.**

**2. To determine if reduced BP responses to standardized behavioral changes across the day and night contribute to the higher overall BP and reduced nocturnal drop in BP in Blacks compared to Whites.** We anticipate that Blacks will have less reduction in BP during a stable period of slow-wave sleep and/or reduced BP response to acute exercise (e.g., less post-exercise hypotension).

**3. To determine if reduced circadian amplitude of BP contributes to the higher overall BP and reduced nocturnal drop in BP in Blacks compared to Whites.** We hypothesize that Blacks will exhibit a reduced circadian amplitude of BP.

Since socioeconomic status and frequent stress exposures (e.g. racial discrimination, societal stigma) can contribute to sleep disparities, these factors will be adjusted for in all analyses.

This study will be the first to distinguish the relative contributions of sleep, circadian and behavioral mechanisms to the non-dipping BP profile in Black adults and will lay the groundwork for optimizing therapies dependent on mechanisms, such as targeting sleep, targeting circadian rhythmicity, or targeting behaviors, and raising the possibility that ideal therapy for HTN may differ

by race. This research will ultimately help to improve health and survival in black populations with HTN.

As a sub-aim and as part of Dr. Nicole Bowles' OCTRI career development award, Medical Research Foundation: New Investigator Grant; and the Faculty Initiative Pool this protocol will also allow us to:

Sub-Aim 1: Test the hypotheses that the change of circulating endocannabinoid (eCBs), lipid messangers, from baseline in response to an acute stress: (a) will correlate with changes in mood; and (b) is dependent upon circadian phase.

Sub-Aim 2: Test the hypothesis that changes in circulating eCBs in response to a mild exercise stress: (a) will correlate with changes in mood; and (b) will be dependent upon circadian phase.

Sub-Aim 3: Test the hypothesis that gene expression and activity levels of eCB synthesizing enzymes in peripheral blood mononuclear cells (PBMC): a) exhibit an increased circadian amplitude in obese (BMI $\geq$30) compared to non-obese adults; and b) correlate with the circadian rhythm of circulating eCBs.

Sub-Aim 4: Examine the contribution of the circadian system to diurnal oscillations of the microbiota in humans.

## 2. BACKGROUND

Black- compared to White-Americans have reduced sleep depth and a shorter circadian phase. Many large cohort studies consistently report subjective short sleep duration and poor quality sleep in Black- compared to White-Americans [2-6]. Similarly, objective measures of sleep via Actigraphy show shorter sleep duration and poorer sleep efficiency in Black-Americans; a relationship that remains after adjustment for sociodemographic and health behavior characteristics [7-9]. In addition to sleep disparities, there is also variation in circadian rhythms between Blacks compared to Whites. Recently two studies found a shorter circadian period in Black compared to White participants of 0.2-0.3 h [10, 11], which is larger than the standard deviation in human circadian period of 0.13 h [12]. These findings may explain increased prevalence of a morning-type chronotype (i.e. lark) and subsequent short sleep in Black compared to White study volunteers [13, 14].

Sleep and circadian rhythms are increasingly acknowledged as contributors to cardiovascular health. Although less commonly studied than the traditional risk factors of hypertension (HTN) (e.g. obesity, high-salt diet), sleep duration and quality are increasingly recognized as contributors to increased BP [15]. BP usually decreases during nocturnal sleep and increases during daytime activities [16, 17]: disturbed sleep can reduce the normal 24-h diurnal fluctuation profile of BP [18]. Lying down at night and initiating sleep leads to reduced BP, and decreased sympathetic activity and cardiac output [19, 20]. Several studies now suggest that <10% drop in the average nocturnal vs. daytime BP (non-dipping) is associated with more adverse CV events and increased mortality [21-24]. Non-dipping blood pressure (NDBP), both normotensive and hypertensive, occurs in ~25% of the population and is associated with increased cardiovascular (CV) events, stroke, renal insufficiency, and all-cause mortality [25-30].

Apart from sleep disruptions, frequently varying the time of behaviors relative to circadian phase as with night shift work, jet lag and every weekend ("social jet lag") [31] can have deleterious health effects [32-41]. In three circadian protocols in humans, we discovered a robust endogenous circadian rhythm in BP, with a drop across the night that is unrelated to level of activity or the presence of

sleep [42]. In addition, we recently discovered a phase advancement of BP rhythms in people with obstructive sleep apnea (OSA) compared to controls without OSA, and we are now studying this phenomenon under IRB00010101 *Thus, we propose to determine the contributions of sleep vs. the circadian system in the nocturnal drop in BP in Black and White volunteers.*

Disparities in hypertension between Black and White-Americans are a fundamental challenge. The US Black population has the highest age-adjusted CV disease mortality [43] attributed to the increased prevalence of HTN [43]. Moreover, compared to Whites, Blacks commonly have earlier onset and more difficultly controlling HTN [44-47]. Elevated BP even at levels below traditional HTN cut-off points (i.e. systolic BP >140 mmHg) results in increased target-organ damage and increased risk in mortality from CV disease in Blacks compared with Whites [48-50]. Blacks have not only an increased prevalence of HTN, but also an increased risk of NDBP compared to Whites [51-55].

Blacks and Whites have differential BP responses to exercise stress. In Blacks, there is a heightened BP response to an acute bout of exercise [56, 57]. In the presence of poor vascular compliance [58], poor microcirculation [59], and decreased elasticity of central vessels [60, 61], increased pressure puts Blacks at a risk for adverse CV events. *Since Blacks have compromised CV function in response to stress including that of exercise, and an altered 24-h BP pattern, it is imperative to study the separate effects of the endogenous circadian system and of the behavioral responses in this population, as proposed herein.*

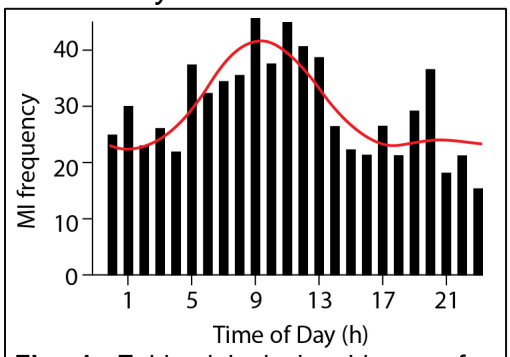

**Fig. 1**. Epidemiological evidence of a morning peak in MI incidence. From [1].

The mechanisms of NDBP and increased risk of CV disease and mortality in Black Americans with HTN remain elusive. Reduced sleep depth, a shorter circadian phase, and different reactivity to exercise may predispose Blacks, particularly those with HTN to NDBP and an increased risk of CV disease. *However, the mechanisms of NDBP and the impact of the physiological response to moderate stress as experienced each day remains unclear.* Thus, we propose aims to systematically test the contributions of sleep, circadian rhythms, and response to activity in Black vs. White individuals in a highly controlled environment. To determine which underlying mechanisms could be responsible for NDBP, we also propose to measure the important regulators of BP including sympathetic and parasympathetic activity, aldosterone, cortisol, plus vascular endothelial function. Understanding these mechanisms is vital to enable us to develop the best chrono-pharmacological or behavioral therapies for treatment of non-dipping HTN.

## 3. STUDY DESIGN

The primary purpose of this study is to determine the contribution of endogenous circadian rhythms and behaviors (daytime activity and nocturnal sleep) to the increased prevalence of HTN in Black Americans. To meet this goal, we will evaluate endogenous circadian rhythms, responses to behaviors and the interaction of behaviors and endogenous circadian rhythms in BP and primary physiological regulators of BP, in people with untreated HTN and controls. We will accomplish this by scheduling sleep, wake, behavioral stressors on a recurring artificial day length of approximately 18 h to ensure that all behaviors and dependent CV outcomes occur at all phases of the endogenous circadian cycle. This 'forced desynchrony' protocol begins on Day 3 of a 7 day study protocol (Fig. 2). After participants are admitted to the laboratory facilities they will have a full 24-h period to acclimated to facilities before baseline measures commence (Day 1 and 2, respectively). This 24-h acclimation period is an effort to minimize the first night effect (increase awakenings) in order to measure a more restful night of baseline sleep [62]. In this way we can more accurately determine differences in sleep architecture between study groups by

comparing polysomnographically scored sleep quantity and quality between groups on night 2 of the lab study. The forced desynchrony protocol, originally designed by Kleitman [63], desynchronizes the sleep-wake schedule from the circadian pacemaker [64-67], which in combination with dim light during wake periods (<3 lux), allows the circadian pacemaker to 'free-run' at its intrinsic rate of ~24.18-h [64]. Participants will be isolated from external time cues, including clocks, radios, personal computers, visitors, and sunlight, but will maintain contact with staff members. We will provide the lab contact information to the participant to give to a contact for time cue free communication or in the case of an emergency. Room temperature will be maintained at ~20-25C. Our lab suites have a light-tight porthole for 24-h blood sampling without disturbing sleep. Investigators/nurses are present in the lab or in a central control room 24-h per day to monitor data acquisition, collect biologic specimens, perform tests, and record sleep episodes. An extensive series of written protocols and checklists are used to ensure uniformity in the execution of standard procedures. Repeated measurements of BP and related variables throughout the 18-h wake/sleep cycles allows assessment of independent effects of circadian rhythms and of scheduled behaviors. Due to repeated measures, we selected clinically relevant CV variables that are reliable, relatively noninvasive, and reasonably independent. Moreover, due to the intensive nature of this protocol, whenever possible two particapants will be studied simultaneously in rooms that connect to our monitoring room. If a prospective participant does not wish to complete the forced desynchrony intervention, an option to only participate in meal tracking, 24-h urine collection, and ambulatory blood pressure after a first screening visit may be offered. The prupose of this option of to assess the potential interaction of habitual eating behaviors (including timing of meals and their composition) and urinary excretion of electrolytes on blood pressure in participants' home environments without this process being contingent on willingness to complete a laboratory stay.

The primary anticipated outcome of this study is optimized treatments for non-dipping hypertension which account for racial disparities. As such, the implementation of treatments which vary on a racial basis necessitates a qualitative understanding of community perspectives on racial biases in medical treatment. This will be obtained through supplemental focus group interviews. Volunteers will be recruited from the general population through our established methods at the same community locations where we will recruit for the main study; these interviews will therefore also spark community interest in the laboratory component of the study.

## 4. STUDY POPULATION

We will recruit participants between 30-60 years since this is the age group where pharmacological and behavioral treatments against CV disease might be most effective. We will compare self-identified Black (n=26) and White (n=26) volunteers. Since BP is a continuum, albeit with clinical cutoffs for classification of HTN, and since we have no reason to suspect that the effects of sleep or circadian rhythms on BP differ across this BP continuum, we will recruit across a wide range of BP as exists in the general population, including normotensive and hypertensive individuals and only exclude severe hypertension for ethical reasons. 20 Black participants (10 female) will be invited to participate in a focus group interview.

Inclusion and Exclusion Criteria:

a. Interviews: There will be no exclusionary criteria for focus group interviews aside from our particular interest in self-identifying Black Americans because insight provided by these sessions is hypothesized to improve patient-provider relationships in the treatment of hypertension in Black Americans, thus resulting in the mitigation of the disparity in prevalence of hypertension relative to their White counterparts.

b. Hypertension: Participants will be segregated into 'normotensive' (resting systolic blood

pressure [BP] <140/90 mmHg) and uncomplicated stage 1 'hypertensive' (systolic BP between 140 and 160 mmHg or a diastolic BP between 90 and 100 mmHg). The classification is based on the average of two or more properly-measured, seated BP readings on each of two or more office visits. Further classification of dipping or non-dipping blood pressure will be made based on ambulatory BP measurments (detailed below). Only participants not currently treated with pharmacologic agents for hypertension will be considered for participation in the study. An upper cut off of 160/100 mmHg will be used as we will study non-medicated hypertensive participants, and we do not wish to withhold treatment in patients with severe hypertension. If unaware of their hypertensive status, participants detected to have stage 1 or higher hypertension (systolic BP above 140 mmHg or a diastolic BP above 90 mmHg) will have an interview to explain the risks of hypertension and the therapeutic options including life style modifications in accordance with AHA guidelines. In addition, such participants will be encouraged to inform their primary care physician of their BP readings, and to potentially seek treatment. It is anticipated that withholding medication for 1-2 months to participate in this study for newly diagnosed participants with mild hypertension will not substantially increase risk. Rather, the frequent blood pressure monitoring may help with future management of a participant's hypertension.

c. Drug/alcohol use: Volunteers must be free of all prescription and non-prescription drugs (including caffeine, nicotine, alcohol and herbal medications) for the first screen, duration of the at home screening and 7-day study period, with no history of drug or alcohol dependency. All participants must be current non-smokers. Participants will be excluded if they have smoked within the last year. A comprehensive toxicological analysis of blood and urine for prescription medication, non-prescription medication, and drugs of abuse will be carried out for verification of reported non-use during the initial screening and on the day of admission to the laboratory.

d. Assessment of prior shift work: Volunteers must have no regular night work or rotating shift work for the three months prior to the study. Participants must not have traveled across more than three time zones during the three months prior to the study.

e. Evaluation of Medical Suitability: Participants must be free from any acute, chronic or debilitating medical conditions, other than mild hypertension (140<SBP<160 or 90<DBP<100 mmHg) and severe renal disease (glomerular filtration rate <30) due to a notable association between NDBP and renal disease in Black-Americans. Suitability will be established on the basis of a medical history questionnaire, sleep questionnaires, clinical history, electrocardiogram, clinical biochemical screening tests of blood and urine, and a physical examination. Any participant with symptoms of acute or active illness (e.g., fever, and leukocytosis) will be excluded from study. Since sleep disparity between Blacks and Whites persists after excluding clinical sleep disorders, we will exclude subjects with moderate to severe OSA based on an overnight sleep screen (see below). We will also exclude subjects with clinically significant insomnia based on the Pittsburg Sleep Symptom Questinnaire_Insomnia (PSSQ_I), also known as the Insomnia Symptom Questionnaire (ISQ).

f. Evaluation of Psychiatric/Psychological Suitability: Each participant's psychiatric/psychological suitability will be determined by the Beck Depression Inventory II questionnaire and a structured interview (Mini International Neuropsychiatric Interview) with study staff approved by an OHSU physician. Individuals with a history of severe psychiatric illnesses or psychiatric disorders will be excluded, including alcoholism, drug dependency, major depression, manic depressive illness, schizophrenic disorders, panic disorder, generalized anxiety disorder, post-traumatic stress disorder, agoraphobia, claustrophobia, paranoid personality disorder, schizoid personality disorder, schizotypal personality disorder, borderline personality disorder, and antisocial personality disorder. Finally, individuals who are unaware of specific psychiatric diagnoses and who have a history of having been treated with neuroleptic medications or major tranquilizers for more than a month will be excluded from study. However, a personal history of limited prior counseling or psychotherapy (e.g., for adjustment reactions) will not necessarily be exclusionary. Participants must demonstrate a full understanding of the requirements and

demands of the study.

g. Meal-tracking eligibility: Meal tracking will be performed by individuals who have a first-screening visit but not eligible for (or interested in) the laboratory portion of the study.

Setting: Research will be performed in the research facilities at Oregon Clinical and Translation Research Institute (OCTRI) in the Hatfield Research Center at OHSU. Participant screenings occur in the Outpatient Research Unit and in-lab stays occur on 10D. Screenings may also take place at our research suite RJH0504. Optional focus groups will be held in accessible community locations. An additional online survey questionnaire will be provided to participants that declined participation after initial completing a screening questionnaire in order to improve recruitment.

Recruitment Methods: Primarily, participants will be recruited from the general population using our established methods via flyers across the OHSU campus, community bulletin boards, internet advertisements (ResearchMatch.com, OCTRI's research Data Warehouse, clinicaltrials.gov, Craigslist, Facebook-using lab or The Oregon Institute of Occupational Health Sciences accounts), and booths at community health fairs. The PreSERVE coalition has agreed to assist in outreach and study recruitment. This coalition works with African-Americans in Portland to promote: healthy lifestyle changes to improve health; education; and research that reflects all populations in Portland. In turn PreSERVE works closely with many other community groups across Portland including the Men's Health Coalition, AARP, and Urban League. A REDCap online database and/or screening phone call may be used to collect answers to pre-screening questions and contact information of interested volunteers. We will evaluate our community-based recruitment activities by offering a survey to volunteers and by providing them with an information sheet detailing their participation in recruitment events and the our of data.

We will also use family medicine clinics at OHSU and OHSU's Cohort Discovery system, a web-based tool that allows OHSU reserachers to discover patient cohort counts from the electronic medical record. Participants will be free of tobacco, alcohol, and drug use, and without a history of night shift work in the prior 12 months. Medical suitability will be established on the basis of phone screens, medical history, biochemical and toxicology screens of blood and urine, a complete lipid panel and HbA1c (>6.5% will be excluded as diabetes), and a physical exam conducted by an OHSU physician. We will exclude participants displaying more than mild OSA (apnea/hypopnea index $\geq$15/hour of sleep) as determined by questionnaires and a home screen using a Watch-PAT system (Itamar Medical, Israel) [68]. Based on experience, we expect numerous participants with newly diagnosed HTN will be identified as part of the screening. Only participants not taking any HTN medication will be considered. An upper cut off of 160/100 mmHg will be used as it would be potentially unethical to withhold treatment in patients with severe HTN. All participants detected to have HTN will be notified by our study physician and encouraged to inform their primary care physician of the newly diagnosed HTN, and to potentially seek treatment. Written informed consent will be obtained from each volunteer before study participation.

## 5. CONSENT PROCESS

There are two written consents and a verbal consent involved in this study. The verbal consent is to allow the researchers to screen for the exclusion criteria in a phone screen. At the time of the phone screen prior to collecting any data, we will ask potential participants for verbal consent to keep their answers to the phone screen questionnaire for purposes of future recruitment (STUDY00015966). We will inform the potential participant that not giving consent will not affect his/her consideration for participation in the current study or their relationship with OHSU. Repository consent may also be obtained online if the participant is initially screened using the REDCap survey. We will also state that, should the participant wish to withdraw consent at any

time, he/she could do so by contacting the repository guardian or study staff via phone, email or written correspondence and request to no longer be approached for research purposes by our group.

For community-based recruitment activities, prospective participants will be provided with an information sheet detaling the purpose and risks of their participation; such risks include our use of survey results for publication in order to help other researchers learn from our efforts, . Regardless of elegibility for or interest in the main study, prospective volunteers who identify as Black may be invited to participate in a focus group interview. An information sheet addressing the purpose, interview structure, and potential risks will be provided. Remaining in the focus group and completing an anonymous demographic form will confirm a participant's consent to participate in the interview.

Once deemed eligible by the phone screen, participants will receive a summarized explanation of the purpose, procedures, risks and discomforts involved in the proposed study in their first screening visit. The investigator will answer any questions in plain English. Participants will be informed that they will not be permitted any timepieces during their stay in the laboratory. Every attempt will be made to acquaint each prospective participant with all of the procedures involved in this study in order to minimize the possible effect of uncertainty about the experimental procedures.

COVID-19 symptom screening will be conducted before scheduling of participant screening appointments, the day before their designated screening appointments, and upon arrival to our facility. If the participant expresses they have symptoms they will be refereed to contact their primary care provider and their screening/in-lab stay will be canceled. The screening of COVID-19 symptoms will continue until OHSU returns to Level 0 operations

Before undergoing the initial screening tests, the Screening Consent form, summarizing the information given above, will be reviewed with the prospective participant. When the prospective participant is satisfied with his/her understanding of the study, and fully comprehends his/her freedom to withdraw at any time, the potential participant will be asked to sign the screening consent form if they wish to do so.

If they meet eligibility requirements and volunteer for the main study, an informed consent (for the intervention) will be presented to them. This consent discussion will most often take place on the second day of the screening phase of the study. Investigators will go over each aspect of the consent form in plain English and answer any questions the participants may have. When the participant is satisfied with his/her understanding of the study, and fully comprehends his/her freedom to withdraw at any time, the potential participant will be asked to sign the research consent form if they wish to do so.

In the event that a prospective participant does *not* meet eligibility requirements and/or they no longer wish to volunteer for the main study, they will be given the option to participate in a simplified version of the at-home screening procedures involving only a food diary, sleep diary, 24-hour urine collection, and ambulatory blood pressure monitoring. These procedures and their associated risks will be reviewed with the prospective participant. When the prospective participant is satisfied with his/her understanding of the study, and fully comprehends his/her freedom to withdraw at any time, the potential participant will be asked to sign the simplified consent form (instead of the main study consent form) if they wish to do so.

The investigators will take all necessary steps to answer questions raised by volunteers pertaining to the nature, purpose and risks of the study. An investigator will be available at all times to answer questions the participant may have. By both verbal and written instruction, the participant will be explicitly informed of his/her freedom to withdraw consent and discontinue participation in the project at any time.

## 6. PROCEDURES INVOLVED

a. Focus group: These optional interviews will take 90 minutes for each session. 30 minutes will be spent on introductions and a light snack followed by a one-hour discussion of 5-6 prepared questions regarding racial biases in medical treatment.

Interview framework:

1. Describe the relationship between you and your primary care physician.
2. When you see your doctor, do you feel in control of your appointment and the discussion of your health? Describe any relevant experiences.
3. In what ways has your race, or your physician's race, affected your experience with healthcare, if at all?
4. Please discuss, to whatever extent you feel comfortable, any personal experience with a health condition that people of your race are at a higher risk for developing?
5. If a new drug or other therapy was to be developed specifically for people of a particular race, would this be acceptable in any case? Why or why not?

Between 4-8 different volunteers will be invited to each session which will be facillitated by 1-2 study team members. These interviews will be held at New Seasons Market in NE Portland, an accessible community location for study participants or via webex. Questions will be open-ended, short, and worded to remain neutral (e.g. "describe a time when you obtained a second opinion on a recommendation from your primary care physician" rather than "do you feel that your primary care physician is likely to make premature recommendations before adequately investigating your symptoms?"

Screening: Screening will involve at least two visits. During the first visit, height, weight and blood pressure will be measured. BMI and waist circumferance will be calculated. Participants will have a 12-lead ECG. A blood draw and a urine sample will be collected for toxicology screening. Participants will fill out sleep and psychological questionnaires either in person or electronically via REDCap, as appropriate. All individuals who pass this initial screening will undergo two sessions of 48-h ambulatory BP recording with BP measurements automatically taken every 20-30 minutes across 2 days and nights, and undergo a home screening test for OSA (Watch-PAT, Itamar Medical, Israel). The participants will be notified of significant findings from the blood and urine test results. If they remain eligible for participation after the toxicology and the screening questionnaires, they will be scheduled for a review of completed screening questionnaires and a physical exam with an OHSU physician. The participants will come in on a mutually convenient day for this part of the screening. Once the physician determines study eligibility, participants will be asked if they want to continue and participate in the intervention. The week of the participant's in-laboratory visit we may also ask them to complete a COVID-19 test and self-isolate for the days following the test and leading up to your in-laboratory study.

b. Baseline routine: After the main study consent is signed we may perform 1 DEXA scan of the subject's whole body. DEXA is a way of looking inside the body by using X-rays. X-rays are a type of radiation. The natural environment has some radiation in it. This DEXA will give subjects about the same amount of radiation that they would get from the environment in 2 days. To avoid unnecessary radiation exposure, the DEXA scan will only be conducted after participants have passed all screening steps. The DEXA scan will most often take place at the second visit during the screening phase, after subjects have signed the main study consent.To stabilize circadian rhythms, subjects will maintain a consistent sleep-wake schedule for at least 1 week, but up to 3, prior to admission to the lab. The bedtime will be determined by a participant's habitual bedtime with 8-h time in bed.

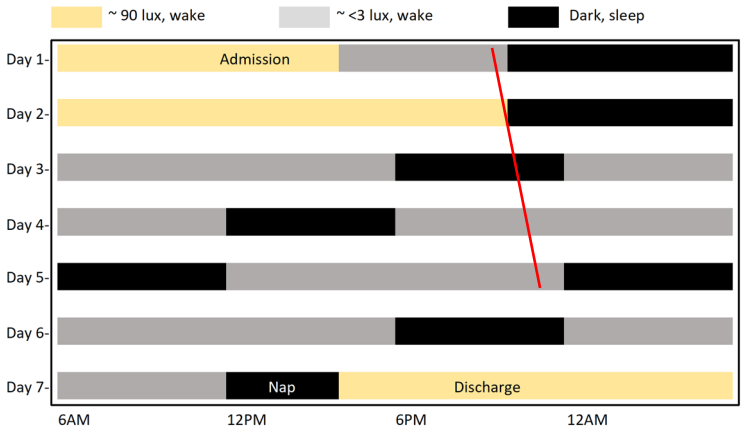


**Fig. 2**. Protocol: Subjects complete a 7-day protocol. They are instrumented for polysomnography, have an acclimatization sleep night at their usual sleep time followed by familiarization of procedures. After an 8-h baseline sleep, subjects live on a 18-h day (12-h wake in dim light [gray bars, <3 lux] and 6-h of sleep in darkness [black bars, <1lux]). All behaviors (e.g., exercise) are identical each wake episode. Blood is drawn regularly (~1-4-h) with additional measures during exercise. A phase marker of 'free-running' circadian rhythm is depicted (red line, DLMO).

To ensure compliance to this schedule, each participant's activity will be monitored by wrist actigraphy (ActiGraph wGT3X-BT, ActiGraph, FL). Actigraphy will provide an estimate of activity levels (participants will be asked to avoid vigorous physical activity), and sleep characteristics in real world settings. Participants fill out a sleep diary every day, and call into a time-stamped voice-mailbox when going to bed and getting out of bed. If more than one deviation (>1 h) from the target times are detected per week, the subject is asked to repeat the screening schedule. To identify daily BP patterns from which % nocturnal dipping quantification will be determined, subjects will wear an ambulatory BP monitor (Spacelabs Healthcare, WA) for 48-h during this home routine. Given that diet is a large contributor of hypertension, we may also ask participants to record all food consumed during baseline routine using a photographic food diary; to ensure the accuracy of this data, a 24-h urine sample may also be collected in order to assess urinary excretion of salt and other electrolytes.

c. Acclimatization and baseline day: Participants will report to the laboratory at OCTRI and admitted to an individual room free of external time cues (e.g., clocks, radios, computers, visitors and sunlight). Participants maintain contact with study investigators. Room temperature is maintained at ~20-25C and light intensity set at <3 lux (dim) during scheduled wakefulness and <0.2 lux (dark) during scheduled sleep opportunities. Participants will be instrumented for full polysomnography, and an EKG Holter monitor. Skin temperature will also be collected using iButtons from the time of admission until discharge. Additionally, participants will be guided on how to collect fecal material from each bowel movement. Our laboratory suites have a porthole for 24-h blood sampling without disturbing sleep and an IV line will be inserted and kept patent by a heparin solution. Investigators/nurses are present in the lab or a central control room 24-h per day to monitor subject health, data acquisition, provide meals, collect biologic specimens, perform tests, and record sleep. A physician is always on call. An extensive series of written protocols and checklists and team practices are used to ensure uniformity in execution of standard procedures. Following a day and night of acclimatization in order to reduce "the first night effect," baseline

measurements will be recorded [62].

d. FD protocol (Fig. 2 above): The FD protocol, designed by Kleitman [63] desynchronizes the sleep-wake schedule from the circadian pacemaker which itself 'free-runs' at its intrinsic rate of ~24.1-h when in dim light (<3 lux) [12]. Repeated measurements of BP and related variables throughout the 5 x 18-h wake/sleep cycles allows assessment of independent effects of circadian rhythms and of scheduled sleep and exercise stress on outcome variables. The FD begins the morning of experimental Day 3 and continues for the next 4 days, ending on the morning of experimental day 7 after the participant has had the opportuntiy for a nap/recovery sleep to minimize sleep intertia. On experimental day 3 after waking at the participant's habitual wake time, a participant's sleep-wake cycle will be scheduled to a period of 18-h with 2:1 ratio of wake:sleep opportunity. Each participant's daily activities will be scheduled in this manner for five, 18-h 'days'. In this way, sleep and wake behaviors are evenly spaced across the entire circadian cycle.

e. After completing the battery of tests, participants will have 'free time' when participants will be able to move about the suite as desired, except that they will not be permitted to lie down, nap, or exercise beyond light stretching. Each participant's activity will be monitored for compliance by closed circuit TV. Isocaloric meals in the form of breakfast, lunch and dinner will be served always at the same relative time since scheduled wake time (i.e., every 18-h). Participants will be asked to finish all meals to maintain metabolic homeostasis. Venous blood may be collected for assays every 1-4-h, and more frequently before, during and after the scheduled exercise stresses.

f. Wake episodes: The testing procedure is identical during each wake episode. Participants are awoken from sleep using a standard auditory tone to minimize different arousal stimuli influences. Participants remain in bed in the supine posture for 20-min (awake, eyes open, supervised by technical staff and EEG monitoring). During this 'constant posture' period, baseline waking hemodynamic, sympathetic activity, endothelial and hemostatic measures are recorded. Sympathetic and hemodynamic (HR and BP) variables, but not endothelial function, will be measured during sleep for comparison (i.e. ∆sleep stage-wake). After these baseline measures, participants will complete an exercise stress, as described further below.

g. Exercise Test: To assess how the BP regulation system copes with a standardized stress we will perform identical exercise tests during each waking period. All team members have experience in performing exercise testing. The power setting of the cycle ergometer will be tailored to induce 50% $HR_{MAX}$ for each individual during their initial exercise test and kept constant for subsequent tests to enable us to quantify changes across the circadian cycle. We have previously used this test routine in a 18-h FD protocol as well as in our current 5-h 20 min FD protocol (IRB00010101). This power setting is chosen to reflect the type of workload placed on the CV system during normal daily activities (e.g. walking). This level elicits characteristic hemodynamic responses without causing an anaerobic state or training effects [69, 70]. The procedure is: (i) sit quietly on the ergometer for 10-15-min for baseline recordings; (ii) cycle for 10-20 min at 50% HRmax, and (iii) remain on the ergometer, stationary and resting for a 10-15-min recovery period.

h. Energy Expenditure: We will measure energy expenditure via indirect calorimetry for assessment of metabolic rate and macronutrient oxidation during the cycle ergometer exercise testing portion of the in-laboratory protocol. O2 consumption and CO2 production are used to calculate metabolic rate and the oxidation of carbohydrate and fat [71].

i. Tilt Test: To assess how the BP regulation system copes with the stress of a postural change (simulating the abrupt change from supine to standing upon awakening) we will perform identical tilt test during each waking period. After a period of 20 min of supine rest on a tilt-table, participants will be inclined to a position of 60 degrees for up to 15 min. During the head-up tilt portion of the test, participants' blood pressure and heart rate will be continuously monitored.

Participants will also be asked to report on feelings of dizziness, nausea, and discomfort. Following the head up portion of the test, participants will be given up to 20 min to recover in a supine position.

j. Venous blood collection and processing: On admission to the laboratory an intravenous catheter is inserted into a vein in the arm. The catheter will be placed peripherally using standard hospital procedures. Alternatively, a midline catheter may be used. Ultrasound imaging and local anesthetic may be used to assist in catheter placement. Occasionally, finger prick method may be used for blood sampling to test for hemoglobin and hematocrit. Blood collection will start immediately on admission. Standard saline and heparin may be infused continuously to maintain patency. Blood will be transferred to vacutainer tubes and centrifuged; the resulting plasma or serum will be pipetted out and frozen at -80°C until analysis. The participant's hematocrit and hemoglobin will be measured before admission, and every 24 hours during the inpatient stay; blood collection will be stopped if hemoglobin levels 1) fall below limits established by the OCTRI and 2) fall below 1.3 gm/dL hemoglobin levels measured immediately upon admission.

k. PBMCs will be isolated from whole-blood collected in lithium-heparin tubes by density-gradient centrifugation using Ficoll-Paque PLUS (Sigma), washed, and frozen at -80°C until processing for enzyme expression and activity levels. In order to reduce the amount of blood needed for isolation of mRNA from the PBMCs we will first pilot test both the amount of blood required and the type of mRNA buffer. To reduce additional participant burden and to move the test procedure forward given COVID-19 related research delays, 20-30ml of blood during a one time draw will be obtained from volunteering co-workers (3-5). Participants for this pilot portion of the study will provide consent.

l. Fecal material will be collected using a stool collection kit https://www.abbexa.com/feces-catcher. Participants will place a small sample in a pre-labelled tube that includes an ethanol stabilizer. https://www.sigmaaldrich.com/US/en/product/mm/111727 Samples will be frozen at -80 until processed for untargeted, semi-quantitative metabolomics and lipidomics on all study samples (~100) and amplicon sequencing. Participants for this pilot portion of the study will provide consent.

**M. Follow-up surveys:** Participants who have completed the initial REDCap or phone screen may be contacted to complete surveys that assess reasons and obstacles for participating or opting out of our research study. The survey includes five comment boxes in response to the following questions/prompts: 1) "Please describe why you opted not to participate in this study including any obstacles to participation (for example: a lack of interest; unable to take the time off from work; family demands); 2) Please describe accommodations we could make to encourage your participation for future studies?; 3) What more could the recruiter have done to improve your experience?; 4) Transparency and clear communication with volunteers is a priority for our team. If you were unclear on anything related to our study, please describe below (and feel free to provide contact information if you would like us to follow up); 5) As a research volunteer, what is your preferred communication method for getting information and contacting the study staff? (for example: email; phone call; text)". Additionally, the survey includes questions that assess trust in science and scientists using 5 point likert scales.

## 7. INDEPENDENT VARIABLES

*a.* Contextual factors: Studies suggest that despite controlling for environmental factors the underlying differences in sleep architecture in Blacks and Whites might result from perceived stress across one's life including racial discrimination as well as lower socioeconomic status [6, 72, 73]. We will use Nancy Adler's concept of *subjective social status*, essentially a person's belief

about his or health location in a status order, which has been shown to reflect one's socioeconomic status as well as lifetime stress exposure [74, 75]. During a screening visit or the first day in the laboratory participants will be asked to rank their socioeconomic hierarchy relative to other people in society based on their income, education, and occupation by placing a mark on a ladder with 10 rungs. Thereby providing a single composite value that will be accounted for in statistical analysis. Additional measures of perceived stress, social support, and social economic status will also be collected in order to full characterize the study population. *Two brief surveys will be administered to investigate personal characteristics that are known to contribute to CV reactivity and thus potentially eCB reactivity [76, 77] and, importantly, may be modified in a stress management intervention [78, 79]. The first is the Resilience Scale (25-item, 7-point scale, Cronbach's α= .87- .95) [80, 81]). The second is the 10-item General Self-Efficacy Scale (4-point scale, Cronbach's α= .76- .90 [82-84]), which measures optimistic self-belief or one's ability to perform a novel task [85]. To the extent that one's exercise history might impact performance on the bike physical stress, participants will be asked the following questions: 1) When was the last time you rode a bike?; 2) When was the last time you worked out to a sweat?; and 3) On a 0 – 100 visual analogue scale, how confident are you in your ability to ride a bike for at least 10min? Contextual stress questions will be asked on the first day of the study.*

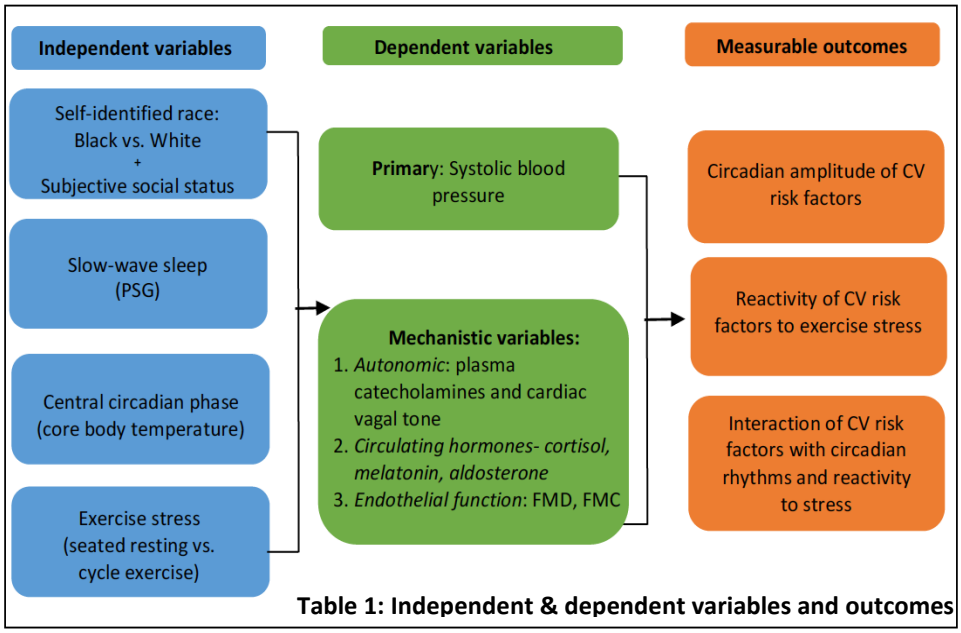

**Table 1: Independent & dependent variables and outcomes**

b. Smoking Status: During screening visit we will record participants prior smoking history using 'pack years.'

c. Circadian Phase: Analysis of dim light melatonin onset (DLMO) measured in the saliva across the study protocol yields an accurate estimate of circadian phase. Using mathematical techniques that we have previously used [42, 86-88] (see statistical techniques) the phase ($\phi$), period ($\tau$) and amplitude ($\omega$) of the DLMO rhythm will be established.

d. Sleep: Sleep episodes will be polysomnographically recorded. Because changes in posture and activity can affect results, participants will be instructed not to get out of bed, even if they should awaken before the end of the scheduled sleep episode. If requested, a nurse will bring the participant a urinal or bedpan during scheduled sleep time. The ambient light intensity in the suite will be <0.2 lux. Blood sampling will continue every hour. Polysomnographic recordings during sleep will include EKG, electroencephalogram (EEG), electro-oculogram (EOG) and submental and bilateral anterior tibialis electromyograms (EMG); cardiac data will include arterial oxygen saturation via pulse oximetry measured on the non-dominant index finger). In addition, continuous beat-by-beat BP will be recorded using two finger cuffs that alternate every 15 minutes. Sleep recordings will be scored in 30-sec epochs for sleep stage, arousals, and any periodic limb movements and respiratory events according to scoring criteria of the American Academy of Sleep Medicine [89].

## 8. DEPENDENT VARIABLES

a) Blood pressure: While in the lab, we will monitor BP every 30 min during wake and every 15 minutes during sleep (Spacelabs Healthcare, Washington, USA), as is standard for ambulatory BP assessments (52). For greater time resolution during scheduled behaviors (relaxed wakefulness, sleep and exercise), we will also measure beat-to-beat BP using a non-invasive device employing the volume-clamp method with hydrostatic correction with measures on alternating fingers every 15 min (NIBP). We have previously used this technique during recordings over extended in-lab stays (up to 12-days) with success in wake and in sleep. In addition to the finger-cuff BP, an automated calibrated sphygmomanometer will be used to record sporadic BP at intervals during the exercise stress for safety and cross-reference to (calibration of) the finger device.

b) Heart Rate: For the duration of the study, 2 channels of EKG are recorded ($R_A$-$V_6$) and stored at a sample frequency of 256 Hz, on BioPac recorders using Acknowledge software. This software will be used for peak detection (R-wave detection and subsequent HRV analysis to estimate cardiac vagal tone).

c) Sympathetic activity: Primary estimators of sympathetic output/effect may include (1) venous epinephrine (adrenal cortex contribution), (2) venous norepinephrine (sympathetic nerve activity), (3) saliva cortisol (sympathetic potentiating hormone), (4) saliva melatonin (sympathetic attenuating hormone), and (5) venous aldosterone (end point of renin-angiotensin, sympathetic nerve activation), and (6) venous endocannabinoids (measures of stress habituation).

d) Parasympathetic activity: We will use the high frequency power (HF) of the HRV power spectrum to estimate cardiac parasympathetic activity as modulated by respiratory sinus arrhythmia [90, 91].

e) Endothelial Function: We will measure endothelial function determined by flow mediated dilation (FMD), starting 10-min after each awakening in a constant posture following an overnight fast. Brachial artery flow-mediated dilation will be measured in the supine rested position using the standard guidelines and protocol [92]. We have expertise in this technique, especially in longitudinal measures within an individual [93]. We will repeat this measure before bed with the addition of a grip strength test (following 10-min of supine rest) in order to measure blood flow following an exercise [94].

f) Skin Temperature Assessment: Skin temperatures will be continuously assessed using wireless Thermochron iButtons (Maxim Integrated Products, Sunnyvale CA). These iButtons are small (1.6×0.6 cm), independent temperature sensors and use data loggers enclosed in a watertight stainless steel package. The iButtons will be taped to the skin with thin, air-permeable adhesive surgical tape in up to 9 different locations and programmed prior to placement via a USB computer interface (DS1402D-DR9 Blue Dot Receptor; DS9490R 1-Wire to USB Adapter, Maxim Integrated Products, Inc., Sunnyvale CA USA). Data will be recorded every minute. “Proximal skin temperature” is the averaged skin temperatures of infraclavicular region (mean of left and right) and sternum; “distal skin temperature” is the averaged skin temperatures of the wrists and ankles. We have used these techniques successfully in the past in similar protocols

g) Timing and content of caloric intake: MealLogger™(Wellness Foundry, New York, http://www.meallogger.com/) is a free downloadable phone application that allows participants to take a time-stamped photograph of their meal, include a detailed description of the meal content and where the meal was prepared (e.g. home vs restaurant), identify which meal they are eating (e.g. breakfast, lunch, dinner, or snack), and specifics of the meal (e.g. type of salad dressing). The application will also allow participants to retroactively include a description and timing of a meal or beverage if they forget or are unable to document the meal at the time of consumption. Study staff will have access to the food diary via secret groups on the MealLogger™ application. Data will be reviewed with the participants for completeness during and at the end of the study. Participants will be provided detailed instructions on how to use the app to document their food

intake. We will be able to use data from participants who do not complete all days of the study

h) *Cognitive performance*: A few hours after awakening, we will assess cognitive performance using PVT to assess sustained attention [95] and/or the Paced Auditory Serial Addition Test (PASAT) for processing speed and working memory. With participants' permission, we may audio record during the cognitive batteries for scoring purposes. Audio recordings will be deleted following scoring by research personnel. Additionally to prevent cortisol habituation the PASAT may be accompanied by a cold pressor test, which will involve placing one hand below the wrist into ice cold water (0-4 degrees C). This test will start 15 seconds before PASAT and participants will be asked to keep hand in for as long as possible, or until the end of the test [96]. This audio arithmetic math test, in conjuction with a social evaluation by study staff (study staff look at participant with clip board in hand to mimic evaluation), and 3 minute hand emersion in 0-4°C ice-water will cause a brief bout of both mental and physical discomfort with no overt risk to health. Again this procedure prevents habituation of cortisol which allows the response to be measured across the circadian rhythm. Participants can remove their hand from the water if they cannot continue for the entire 3 minutes.
i) *Emotional regulation:* Participants will be asked to evaluate their mood upon awake, mid 'day', prior to sleep, and before and after each stress test using the Profile of Mood States (POMS) and Positive Affect Negative Affect Schedule (PANAS) [97, 98] questionnaires.
j) *Peripheral eCB system enzyme expression and activity*: The enzymes responsible for the synthesis and degradation of endocannabinoids will also be assessed from PBMC extracts using flurometric assay kits and qRT-PCR.
k) *Global lipodomic and metabolomic analysis*: To broadly assess how related classes of lipids (i.e., monounsaturated fatty acids: saturated fatty acids as indicators of lipogenesis; N-acyl amides and prostaglandins as indicators of the endocannabinoidome) shift in relation to changes of the eCB system across circadian phases and in response to obesity, 100ul of separated plasma will be stored at −80 °C in polypropylene tubes and sent as a batch to the University of Florida (UF) Southeast Center for Integrated Metabolics Center or PNNL. PNNL will also measure untargeted, semi-quantitative metabolimcs and lipidomics on saliva and fecal samples.
l) *Microbiomics:* To measure the trends and potential overlap between the ecosystems of bacteria in saliva and fecal matter across circadian phase, we will assess the microbiota from 500ul of saliva and 1ml (~1g) (samples stored at -80 °C) and sent to PNNL. Findings here will help to determine if both saliva and fecal material are required for future protocols and funding.

## 9. DATA INTERPRETATION, STATISTICAL ANALYSIS, AND EXPECTED RESULTS

Analysis of circadian rhythmicity: FD data will be composed of circadian effects (intrinsic period ~24-h), evoked effects (18-h behavioral cycles-sleep, exercise, meal intake) and 'noise'. The intrinsic period of the circadian pacemaker will be assessed with standard techniques as used for over 20 years by the PI [42, 88]. Analysis then proceeds in two ways. (1) Binning: Data for each variable and each participant will be 'binned' according to circadian phase and an average value per bin will be calculated. Data will then be averaged across participants. Bin size will be determined by the nature of the data being analyzed; in most cases, we have found that a bin size of 60º (~4 h) allows sufficient detail while at the same time provides data for each subject in each bin. (2) Cosinor analysis to estimate rhythm amplitude and phase [99]: Using regression techniques, time series data, not necessarily evenly spaced, can be fit to the function. This can be done within-subject, so standard deviations of amplitude and phase can be estimated [100]. In this way, the above noted dependent variables (**Table 1, above**) will be: (1) assigned circadian phases; (2) compared across phases (estimate circadian amplitudes); (3) compared between

supine rest but awake, sleep or exercise to estimate reactivity across circadian phases; (4) then the circadian rhythm amplitudes and physiological changes across states (reactivity) will be compared between Black and White groups.

Analysis of qualitative group interviews:
Data collected after the completion of the focus groups will be transcribed to a word document as soon as possible through the recordings collected using Audacity. These notes will then be organized into meaningful subsections by drawing upon grounded theory. These subsections will then be coded by identifying meaningful phrases and quotes. These phrases and quotes will then be organized by key themes to allow for the searching of patterns within and across the original subsections using NVivo software for qualitative data interpretation.

Power / Sample Size Justification: To answer our main aim regarding percentage change in BP from resting wakefulness to a standardized sleep stage (N3 sleep) in Blacks vs. Whites, preliminary data suggest the average systolic BP during sleep (night) is 90% of the average during periods of wake (day) and that the SD of this relative effect is not more than 7 percentage points. Assuming the combined effects of age, sex, BMI and stress will account for 20% of the variance in relative night/day response, then a sample size of 52 (26 white; 26 black) gives us an 78% chance of detecting a genuine 5 percentage point difference in the change in BP with sleep (e.g. 90% of average vs 95% of average) at the 0.05 level of significance between races, after adjusting for these covariates. To test for differences in circadian amplitude of BP, our preliminary data suggest the SD of the change in systolic BP across the circadian cycle is not more than 9.8 mm Hg. We again expect effects of age, sex, BMI and stress will explain 20% of the variance in the response. Using an overall sample size of 52 (26 black; 26 white) provides 83% power to detect a 7.5 mm Hg difference in average change in circadian amplitude between black and white subjects at the 0.05 level of significance. Since the qualitative interviews are intended to be exploratory only 20 participants will be recruited for focus groups.

## 10. PRIVACY, CONFIDENTIALITY, AND DATA SECURITY

Standard institutional practices will be followed as described in the OHSU Information Security and Research Data Resource Guide (http://ozone.ohsu.edu/cc/sec/isg/res_sec.pdf) to maintain the confidentiality and security of data collected in this study. Study staff will be trained with regard to these procedures.While the participant is in the lab, the audio recordings will be on a password protected computer or iPod/iPad. Audio recordings will be transferred to a password protected database on a network drive behind the OHSU firewall until deleted. The PI shall prepare and maintain complete and accurate study documentation in compliance with good clinical practice standards and applicable federal, state and local laws, rules and regulations. Study documentation shall be made available at the investigator's site upon request for inspection, copying, review and audit at reasonable times by any regulatory agencies. The investigator agrees to promptly take any reasonable steps that are requested as a result of an audit to correct deficiencies in the study documentation. Confidentiality of data will be maintained. However, legal regulations regarding access to data by those other than the investigator and his/her representatives will be followed. All information will be entered into the study dataset by coded entry (de-identified data) only. The code linkage data will be kept in a locked file available only to the PI or his/her authorized designate unless requested by the appropriate regulatory authority

Electronic Data Handling: While study is active, all PHI will be stored in a web-accessible REDCap database housed on a secure OHSU server or on a password protected database on a network drive behind the OHSU firewall. PHI will be accessible to only investigators, and research staff listed on the

protocol. At the end of the study PHI will be destroyed by removing all 18 identifiers for those who did not give verbal consent to have their information stored in a recruitment repository (IRB 10147). For those who gave verbal consent to have their information stored in a recruitment repository, PHI collected on the 'Phone Screen Questionnaire' will be transferred to a password protected database on a network drive behind the OHSU firewall. No PHI will be stored or transferred via USB or other portable drives. We will obtain a waiver of the HIPPA authorization requirement for PHI collect at the time of the phone screen.

At the initial phone-screen, all participants will be assigned a unique code that will be used to identify them on documents residing outside of the REDCap database. Upon enrollment, participant study data will be recorded and stored in a password protected Access database and will be identified only by the unique code assigned at the initial phone screen. The Access database will reside on restricted and firewall protected OHSU network drive. Only persons listed on the IRB approved protocol will be given access to the REDCap and Access databases.

Interview recordings and transcription: The information and data collected from the focus groups will contain personal information from each participant based on demographic information. Each participant's information will be coded, for example as DIP_0001, to ensure anonymity of their personal information. This data will be stored on an OHSU-encrypted and password protected laptop, as well as being stored on a hard paper copy in a locked cabinet at OHSU. Upon the completion of the qualitative analysis of the data, the audio file recordings will be destroyed, and the transcribed focus group discussions and analysis will be saved for a minimum of 7 years based on NIH standards. A modification will be submitted to the IRB should we decide to retain the data beyond 7 years.

Research Electronic Data Capture (REDCap): Data for this project will be stored in OCTRI's installation of REDCap, a highly secure and robust web-based research data collection and management system. Features of REDCap that protect participants' privacy and data security include:

1. Physical Security: OCTRI's REDCap software is housed on servers located in ITG's Advanced Computing Center providing locked physical security
2. Electronic Security: The REDCap servers are housed behind both the OHSU firewall and a second ACC firewall. All web-based data transmissions are encrypted with industry-standard SSL methods.
3. Controlled User Access: REDCap is employs a robust multi-level security system that enables researchers to easily implement "minimum necessary" data access for their research staff, including specification of data fields that are identifiers. This feature includes "single click" ability to provide completely deidentified (removing all identified data fields and shifting dates) for analysis or other purposes. User activities are logged to enable auditing of all data access. Access is integrated with OHSU's network such that users who are also OHSU employees are authenticated against their OHSU network credentials.
4. Data Integrity: REDCap is jointly managed in accordance with OHSU Information Security Directives by ACC staff and members of OCTRI's Biomedical Informatics Program, ensuring fidelity of database configuration and back-ups. User activities are logged to enable auditing of all data changes.

a. Paper Data Handling**:** All paper files (e.g. signed consent forms, participant sleep and activity diaries) will be stored in locked filing cabinets in restricted access offices at OHSU. Whenever possible, original records will be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible and exact duplication of the original

document. Access to these files will be limited to study personnel listed on the IRB approved protocol.

b. Specimen Handling**:** Blood and urine samples will be identified only by the code assigned at the initial phone screen. Processing and handling of samples will be done by the OCTRI nursing and core lab staff. Samples will be stored and maintained by the OCTRI core lab -80 degree freezers. Access to study samples at OHSU will be limited to OCTRI core lab staff and study personnel listed on the IRB approved protocol. 24-h urine samples will be collected from participants during the baseline day of their in lab study or during their at-home routine after which the samples will be processed by the OHSU Core Lab and then discarded. Coded biological samples may be sent to outside labs for analysis.

## 11. PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

a. Safety Monitoring**:** Dr. Steven A. Shea, the PI, and study physician(s) will monitor this study for all patient safety issues. The data to be monitored for safety include hematocrit/hemoglobin levels and response to experimental events. Vitals, hemoglobin, computerized tests of mood, and post-sleep questionnaires are monitored daily by project leaders and RN staff. Attending physicians monitor these data while volunteers are participating in the inpatient portions of the study. Participants will be given a 24-h access number to reach Dr. Shea, the project leader, and the responsible recruiter/coordinator during inpatient and outpatient portions of the study.

b. Protocol Issues**:** These studies will be conducted in a well-supervised hospital facility and participants will be under constant observation by skilled professionals highly qualified to perform the study procedures. A physician and nurse will be available at all times. Procedures will be terminated immediately if potentially serious side effects develop. In the event of adverse reactions, medical care will be available.

c. Outcomes Monitoring**:** Dr. Steven A. Shea or his doctoral level authorized designate will be responsible for reviewing and reporting all data and safety measurements. He or his authorized designate will review all screening and safety data. All abnormal values will be handled in one of two ways. (1) If minor, the test is repeated. If still abnormal, the participant is either disempanelled or not enrolled. (2) If major, the participant is either disempanelled or not enrolled. In both cases the participant will be encouraged to follow up with their personal physician and we will be willing to converse with their physician about the test results. If the participant does not have a physician, we will offer to help the participant find a physician. A yearly progress report will be submitted to the Institutional Review Board (IRB).

d. Reportable New Information Guidelines**:** All Reportable New Information (RNI) will be reported to the OHSU IRB within 5 business days in compliance with the RNI Prompt Reporting Requirements.

## 12. RISKS TO PARTICIPANTS

a. Blood sampling:

i. There may be some discomfort or bruising on initial insertion of the catheter into a vein, but wearing the catheter should not be painful.

ii. There may be some soreness of fingers secondary to an occasional finger prick.

iii. Occasionally, mild discomfort may occur from the tube in the vein. If this happens, it can be repositioned or removed, asking the participant's permission before any subsequent reinsertion.

iv. To help keep the venipuncture site clean, we may ask permission to shave the forearm

hair of the participant prior to insertion of the IV.

v. There is a rare possibility of developing a small blood clot, inflammation, or local infection around the vein where the catheter is inserted, or in rare cases a generalized infection spread through the bloodstream as a result of the IV catheter.

vi. Occasionally, there is a black and blue mark at the site of the IV insertion, which may last a couple of weeks; and, rarely, a small scar may remain permanently at the venipuncture site.

vii. There is the possibility that the participant may faint during or after the IV insertion procedure. Therefore, we ensure that the catheter insertion is made when the participant is supine.

viii. A member of the IV team who has been trained in midline catheter placement will place the midline catheter.

ix. Ultrasound imaging and local anesthetic may be used to aid catheter placement.

x. There may be a minor skin rash or reaction to the sterile tape (contact dermatitis) used to hold the catheter in position. Hypoallergenic tape will be used as necessary.

xi. There may be some side effects from the use of heparin, such as bleeding, heparin-induced thrombocytopenia, and/or allergy.

xii. The amount of blood drawn should not significantly alter blood volume, although there may be a small decrease in the hematocrit. We will only recruit participants with normal levels of hemoglobin, hematocrit, and ferritin. The participants' hemoglobin and red blood cell concentration will be carefully monitored throughout the study as needed, and blood drawing will be discontinued if at any time the hemoglobin and/or red blood cell concentration should fall below established guidelines in addition to falling more than 1.3 gm/dL below their hemoglobin levels at admission. In this case, upon discharge, participants will be given information on their hemoglobin levels and will be encouraged to see their primary care provider. In addition, participants are encouraged to eat iron rich food before and after the study.

b. Polysomnography: The tape and special paste used to attach the EEG electrodes may cause some minor discomfort, skin irritation, and the glue used to hold electrodes to the scalp may leave a flaky residue for several days. The adhesive ECG pads may cause some skin irritation; the participant will be instructed to ask for a change in their placement if this occurs. To prep skin for ECG electrodes, participant skin may need to be shaved which may cause some minor discomfort and skin irritation.

c. Flow mediated dilation (FMD)**:** The blood pressure cuff on the forearm is inflated to suprasystolic pressure. This may cause numbness below the site of inflation, and slight pain at the cuff site. In our experience, this discomfort does not last for more than 5 minutes after deflation of the cuff. Standard guidelines will be followed to measure FMD in a safe manner. The gel used for the ultrasound imaging may cause some skin irritation and redness. In case participants are allergic to one particular gel, a different gel will be used. The gel will be wiped off after the measurement.

d. In-lab Protocol:

i. The participant may undergo some weight loss over the course of the study. This is typically due to a loss of fluid as a result of differences in the sodium content between the laboratory diet and the participants' regular diet. However, it is also possible that participants may experience a modest loss in actual body mass.

ii. The participant's mood, appetite and weight will be monitored.

iii. Participants will be visited by RN staff at least once per day while in the OCTRI to

evaluate overall physical and mental health. The participant's vital signs will be checked at least once per day. This will include pulse rate, respiratory rate, systolic and diastolic blood pressure.

iv. The participant may become sleepy during some segments of the study. The participant will be asked to remain awake during the entirety of their scheduled wake times. Should the participant feel that he/she is unable to remain awake, he/she is free to withdraw his/her consent to participate in this experiment and then go to sleep.

v. The light level in the room is dim (~3 lux) for the "awake" portion of the experiment. Though it may be difficult for participants to read and stay awake, this has not been a problem.

vi. Volunteer participants are studied in the OCTRI research laboratories at OHSU. In the unlikely event that emergency treatment is required during the participant's stay in the research facility, this treatment will be provided.

vii. During the in-laboratory part of the study, if the resting SBP ≥180 mmHg or the DBP ≥110 mmHg on three consecutive measurements taken at least 5 minutes apart during rest, the physician will be called to decide on continuing testing.

e. Exercise Test**:** In a population of individuals with no history of cardiovascular disease, the risk for complications during mild bicycle exercise at a level that raises the heart rate to approximately 50% of maximal heart rate for 15 minutes is negligible. Even though participants with HTN are at risk for cardiovascular disease, the exercise intensity used in our protocol is less than moderate intensity and simulates physical activity during activities of daily living. If the BP rises to ≥220 mmHg SBP or ≥110 mmHg DBP associated with the initiation of the exercise test, the exercise test will be terminated and the physician will be contacted. The participant will be monitored continuously until further orders from the on-call physician.

f. Tilt test: The participant may experience some presyncopal symptoms (lightheadedness, nausea) when they move from the supine to tilt position. If we encounter any of these symptoms during the test battery, we will return the participant to a supine position. Typically, such symptoms disappear within a few minutes following lying down. Blood pressure, EKG and heart rate are monitored as part of the test, and any unusual physiological responses will be reported immediately to the physician on call and appropriate action instigated.

g. Blood Pressure Tests: The supra-systolic cuff pressure used during measurement of blood pressure may be uncomfortable for a brief period (30 seconds), but this is not dangerous. The finger cuff device can also be mildly uncomfortable but will be removed if it becomes painful.

h. Energy Expenditure: Measurement of energy expenditure can cause mild discomfort. Some participants may also find the mouthpiece or hood to be slightly claustrophobic.

i. Sleep Disturbances: At the end of the experiment, the participant may find that they are no longer going to sleep and waking up at the same time that they ordinarily did before the study. In fact, it may take them several days to readjust to the regular routine. This is very similar to jet lag. Some commonly reported symptoms include upset stomach and/or digestive disorders, insomnia, irritability, and/or excessive daytime sleepiness. These symptoms may last for 1-2 weeks, although most people report readjustment after only a few days. Participants may not sleep as well in the laboratory as they do when at home. While dangerous accidents due to sleep loss are unlikely in the controlled environment of the laboratory, we have less control when participants leave the laboratory. Thus, we will ensure that the participant fully appreciates this risk and they will be given the opportunity to have recovery sleep after completion of the protocol before leaving the laboratory. If needed transportation will be arranged so that participants can return home safely.

j. Electrical risks: All amplifiers connected to the transducers and electrodes have been designed and constructed to clinical safety standards, and are tested and approved by the Bioengineering Group at the Oregon Clinical & Translational Research Institute. There is always a possibility of electric shock or burn but this risk is extremely remote and is further minimized by isolation of amplifiers from ground, and routine mandatory inspection for leakage currents. Amplifiers designed for safety in human studies will be used.

k. Skin Temperature Assessment: Tape will be used to place the Thermochron iButtons measuring skin temperature. The tape may cause some minor discomfort and skin irritation.

l. Handgrip Test: The handgrip test may result in some hand cramping. Stretches will be preformed before and after the test to reduce possible risk and participants will not be asked to perform beyond maximum capacity which will be established on the first day in the lab.

m. PASAT/PVT Test: During the study there are questionnaires on a computer and or using a pencil and paper. These tests may become boring, but there are no known short term or long term side-effects from these tests.

n. Cold pressor Test: This may be performed during PASAT. Keeping the hand in cold water may become painful and may increase blood pressure as a result of contracting blood vessels. Hands may become red after removal from water as blood vessles dilate and blood flow increases. These are normal responses and are short term effects.

o. Focus group interviews: Emotional upset is a possible risk due to the possible personal nature of the focus group discussion that may or may not elicit strong emotional feelings.

p. Fecal matter collection: Participants may feel uncomfortable with depositing their stools in a collection container and using a spatula to collect the sample in the collection tubes.

## 13. POTENTIAL BENEFITS TO PARTICIPANTS

Although there will be no direct physical benefit resulting from participation in this study, we will make known to the participant some of the information we have gathered from the physical exam, physiological testing during screening and in the laboratory. There is a chance that the pre-study screening or the various blood and urine samples taken during the study will reveal some medical abnormality. This information will be conveyed to the participant, together with a recommendation of a local clinic or physician from whom to seek treatment.

Participant remuneration: Monetary compensation for participation in the study will be based on the following criteria.

| | |
|---|---|
| Optional completion of focus group interview | = $25 |
| Completion of initial screening visit | = $25 |
| Completion of physical exam and psychological screening | = $25 |
| Home monitoring portion at $25/week | = up to $75 |
| Returning home activity monitoring equipment | = $25 |
| Wait time for possible covid-19 testing(including drive thru) | =25/hr (up to $50) |
| Collection of 24-h urine sample | = $25 |
| Collection of stools across the in lab study | = $100 |
| 6-night in-laboratory stay at $300/night | = up to $1800 |
| Entire study completion bonus | = $600 |
| Follow up survey | =$10 |
| **TOTAL** | = $2,710 |

$40 for travel cost reimbursement may be provided.

## 14. DRUGS OR DEVICES

Throughout the in-lab portion of the study, heparin may be infused continuously to maintian patency. If used, heparin use is the same as the FDA approved indication, population, dose, and route of administration.

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