Body Weight, Sleep, and Heart Health

ClinicalTrials.gov ID: NCT03388788

Oregon Health & Science University

IRB Protocol: 17489

OREGON HEALTH & SCIENCE UNIVERSITY
OHSU

IRB MEMO

Research Integrity Office
3181 SW Sam Jackson Park Road - L106RI
Portland, OR 97239-3098
(503)494-7887 irb@ohsu.edu

APPROVAL OF SUBMISSION

November 5, 2025

Dear Investigator:

On 11/5/2025, the IRB reviewed the following submission:

IRB ID:	STUDY00017489	MOD or CR ID:	MODCR00033153
Type of Review:	Modification and Continuing Review		
Title of Study:	Circadian Mechanisms of Cardiovascular Risk in Obesity		
Title of modification	This is a continuing review		
Principal Investigator:	Steven Shea		
Funding:	Name: DHHS NIH Natl Ctr for Advancing Translational Sciences, PPQ #: 1010312; Name: Sleep Research Society Foundation, PPQ #: 1013221; Name: DHHS NIH Natl Heart, Lung, and Blood Inst, PPQ #: 1010921; Name: Ford Family Foundation, PPQ #: 1012078		
IND, IDE, or HDE:	IND #138055; IND #138056; IND #138054		
Documents Reviewed:	• Approval for 138055 • Regadenoson packet insert • Ad_flyer • Approval for IND 138054 • Screen_PET Scanner Pictures • REDCap Screening Survey • Screen_BDI-II • Memo_2018-03-21 • driving-wavier • Ad_Craigslist_2021-03-24.pdf • Screen_PROMIS-sleep • Questionnaire_Stanford • Questionnaire_owl-lark • MOU-Emens-2017 • CHOP_Ad_flyer-halfsheet • Screen_Primary care provider release form • SRS Career Development Award Application • IND # 138056 for S-(4-[3-(tert-butylamino)-2-		

	hydroxypropoxy]-1,3-dihydro-2H-benzimidazole-2-[11C]-one or S-[11C]CGP12177 • CHOP_hipaa-woa_2021-04-06.docx • Thosar_KL2 funded grant • reason for holding IND • Perflutren packet insert • DSMP • DSMB-Appendix-A • NHLBI-grant-submission • Questionnaire_Poms • IND # 138055 for [11C]-meta-hydroxyephedrine or [11C]mHED • Questionnaire_appetite • IND # 138054 [15O]water or Water O15 Injection • Screen_info-release • NIH NHLBI grant application • Screen_SDQ • Test_pvt • REDCap_Morning Sleep Diary • REDCap_Evening Sleep Diary • Questionnaire - MINI Interview screen • Participant-contact-card • sst-draft-20190913-response to ncats (REFERENCE ONLY) • Questionnaire_PANAS • Approval for 138056 • Test_math • Ad_research-match • Screen_demographics • Questionnaire - MINI Interview 5.0.0 • CHOP_visual analog scales • Questionnaire_exit • CHOP_COVID-questions_2020-06-29.xlsx • Ionizing-radiation-form • Sleep-diary • CHOP_protocol_2022-03-04.docx • Consent and Authorization-Main 3.24.21 • Consent and Authorization-Screening • Phone Screen Script

The IRB granted final approval on 11/5/2025. The study is approved until 11/4/2026.

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

IRB #17489 Protocol

1. **Protocol Title:** Circadian mechanisms of cardiovascular risk in obesity

2. Objectives

Endogenous circadian rhythms of cardiovascular (CV) risk factors are rarely studied in obese humans. Thus, we aim to determine whether obese individuals have elevated morning CV risk because of normal CV rhythms on top of their existing increased CV risk (e.g., hypertension and increased sympathetic activation), or whether obesity disrupts CV rhythms and thereby furthers CV risk. Using an established multi-day circadian protocol in humans performed in dim light with behaviors scheduled evenly across the entire circadian cycle, our aims are to determine: (1) if obese individuals have normal or abnormal basal circadian CV rhythms, and (2) if obese individuals have normal or abnormal circadian rhythms in CV reactivity to stresses.

Specifically, we aim:

a. To test if the circadian amplitudes and phases of important obesity-related CV risk markers are affected by obesity. We hypothesize that there will be endogenous circadian rhythms in CV risk markers including important hemodynamic variables (blood pressure, heart rate, vascular endothelial function), plus circulating markers of prothrombotic state, oxidative stress and inflammation in both obese and lean individuals. We expect the mean levels of these markers to be impaired in obese individuals along with changes in circadian rhythm amplitude contributing to increased CV risk during the morning period of CV vulnerability.
    - Sub aim a1: Determine in lean healthy humans if CMF, measured as coronary microvascular blood flux, has an endogenous circadian rhythm with lowest function in the morning.
    i. Sub aim a2: Test the hypothesis that people with obesity have impaired coronary microvascular blood flux compared to lean individuals, with the exaggerated impairment during the morning.

b. To determine how obese individuals differ in their rhythms of CV responses to standardized stresses (i.e. change in posture and exercise) compared to lean controls, with special attention to the morning period of CV vulnerability. We hypothesize that impaired sympatho-vagal balance (2) and impaired vascular function (3) that are characteristic of obesity will lead to exaggerated CV responses to standardized stressors. For example, the morning increase in cortisol is higher in people with increased abdominal obesity (4) and the morning surge in blood pressure (BP) is higher with increasing central obesity (5) and increasing serum cholesterol levels (6). We anticipate replicating this finding, and here we will determine whether this is specific to the subjective morning as encoded by the internal circadian clock.

c. Although obesity increases efferent sympathetic activity, it is unknown how effector organs like the heart adapt to chronically increased sympathetic tone especially with respect to changes across the day. We will use short-lived positron emission tomography (PET) ligands to measure in the cardiomyocytes the balance between sympathetic pre-synaptic function (norepinephrine) and postsynaptic function (beta-adrenergic receptor density) across the circadian cycle in lean and obese individuals.

Overall, these studies will help us answer whether CV rhythms predispose obese individuals to increased CV disease risk - particularly around the vulnerable morning period. The results will serve as a foundation for clinical

trials of appropriately timed dosing of medications targeting aspects of the CV system in obesity that increase effectiveness while decreasing side-effects.

3. Background

Obesity and cardiovascular risk. The prevalence of obesity in the US has increased dramatically over the last 25 years such that now over a third of adults are obese, and this is projected to reach 50% by 2030 (7-9). Obesity increases the risk for cardiovascular (CV) disease, the leading cause of death in both males and females (10-12). Increased body fat, in particular central adiposity, contributes substantially to circulating levels of leptin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) [leading to CRP release from the liver], plasminogen activator inhibitor-1 (PAI-1), and angiotensinogen leading to a relatively pro-thrombotic state with chronic inflammation, increased sympathetic nervous system (SNS) activation, hypertension (HTN) and obstructive sleep apnea (OSA) – all notable CV risk factors (13-18). There are also adaptations in cardiac structure and function with increased adiposity even in the absence of HTN or other pathological states (19,20). To meet increased metabolic needs with obesity, blood volume and cardiac output increase, leading to heart chamber enlargement, left ventricular hypertrophy, reduced diastolic compliance, increased left ventricular filling pressure and in some cases, heart failure. Obesity related systemic HTN combined with other cardiometabolic risk factors leads to coronary heart disease (19). Ultimately, there is a fivefold increased risk of death from CV disease in obese individuals (21,22).

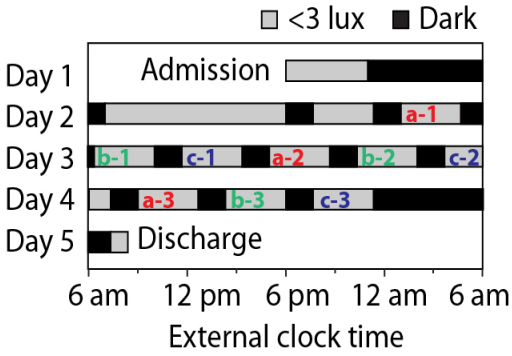

Fig. 1. The FD consists of a baseline night and day, then ten 5 h 20 m cycles of sleep (black) and wake (gray). Stressors are scheduled each wake period. Lights are dim (≤3 lux). Letters indicate PET scanning for cardiac sympathetic function. Each participant undergoes up to 3 scans across the entire FD, 16 h apart across the circadian cycle, in a randomized order (a1, a2 & a3, or b1, b2 & b3, or c1, c2 & c3, balanced among participants).

Circadian pattern of adverse CV events. Overall, obesity increases the risk of adverse CV events including myocardial infarction (MI) (23,24), sudden cardiac death (SCD) (12,25,26), and stroke (27). These adverse events do not occur randomly across the day (28) but are clustered around a morning vulnerable period (29-31). This daily pattern of vulnerability likely emerges from three sources. First, CV responses to rapid changes in behavior during the morning—including waking from sleep, posture changes, and sudden physical activity—precipitate adverse events (17,18,32-34). Second, the time of day itself represents a time of vulnerability to CV events due to the function of the circadian clock that times 24 h rhythms in physiology and behavior (Clock-Risk Model) (35,36). The likeliest scenario, is a mixture of these, in which a behavior like exercise is harmless at most times of day, but may trigger an adverse event in this clock-defined window of vulnerability (Clock-Reactivity Model). Thus, we aim to determine how obesity affects: (1) circadian rhythms of CV function and (2) the time-of-day dependent reactivity of the CV system to typical physical stressors i.e., posture and exercise.

Studying circadian rhythms in humans. To separate the endogenous circadian and behavioral influences on CV function in humans, our research team has applied 'Forced Desynchrony' protocols – as proposed in the current study. This separation is achieved by scheduling all behaviors evenly across all phases of the circadian cycle via numerous recurring days that have non-24 h periods while the participant remains in dim light to avoid circadian entrainment to light (37,38). Using this technique, we discovered in healthy lean individuals that certain CV risk factors surge around the vulnerable morning period for increased MI and CV deaths, including circulating cortisol (39), sympathetic activation, cardiac vagal withdrawal, heart rate (HR) (39), (40), platelet aggregability (41) and the pro-thrombotic plasminogen activator inhibitor-1 (PAI-1) (42). These circadian rhythms are presumably beneficial in healthy people as they prepare individuals for rapid physiological responses associated

Revised: 11.DEC.2018

with anticipated rigorous daytime behaviors. But these same rhythms might be deleterious in obese individuals due to their increased CV risk. Despite the information on CV rhythms in healthy and generally lean subjects, how obesity alters the timing or amplitude of these rhythms is unknown.

4. Study Design

The goals of this study are to elucidate circadian rhythms in CV risk markers in obesity. To meet this goal, we will evaluate endogenous circadian rhythms, responses to behaviors and the interaction of behaviors and endogenous circadian rhythms in hemodynamic variables (BP, HR, vascular endothelial function) and primary physiological regulators, in obese and lean individuals. To study independent effects of the circadian clock, we will conduct a 5-day forced desynchrony protocol (FD, **Fig 1**) where all behaviors such as arousal from sleep, eating a meal, performing an exercise test, and sleep will occur evenly spaced across the entire circadian cycle by scheduling 10 recurring 'day' of 5 h 20 min. Repeated measurements of CV variables throughout the 10 wake/sleep cycles allow for assessment of independent effects of circadian rhythms and scheduled behaviors. Due to repeated measures, we propose to measure selected clinically relevant CV variables that are reliable, relatively noninvasive, and independent.

Baseline routine: To stabilize circadian rhythms, participants will maintain a consistent sleep-wake schedule for at least 1 week prior to admission to the laboratory. The bedtime will be determined by a participant's habitual bedtime with 8-h time in bed. To ensure compliance to this schedule, each participant's activity will be monitored by wrist Actigraphy (ActiGraph wGT3X-BT, ActiGraph, Pensacola, FL). Actigraphy will provide an estimate of daily activity levels 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, subjects will wear an ambulatory BP monitor (Spacelabs Healthcare, WA) for 48-h during this home routine.

Acclimatization and baseline day: Participants report to the laboratory at Oregon Clinical & Translational Research Institute (OCTRI) on day 1 and are 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 21-22.2 °C and light intensity set at $\leq$3 lux during scheduled wakefulness and <0.2 lux (darkness) during scheduled sleep opportunities. Participants will be instrumented for full polysomnography, and a Holter monitor throughout the study. 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 in a central control room 24h/day to monitor subject health, data acquisition, provide meals, collect biologic specimens, perform tests, and record sleep. A physician is always on call when a participant is in the laboratory. An extensive series of written protocols and checklists and team practices are used to ensure uniformity in execution of standard procedures.

FD Protocol (**Fig. 1**): Designed by Kleitman (37), the FD protocol desynchronizes the sleep-wake cycles from the circadian pacemaker and is performed in dim light to ensure the circadian pacemaker 'free-runs' at its intrinsic rate of ~24.1 h (43). At ~6 PM on day 2 after having been awake for 10 h, a participant's sleep-wake cycle is scheduled to a period of 5 h 20 min with a 2:1 ratio of wake: sleep-opportunity.

Imaging: PET imaging of the heart (detailed below in section 6h) will occur up to 3 times for each subject across the FD protocol (**Fig. 1**). The scans will be a minimum of 16 h apart (**Fig. 1**) and represent up to 3 different circadian phases.

Cardiac ultrasound: Myocardial perfusion imaging (detailed in section 6i) may occur up to 3 times for each

participant across the FD protocol.

5. Study Population

a. Number of Subjects

To determine the effects of obesity and specifically abdominal fat on our outcomes of interest, we will compare 2 groups of participants, ages 25-65: (1) healthy obese [30≤BMI<40 kg/m^2] and (2) healthy lean controls [18.5<BMI<25 kg/m^2]; n=14 in each group. To ensure some degree of stability and avoid recent changes due to significant recent weight gain, all obese participants must report the presence of obesity with abdominal adiposity for >5 years. Participants must also be free of medication use. Based on experience in these protocols, we estimate that we will medically screen 120 participants in order to complete 28 participants.

b. Inclusion and Exclusion Criteria

In order to control for additional varation among obese individuals such as worse sleep at night or reduced daytime activity we will screen and match for insomnia, sleep apnea and overall habitual activity among our participants. Thus, we will exclude participants with moderate to severe obstructive sleep apnea based on an overnight sleep screen (Watch-PAT, Itamar Medical, Israel), we will exclude subjects with clinically significant insomnia (sleep efficiency less than desired, and residual daytime sleepiness based on the PROMIS Sleep Distrubance and Sleep-Related Impairment surveys).. The healthy control group will also be matched for age (+/- 5 years) and sex.

Diabetes. For participants who have a fasting blood glucose level of ≥ 100 mg/dL we will measure hemoglobin A1c to exclude for diabetes (HbA1c>6.5%).

Hypertension. An upper cut off of 160/100 mmHg during an office BP measure will be used as an exclusion criterion as it would not be adviseable to delay initiation of therapy in patients with severe hypertension.

Cardiovascular disease. Individuals actively treated or previously hospitalized for heart failure, arrhythmia or coronary artery disease, a history of coronary artery bypass grafts and coronary stents, and those that use a pacemaker or defibrillator will be excluded.

Psychiatric/psychological suitability. Each participant will undergo a structured interview (Mini International Neuropsychiatric Interview) with study staff approved by a qualified OHSU physician. This physician will supervise the administration and scoring of a Beck Depression Inventory II (BDI-II) questionnaire for each potential participant. Individuals with evidence of psychopathology on the BDI-II, or in a structured clinical interview with the physician, will be excluded from study. Individuals will be excluded if they have a history of severe psychiatric illnesses or psychiatric disorders such as 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 antidepressants, neuroleptic medications, or tranquilizers will be excluded. 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.

Drug/alcohol use. Volunteers must be drug-free (including caffeine, nicotine, alcohol and herbal medications) for the duration of the screening and study period, with no history of drug or alcohol

dependency. All participants must be current non-smokers, and are required to have a history of less than 5 'pack years' of smoking. A comprehensive toxicological analysis of blood and urine for prescription medication, non-prescription medication, herbal remedies/medications 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.

Medication/drug use. Volunteers must not be taking any prescribed medications. Over the counter medications will need to be avoided throughout the at-home period and the in-lab study. As above, compliance is verified by comprehensive toxicological assessment before admission. Oral contraceptives are allowed, and women will continue to take these throughout the experiment.

Prior shift work. For stability of endogenous circadian rhythmicity, volunteers must have no history of working irregular day and night hours, regular night work, or rotating shift work for the 6 months prior to the study. They must not have traveled across more than two time zones during the month prior to the study.

All candidates who are excluded from study for medical and/or psychological reasons are so informed. In addition, they will be encouraged to inform their primary care physician of any elevated blood pressures, increased blood glucose levels or obstructive sleep apnea, and to potentially seek treatment.

c. Vulnerable Populations

No vulnerable populations will be studied.

d. Setting

All research is 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.

e. Recruitment Methods

Using established methods, participants will be recruited from the general population via internet (ResearchMatch.com, OCTRI's research Data Warehouse, advertisements, clinicaltrials.gov), flyers across the OHSU campus and at local organizations, as well as Epic Reporting Workbench and OCTRI RVR. Medical suitability will be established on the basis of phone screens, medical history, biochemical and toxicology screens of blood and urine, and a physical exam conducted by an OHSU physician. Written informed consent will be obtained from each volunteer before study participation.

f. 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. 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. Additionally, we will collect basic demographics, gender/sex, and COVID-19 screening information during the phone screen.

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.

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 volunteer, an informed consent (for the intervention) will be presented to them. 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.

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. **Screening:** Screening will involve at least two visits. During the first visit, height, weight, waist circumference and blood pressure will be measured. BMI will be calculated. Participants will have a 12-lead ECG. A blood draw will be collected for testing basic metabolic panel, lipid levels, plasma glucose and HbA1c and a urine sample will be collected for toxicology screening. Participants will fill out sleep and psychological questionnaires.

   All individuals who pass this initial screening will undergo up to two sessions of 48 hour ambulatory BP recording with BP measurements automatically taken every 20-30 minutes across 2 days and nights, and undergo a home screening test for Obstructive Sleep Apnea (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 a study 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 study.

   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. **Establishing ambulatory baseline:** It is essential to ensure initial stability of circadian rhythms and sleep. Hence, participants will maintain a consistent sleep-wake schedule for at least 1 week prior to admission to the laboratory. The bedtime will be determined by the participants' habitual bedtime with 8-h time in bed. To ensure compliance to this schedule, each participant's activity will be monitored by wrist Actigraphy (ActiGraph, Pensacola, FL). Additionally, participants will call a time-stamped voice-mailbox both when going to bed and getting out of bed. For the first and last two days of the acclimatization, the participants may be asked to wear an ambulatory BP monitor (Spacelabs Healthcare, WA) to get accustomed to the regular inflation-deflation cycles. Actigraphy

and ambulatory BP measures will provide an estimate of daily activity levels and sleep characteristics in real world settings. Actigraphy is widely accepted as a noninvasive means to estimate sleep patterns under non-laboratory conditions (44). The estimated sleep from at least 1 week of home recordings with timed sleep opportunities will also provide an estimate of sleep duration, which will complement the results from the polysomnographically scored sleep on the first night in the lab.

c. **Acclimatization and baseline days:** Participants will be admitted to the OCTRI research laboratory on experimental day 1. Saliva will be collected with Salivettes for melatonin and cortisol assays and genetic testing at regular intervals. Sleep and cardiac variables will be continuously recorded by polysomnography. A venous catheter will be placed where the tip of the catheter is peripherally in an arm vein, or in some cases closer to the armpit (a 'midline' catheter) using standard hospital procedures. Ultrasound imaging and local anesthetic may be used to assist in catheter placement. The catheter will be kept patent using a solution of sodium and heparin. BP will be measured throughout the in-lab stay (e.g., every 30 min during the wake periods with sphygmomanometry and continuously during sleep using a finger plethysmography device). Light intensity will be set at 150 lux (normal indoor room light) during the baseline wake period and reduced to <1 lux (darkness) during sleep.
d. **Cardiac Computed tomography (CT) examination:** Subjects enrolled in this study will undergo cardiac computed tomography scan for the detection and quantification of coronary artery calcium. The presence and amount of coronary artery calcium as quantified by Agatston score (CAC score) as a predictor of major adverse cardiovascular events. The CT examinations are designed to efficiently and accurately provide volumetric CT image data for measuring coronary artery calcium (CAC), an accurate measure of coronary atherosclerosis burden (45,46). The CT examination for CAC consists of scout images and one cardiac prospectively ECG-triggered series of the heart to measure CAC. On average, 15 minutes of participant time will be spent within the CT scan suite; this includes instructions, setup and imaging. Participants will have ECG electrodes attached for cardiac gating and be instructed to use a standardized breath hold technique. The CT examination will be performed on a 256-detector row CT scanner (Philips iCT, Philips Medical Imaging, Cleveland, OH). The scan will be performed in prospectively ECG-triggered mode at 75% of RR interval with the tube current of 120 kVp and tube potential of 100 mAs.

e. **Forced Desynchrony (FD) protocol:** See above and **Fig. 1.**

f. **Wake episodes:** Tests are identical on all wake periods, except for additional PET scanning on up to 3 of the 10 cycles (**Fig. 1** and detailed below in section 6h). We may also make cardiac ultrasound on up to 3 cycles. Participants are aroused from sleep using a standard auditory tone to minimize different arousal stimuli influences. Participants remain in bed in the supine posture for ~10 minutes (awake, eyes open, supervised by technical staff and EEG monitoring). During this 'constant posture' period, baseline waking hemodynamic, autonomic, endothelial and hemostatic measures are recorded (details of dependent measures below). Participants complete a test battery consisting of endothelial function assessment, a possible postural stress, cognitive challenges, PET imaging (on up to 3 of 10 wake periods) or cardiac ultrasound (on up to 3 of the remaining wake periods), mild exercise and an isocaloric meal containing 22% of the daily target intake. Participants are to finish each meal to ensure metabolic homeostasis. Participants will then have scheduled 'free-time' where they are able to move about the suite but are not permitted to lie down, nap, or exercise beyond light stretching. Activity will be monitored for compliance by closed circuit TV.

g. **Posture Test:** Standing up after a period of lying down is a large physiological challenge requiring sympathetic activation to maintain perfusion pressure to the brain and avoid syncope. If this test is

performed, participants will be instrumented with EKG and an automated sphygmomanometer. Non-invasive BP recordings on a beat-by-beat basis (Nexfin, Netherlands) and may also be performed. The Nexfin finger cuffs will be placed on the non-dominant hand and held in place by an arm sling so that the BP cuffs will be at heart level. BP and HR will be assessed while participants stand up from a semi-recumbent posture and remain standing for ~5 min. A tilt table may also be used to transition the participant from supine to vertical posture at a controlled rate. For this procedure, participants will be strapped to a hydraulic tilt table with a weight-bearing footboard. Measurements will then be made under 3 conditions: (1) Baseline: Participants lie supine (0-degrees), awake and resting for approximately 20-min (2) 60 degree tilt: within a 3 to 5 sec period the table will be tilted to a 60-degree head-up angle. The table will be held here for a further 15-minutes (except in situations where the participant becomes uncomfortable, dizzy or faint). (3) Recovery: Within a 3 to 5 second period the table will be tilted to a 0-degree angle. The table will be held here for a further approximately 20-min.

h. **Proton Emission Tomography (PET):** PET imaging of the heart will occur up to 3 times across the FD protocol (minimum 16 h between scans), with order randomized for each participant and beginning ~40 min after waking (**Fig.1**).

We will use O-15 water for blood flow, C-11 – meta-hydroxyephedrine for presynaptic norepinephrine transporter function and CGP 12177 for measure of beta adrenergic receptor density.

After the endothelial and posture tests, participants are taken by wheelchair to the Nuclear Medicine Suite (in the same building but on a different floor) while wearing opaque goggles to prevent light exposure and contact with non-study personnel that could provide time cues. One in the scanner suite, the lights will be kept dim, and the participant will lie supine on the imaging table, movement may be restricted with foam or other form of padding, and the participant will be connected for heart rate, oxygen saturation, and blood pressure monitoring. The IV from the circadian lab will already be in place and will be used for periodic blood sampling during the imaging period. A second IV will be put in place and used to inject the radiopharmaceuticals. Once settled, an attenuation image will be acquired using a low dose CT scan. All images will be dynamic acquisition collected in "list mode". Cardiac beta adrenergic receptor density via CGP12177 images: [^{11}C]-CGP12177 (≤0.1 mCi/kg body weight up to 10 mCi of high specific activity), will be injected via IV over ~1 min. Image acquisition will begin one min before tracer injection and continue for ~20 min after which time ≤0.1 mCi/kg up to 10 mCi of low specific activity [^{11}C]-CGP12177 will be administered via IV over ~1 min and images acquired for up to an additional 40 min. Myocardial blood flow may be measured via [^{15}O]-water (≤0.5 mCi/kg body weight) up to 50 mCi injected via IV and images acquired for ~ 5 min. Coronary blood flow is calculated using the Bergmann model (47). The ^{15}O water study will be performed between the norepinephrine and post-synaptic receptor studies. Cardiac presynaptic norepinephrine transporter function will be measured via infusion of [^{11}C]-meta-hydroxyephedrine (mHED: ≤0.2 mCi/kg body weight up to 20 mCi) over ~30 sec with PET images acquired continuously for 40 min. Following completion of the images, IV lines will be disconnected (but not removed) and the participant will return to the circadian lab in a wheelchair while wearing opaque goggles. In wake periods without imaging, participants will remain in bed to match the duration of lying in the scanner. During that time there may be interactions with lab staff and completion of computerized tests that measure vigilance and neurocognitive performance and ensure wakefulness.

i. Perfusion imaging: A commercially-produced FDA-approved microbubble contrast agent (Definity,

Bristol Myers Squibb) will be used. One vial of Definity (1.3 mL) will be diluted to a total volume of 30 mL and infused at 1.5 ml/min intravenously. Myocardial contrast echocardiography will be performed with a phased-array transducer interfaced with an ultrasound system (iE33, Philips Ultrasound). A contrast-specific multipulse amplitude-modulation sequence will be performed at a centerline transmission frequency of 1.8 MHz, a mechanical index of 0.12, and a compression of 60. Images will be acquired in the apical 4-chamber, 2- chamber, and 3-chamber views. End-systolic images will be acquired for 10 seconds after a 5-frame destructive pulse sequence performed at a mechanical index of 1.0. Vasodilator stress will be applied using i.v. regadenoson and echocardiography will be repeated to measure the effect of hyperemia.

j. **Exercise Test**: All team members have extensive experience in performing exercise testing under laboratory conditions. 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. This workload is similar to that in our preliminary data and 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 (48,49). The procedure is: (i) sit quietly on the ergometer for 5-10 min for baseline recordings; (ii) cycle for 15 min at 50% HRmax, and (iii) remain on the ergometer, stationary and resting for a 5-10 min recovery period.

k. **Energy Expenditure:** We will measure energy expenditure via indirect calorimetry for assessment of EE and macronutrient oxidation (calculated using equations of (Jequier, 1987) during different portions of the inpatient protocol. O2 consumption and CO2 production are used to calculate metabolic rate and the oxidation of carbohydrate and fat (50).

l. **Sleep:** Autonomic and hemodynamic (HR and BP), but not endothelial function, will be measured during sleep for comparison with standardized wake periods while in the same posture. Sleep episodes will be polysomnographically recorded. Electronic sensors (Emfit Bed Monitor) may also be placed under the bed to estimate sleep and wakefulness. 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 or technician will bring the participant a urinal, bedpan, or commode during scheduled sleep time. The ambient light intensity in the suite will be <1 lux. Blood sampling will continue every ~2 h. Standard polysomnographic recordings during sleep will include EKG, electroencephalogram (EEG), electro-oculogram (EOG) and submental electromyograms (EMG), and arterial oxygen saturation via pulse oximetry measured on the non-dominant index finger. In addition, continuous beat-by-beat BP will be recorded. Sleep recordings will be scored by a registered polysomnographic technician 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 (51).

m. **Circadian Phase:** Analysis of dim light melatonin onset (DLMO) from saliva collected over the study duration yields an accurate estimate of circadian phase**.** With mathematical techniques that we have previously used (39,41,42,52) see statistical techniques) the phase, period and amplitude of the DLMO rhythm will be established. The phase and period information will be used as phase markers for assigning circadian phase to all other collected data. Saliva will be collected with Salivettes.

n. **Genetics:** Single nucleotide polymorphisms (SNPs) will be measured from saliva or blood to determine how polymorphisms within genes that regulate biological rhythms may vary in obese and lean participants. Additional consent will be obtained for this portion of the study.

o. **Primary dependent variables:**

i. BP: We may measure beat-to-beat BP (using a non-invasive device employing the volume-clamp method with hydrostatic correction (53)). We have previously used this technique during recordings over extended in-lab stays (up to 12-days) with success during rest, posture test, cycle exercise and in sleep. Beat-to-beat BP will be recorded during: (i) each sleep episode; (ii) each constant posture period immediately following awakening; and (iii) throughout each posture test session. In addition to the finger-cuff BP, an automated calibrated sphygmomanometer will be used to record BP at intervals during the waking stresses for safety and cross-reference to the finger device. As a secondary variable, cardiac output will also be estimated using algorithms built into the beat-to-beat BP system. We may also be monitoring ambulatory BP (Spacelabs Healthcare, Washington, USA) every ~30 min during wake and every ~15 min during sleep as is standard in most ambulatory BP assessments (54).

ii. Heart Rate: For the duration of the study, 2 channels of EKG will be recorded (RA-V6) and stored. Software will be used for peak detection (R-wave detection and subsequent HRV analysis to estimate cardiac vagal tone (see below).

iii. Sympathetic activity: Primary estimators of sympathetic output/effect include (1) venous epinephrine (adrenal cortex contribution), (2) venous norepinephrine (sympathetic nerve activity), (3) saliva cortisol (sympathetic potentiating hormone), and (4) venous aldosterone (end point of renin-angiotensin, sympathetic nerve activation). Endocannabinoid analysis from plasma may also be measured as a secondary marker of HPA-axis and sympathetic regulation.

iv. 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 (55,56). The R-R intervals resulting from ectopic beats or beats >3 SD from the mean are deleted from datasets. Thereafter, R-R interval data for sequential 5-minute time segments will be used for HRV analysis in each condition (i.e. semi-recumbent vs. standing, resting vs. exercise, wakefulness vs. stable sleep stages) according to published criteria (57). Frequency domain measures are calculated by interpolating the R-R tachogram with a cubic spline and re-sampling. Power spectral density will be calculated using Welch's technique (58) of FFT. Calculated time domain HRV measures will include mean R-R, SD of all normal-normal R-R intervals (SDNN), and percentage of R-R intervals differing by greater than 50 msec (pNN50).

v. Oxidative stress: We will measure markers of lipid peroxidation from plasma.

vi. Endothelial Function: We will measure endothelium-dependent flow mediated dilation (FMD) and endothelium-independent vasodilation starting ~20-min after each awakening in a constant posture following an overnight fast. Brachial artery FMD will be measured in the supine rested position using the standard guidelines and protocol (59). We have expertise in these technique, especially in longitudinal measures within an individual (60-62).

vii. Inflammation: We will measure plasma TNFα because of its strong association with obesity related CV disease, effect on vascular function, and known circadian variation (34,63,64).

viii. Coronary blood flow (using [^{15}O]-water), cardiac presynaptic norepinephrine transporter function (reuptake; using [^{11}C]-mHED), and beta-adrenergic receptor density (using [^{11}C]-CGP12177) and will be measured as described above in the procedures section.

ix. Anthropometric measures. Dual energy X-ray absorptiometry (DEXA, Model Model GE Lunar iDXA) will be used to assess body fat percentage and fat free mass at during the study. This is currently considered the gold standard for body composition analysis.

x. Mental Stress Test: Approximately 3 h after awakening participants start the mental stress test. Measurements will be made under 3 conditions: (1) Baseline: Participants sit in a semi-recumbent posture (45°), awake and resting for ~20-min; (2) Mental Stress: for ~20 min.

p. **Discharge:** Following the last FD waking period, participants will be given 9 hours of recovery sleep during their extended habitual sleep time. The additional hour beyond habitual sleep time will account for possible sleep deprivation during the protocol and account for the natural drift the participant may experience as a result of a free running circadian system (a result of constant dark or dim light). Upon awakening participants will have the opportunity to shower and eat a breakfast of their choosing. Following the meal, participants will complete the *Stanford Sleepiness Scale* and have a final nursing assessment. If an individual does not rate a score of 1 or 2 (Feeling active, wide awake OR Functioning at high levels), participants will be given the opportunity for additional rest, provided a cab ride home, or picked up by a family member/friend. We may also use a 10-minute psychomotor vigilance test (PTV) initially provided during the second screening visit. Failure to obtain within 10% of their initial score would be similar to having a blood alcohol score above the legal limit. If the participant insists on leaving and driving themselves home, they must sign a waiver stating that upon research and nursing staff, and physician advice they chose to drive home and assume liability. Finally, upon departure participants will be provided a card that once more (originally in consent form) provides staff contact information. The card will also remind participants of possible residual side effects associated with the study.

7. Data and Specimens

a. **Handling of Data and Specimens**

i. **Electronic Data Handling**: While study is active, all PHI will be stored in a database housed on a secure OHSU server. PHI will be accessible to only investigators, and research staff listed on the protocol. The PET images are reconstructed and saved on the tomograph data system until the data are converted and stored as de-identified DICOM files for further data analyses. At the end of the study, participants who gave verbal consent to have their information stored in a recruitment repository (STUDY00015966), PHI collected on the 'Phone Screen Questionnaire' will be transferred to a password protected database on a network drive behind the OHSU firewall. For those who did not give verbal consent to have their information stored in a recruitment repository, PHI will be destroyed by removing all 18 identifiers. 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 secure database. Upon enrollment, participant study data will be recorded and stored in a password protected database and will be identified only by the unique code assigned at the initial phone screen. The 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 databases.

ii. **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. The PET radiopharmaceutical manufacturing batch records are retained in the Center for Radiochemistry Research (CRR) under lock and key with access restricted to authorized CRR personnel, the PIs and to authorized manufacturing auditors. The batch records are retained as required by Federal and State laws for investigational drug manufacturing.

iii. **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. Coded biological samples may be sent to outside labs for analysis.

b. **Sharing of Results with Subjects**

With their consent, we will make known to the participant some of the information we have gathered from the 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 to discuss the results with a physician if appropriate. Specifically, since genetic information obtained does not have medical or treatment importance at this time it will not be shared with the participant.

8. Data Analysis

Analysis of circadian rhythmicity: Forced desynchrony data is composed of circadian effects (intrinsic period ~24 h), evoked effects (5 h 20 min behavioral cycles) and 'noise'. The intrinsic period of the circadian pacemaker will be assessed with standard techniques as used for 20 years by the Principal Investigator (65,66). This intrinsic period, derived from melatonin, will be used to assign a circadian phase (from 0° to 360°) to each minute of the study, with 0° corresponding to the minimum of the waveform fitted to the DLMO data from the FD portion of the study. Analysis then proceeds in two ways. (A) 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 60° (~4 h) allows sufficient detail while at the same time provides data for each participant in each bin. (B) Cosinor analysis to estimate rhythm amplitude and phase (67): 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 (68).

Analysis of Cardiac Computed tomography (CT) examination: The images will be reconstructed with 2.5 mm slice thickness. The gantry rotation time will be 280 ms resulting in temporal resolution of 140 ms. A dataset with a field-of-view focused on the heart for the purpose of the CAC quantification will be reconstructed. The reconstructed datasets will be transferred to Knight Cardiovascular Institute Imaging Core Laboratory. The coronary calcium CT scans will be analyzed on a dedicated workstation (Medis medical

imaging systems, Leiden, the Netherlands). A software application will be used for the semi-automated selection of CAC. The Agatston score (AS) will be calculated to quantify total CAC amount (69).

Analysis of PET Imaging Data:

We will analyze regional radiotracer uptake in the left ventricle. The heart will be segmented into 5 mm thick slices with 8 sectors per slice **(70)**.

Norepinephrine re-uptake: *Retention fraction:* This is the sum of ^{11}C activity (cpm/ml) per sector from 25-40 min post-injection divided by the image-based blood pool activity integrated from 0-40 min and corrected for any metabolites. Images will also be evaluated using a kinetic model on a sector-by-sector basis and the values, summarized in the legend of Fig. 2 are reported which also shows sample time activity curves from two sectors from a single heart-failure patient (9).

Beta-receptor density: Will be analyzed by the graphical method of **(71)**. Two injections of [^{11}C]-CGP are required for the graphical analysis approach to determine regional LV beta adrenoceptor density. The Delforge model provides validated estimates of receptor density (B' max) on a sector by sector basis, expressed in terms of pmol/g **(72).**

Analysis of myocardial perfusion imaging. Image analysis will be conducted offline. The immediate post-destruction frame will be digitally subtracted from all subsequent frames. Time versus background subtracted intensity will be measured on the background-subtracted frames from regions-of-interest placed over the myocardium according to the American Society of Echocardiography's 17-segment model. Data will be fit to the function $y=A(1-e^{-\beta t})$ where y is the video intensity at time t; A is plateau video intensity, an index of capillary blood volume and β is the rate constant which provides a measure of average erythrocyte flux rate. Microvascular blood flow will be calculated by the product of A and β. Since flow reserve and β-reserve can be abnormal from increased resting values more so than reduced stress values, the definition of microvascular dysfunction will also require that complete refill of the microcirculation take >2 postdestructive beats. These data will be further analyzed using mixed model analysis with β-reserve as the primary dependent variable and circadian time as the independent variable.

9. **Power / Sample Size Justification:** To estimate sample size, we have preliminary data from a similar study population (4 obese, 6 lean), in the same age range, and under the same protocol as the current proposal. The variables will be compared across circadian phases, across stresses, and between obese and lean groups in terms of basal circadian rhythm amplitudes (Aim 1) and circadian rhythms of reactivity (Aim 2). The main outcomes of interest are: differences in systolic BP, HR or vascular endothelial function (FMD) between obese and lean individuals upon awakening; changes in these variables with behavioral stresses, and between the most vulnerable (~9AM) and less vulnerable other circadian phases (e.g., 12 h later). The clinically significant or meaningful differences that we aimed to detect between groups, between states and between circadian phases were 10 mmHg for systolic BP, 10 bpm for HR and an absolute 3 point different in % FMD (73-76). The sample size calculations revealed that for all 6 main comparisons, 14 participants per group was sufficient to provide at least 80% power to identify the assumed main effects. To account for multiple comparisons, even after adjusting for six outcomes of primary interest (adjusted level of significance = 0.00833 per comparison), use of 14 participants for each of obese and lean groups, we have 80% power to detect effect sizes of at least 1.41 SD between groups. Thus, we will study 14 participants per group to adequately test our hypotheses. For our sub aims a1 and a2 related to circadian rhythmicity in coronary microvascular function between lean and obese individuals, in order to test the hypotheses with

80% statistical power, out of the total 28 participants, we will study 10 lean and 10 obese individuals.

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. 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

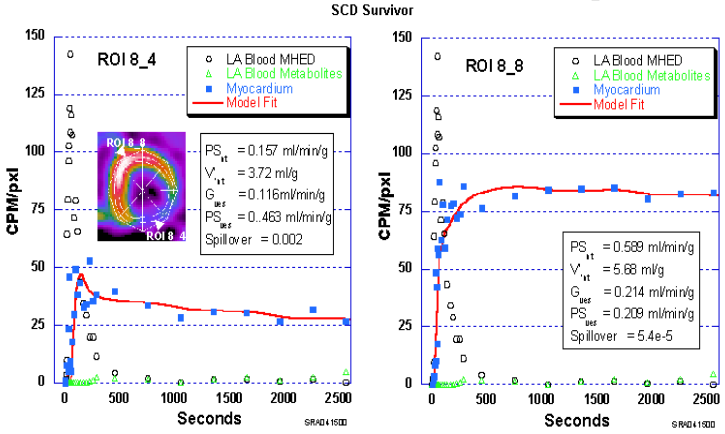

Fig. 12. Model analysis of pre-synaptic NE function in LV sectors from a heart-failure patient. Points in black are the time activity curve points from the left atrial cavity. Green are metabolites in the blood. Blue are the activities in the LV tissue, and the red line is the model fit. The parameters are image spillover from the ventricular blood cavity (spillover), virtual volume of the nerve terminal (Vnt), volume for NE presynaptic vesicular storage (Gves) and transfer into the vesicle (PSves), and our main variable **presynaptic reuptake rate (PSnt)** (1).

10. Provisions to Monitor the Data to Ensure the Safety of Subjects

a. **Safety Monitoring:** Dr. Steven A. Shea and Dr. Jeanne Link, the study mPIs, study physician(s), and an independent safety officer will monitor this study for all patient safety issues. A semi-annual evaluation will be conducted by the independent safety officer to identify any safety or other protocol-related concerns. A detailed Data Safety Monitoring Plan (DSMP) is included in the study materials.

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. PET imaging will be done under the supervision of a nuclear medicine physician who will perform each injection of the radiopharmaceutical and observe the subject for at least 15 minutes for any adverse reaction.

c. **Outcomes Monitoring:** The project leader will review and report all data and safety issues after discussions with the principal investigator. The Center for Radiochemistry Research will have internal and external audits of their records. In consultation with the on-call physician associated with the

study and with the PIs, Steven A. Shea, or Jeanne Link, all abnormal values will be handled in one of two ways:

1. If minor, the test (e.g. blood pressure) is repeated. If still abnormal, the participant is either withdrawn or not enrolled and a letter to the participant's personal physician is sent if the participant requests.
2. If major, the participant is either withdrawn or not enrolled and, with the participant's permission, a letter will be sent to the participant's physician detailing the abnormality. If the participant does not have a physician, we will endeavor 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:** Reportable New Information (RNI) will be reported to the IRB within 5 business days of discovery. RNI not meeting IRB requirements for prompt reporting will be submitted with the Continuing Review. Adverse events or unanticipated problems will be reported to the funder, National Heart Lung and Blood Insitute (NHLBI), according to current guidelines (https://www.nhlbi.nih.gov/grants-and-training/policies-and-guidelines/nhlbi-adverse-event-and-unanticipated-problem-reporting-policy). Full details can be found in the Data Safety Monitoring Plan.

11. Risks and Benefits

a. Risks to Participants

i. Blood sampling

1. There may be some discomfort or bruising on initial insertion of the catheter into a vein, but wearing the catheter should not be painful.
2. There may be some soreness of fingers secondary to an occasional finger prick.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. A member of the IV team who has been trained in midline catheter placement will place the midline catheter.
9. Ultrasound imaging and local anesthetic may be used to aid catheter placement.
10. 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.
11. There may be some side effects from the use of heparin, such as bleeding, heparin-induced thrombocytopenia, and/or allergy.
12. 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, with the exception of the blood drawn

during the 3 PET scanning procedures (1mL each scan, total of 3mL), 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.

ii. **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.

iii. **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.

iv. **In-lab Protocol**

1. 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.
2. The participant's mood, appetite and weight will be monitored.
3. 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.
4. 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.
5. 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.
6. 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.
7. 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.
8. In adherence with OHSU's policies, we will have participants wear personal protective equipment and follow other relevant regulations when appropriate.

v. **Posture Test**: The participant may experience some presyncopal symptoms (lightheadedness, nausea) when they move from the semi-recumbent to standing position. If we encounter any of these symptoms during the test battery, we will ask the participant to lie down until these

symptoms go away. 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.

vi. **PET Imaging**: *Activation or blockade of the cardiac adrenergic nervous system:* In terms of sympathetic activation or blockade: *1)* The tracers, injected in the patients at 1000-fold higher doses than planned, could block either the pre-synaptic norepinephrine reuptake system or post-synaptic beta-adrenergic receptor system creating a potential for increased ventricular arrhythmias, hypotension or hypertension, bradycardia or sinus tachycardia. These risks are exceedingly small. For the 60 healthy control volunteers and 14 patients with serious heart disease that Dr. Link has studied with [^{11}C]-mHED, there have been no symptoms reported and no notable changes in heart rate or blood pressure have been observed. PET studies of [^{11}C]-mHED in diverse patient populations have not reported hemodynamic changes, except for a ~ 5 mmHg increase in BP over a 40 minute study period likely due to IV fluid administration (77-81).

The potential side effects of beta receptor-blockade (decreased heart rate and blood pressure) were not observed in the control patients in the study by Merlet et al (82) using [11C]-CGP12177. In the congestive heart failure patients in our prior study, heart rate decreased 15% without change in blood pressure. Two patients had transient and mild dyspnea. Delforge et al reported no change in heart rate or blood pressure in a series of 5 dogs given 11C-CGP12177 at a dose approximately twice that used in the human studies. Qing et al (83) did not report hemodynamic effects when using [11C]-CGP12177 in patients with asthma. Thus, we do not anticipate adverse effects; nevertheless, heart rate, oxygen saturation, and blood pressure will be monitored throughout the studies. For all studies, a physician will be present in the Nuclear Medicine suite for each injection and 15 minutes following each injection. A trained technician will monitor throughout the imaging period in order to anticipate any rhythm abnormalities or hemodynamic changes, and a physician will be on premises to treat these conditions appropriately.

The subject could develop claustrophobia from the imaging apparatus and discomfort from lying on the imaging table for up to two hours. Only one subject out of over 100 such studies that we have performed has asked to terminate the study early because of discomfort. That subject was a young healthy control.

vii. **Radiation exposure:** Radiation dose carries an associated risk to the patient. The estimated effective radiation dose from a CAC scan is approximately 1.5 mSv (as compared to annual natural background radiation of 3-4 mSv). The effective equivalent (EDE) for one PET + CT imaging session is calculated to be 7.4 mSv for a male and about 7.0 mSv for a female assuming 70 kg per male or female. The radiation dose for 3 imaging sessions, the entire protocol, is 22 mSv for a male and 21 mSv for a female. The critical organ targeted in this study is the kidney with a dose of 17 mSv to male or female of 70 kg weight for one imaging session, the dose to the critical organ is 51 mSv for three imaging sessions. For the population in this study which includes both lean and obese individuals the increased risk of death from their radiation exposure is minimal. There are no methods to reduce the radiation exposure. To minimize risk to the patient, the injected doses will be kept as low as reasonably achievable and to still obtain reasonable counting statistics.

viii. **Myocardial perfusion imaging:** Lipid-stabilized octafluoropropane microbubbles (Definity, Lantheus Imaging) will be used for perfusion imaging. Safety of this agent has been well-established, including post-marketing registry studies involving >1 million doses

administered, and multicenter trials of hundreds of thousands of patients. The safety studies have demonstrated that serious pseudoanaphylactic reaction or any other major cardiopulmonary adverse event is exceedingly rare (1 in 8,000 to 10,000) and much lower than any other cardiovascular imaging contrast agent that has been used in humans. Dose of Definity will be within the limits approved by the US FDA for left ventricular opacification. Although non-serious adverse events (AEs) are very common with coronary vasodilators such as regadenoson that are widely used for pharmacologic stress testing in cardiovascular medicine, most of these AEs are non-harmful side effects of A1-receptor agonism such as flushing, headache, dyspnea and chest pain, and resolve within minutes of discontinuation of adenosine. If participants experience any discomfort, then we will immediately reverse the effect of regadenoson with an antidote, Aminophylline. Additionally, to reduce risk, each subject will be closely supervised for the duration of the study including evaluation of vital signs, monitoring of symptoms, and ECG. The procedures will be conducted on a hospital floor fully-equipped with emergency response kit for dealing with any anaphylactic, arrhythmic, or hypotensive event.

ix. **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.

x. **Energy Expenditure:** Measurement of energy expenditure can cause mild discomfort. Some participants may also find the mouthpiece or hood to be slightly claustrophobic. Participants will be shown the metabolic mouthpiece or hood and provided the opportunity to test the mouthpiece or lay down under the hood at the consent if they express concern with the procedure. Sometimes, the metabolic cart recording needs to be delayed by a few minutes and most participants have no concerns with the equipment.

xi. **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.

xii. **Anthropometric measures:** As part of this study we will perform 1 DEXA scan of the participant'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 participants about the same amount of radiation that they would get from the environment in 2 days.

xiii. **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.

xiv. **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.

xv. **Genetic Testing:** The genetic information examined in this study does not have medical or treatment importance at this time. Therefore, risks to the participant are currently unforeseeable. We will not place information about participation in the genetic study or the results of the genetic study tests in the participant's medical record. There is a small risk of loss of confidentiality. If the results were to be accidentally released, it might be possible that the information we will gather about could become available to an insurer or an employer, or a relative, or someone else outside the study. Even though there are certain genetic discrimination and confidentiality protections in both Oregon law and federal law, there is still a small chance that harm could occur if this information.

xvi. **Emfit Bed Monitor:** The Emfit bed monitor will send de-identified data directly to the study sponsor. While every effort will be made to ensure that the transmitted data is safe and secure, there is a small risk that if the device were lost or stolen, that certain sleep measurement data relating to the participant could be compromised.

b. **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.

Table 1: Participant Remuneration

Screening Phase			
Travel		Based on mileage/public transit	
Toxicology screening		$25	
Psychological screening		$25	
At home screening		$25/week (up to 3 weeks)	
Activity monitor return		$25	
COVID-19 Testing and Wait time for drive-thru		$25/hr up to $50	
Screening possible max		$200 + travel	
In-Laboratory Phase			
Per day stay	$240	x 5 (days)	= $1,200
CT Scan	25	X 1 time	$25
Myocardial perfusion imaging	$50	X 3 times	$150
Completion bonus	$350		= $350
		laboratory total	= $1,550

Screening + In-laboratory Phase	= $1,925 +travel

Drugs or Devices

Throughout the in-lab portion of the study, heparin may be infused continuously to maintain patency. If used, heparin use is the same as the FDA approved indication, population, dose, and route of administration. The PET radiopharmaceuticals will be manufactured and the administered dose will be done under an approved IND for these radiopharmaceuticals, [^{11}C]-mHED, [^{11}C]-CGP12177 and [^{15}O]-water as described in this protocol. For practical reasons, the [^{15}O]-water test may not be performed at each PET session. Perflutren and regadenoson will be used for myocardial perfusion imaging. The study will not proceed until the protocol, consent form, data safety monitoring plan and IND are approved.

1. Caldwell JH, Kroll K, Li Z, Seymour K. Quantitation of presynaptic cardiac sympathetic function with carbon-11-meta-hydroxyephedrine. The Journal of Nuclear Medicine 1998;39:1327.
2. Esposito K, Marfella R, Gualdiero P et al. Sympathovagal balance, nighttime blood pressure, and QT intervals in normotensive obese women. Obesity research 2003;11:653-659.
3. Hashimoto M, Akishita M, Eto M et al. The impairment of flow-mediated vasodilatation in obese men with visceral fat accumulation. International journal of obesity 1998;22:477-484.
4. Wallerius S, Rosmond R, Ljung T, Holm G, Björntorp P. Rise in morning saliva cortisol is associated with abdominal obesity in men: a preliminary report. Journal of endocrinological investigation 2003;26:616-619.
5. Feldstein CA, Akopian M, Olivieri AO, Kramer AP, Nasi M, Garrido D. A comparison of body mass index and waist-to-hip ratio as indicators of hypertension risk in an urban Argentine population: a hospital-based study. Nutrition, metabolism and cardiovascular diseases 2005;15:310-315.
6. Martin C, Cameron J, Head G, Chen S, Eikelis N, McGrath B. The morning blood pressure surge is related to serum cholesterol. Journal of human hypertension 2013;27:315-320.
7. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. JAMA 2016;315:2284-91.
8. Finkelstein EA, Khavjou OA, Thompson H et al. Obesity and severe obesity forecasts through 2030. Am J Prev Med 2012;42:563-70.
9. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS data brief 2015;219:1-8.
10. Yusuf S, Vaz M, Pais P. Tackling the challenge of cardiovascular disease burden in developing countries. Am Heart J 2004;148:1-4.
11. Yusuf S, Hawken S, Ounpuu S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.
12. Eranti A, Aro AL, Kerola T et al. Body mass index as a predictor of sudden cardiac death and usefulness of the electrocardiogram for risk stratification. The American journal of cardiology 2016;117:388-393.
13. Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A, Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. J Am Coll Cardiol 1995;26:1257-63.

14. Grassi G, Seravalle G, Dell'Oro R et al. Comparative effects of candesartan and hydrochlorothiazide on blood pressure, insulin sensitivity, and sympathetic drive in obese hypertensive individuals: results of the CROSS study. J Hypertens 2003;21:1761-9.
15. Grassi G, Seravalle G, Dell'Oro R, Turri C, Bolla GB, Mancia G. Adrenergic and reflex abnormalities in obesity-related hypertension. Hypertension 2000;36:538-42.
16. Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F, Esler MD. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. Circulation 2003;108:560-5.
17. Cigolini M, Targher G, Bergamo Andreis IA, Tonoli M, Agostino G, De Sandre G. Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. Arterioscler Thromb Vasc Biol 1996;16:368-74.
18. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.
19. Poirier P, Martin J, Marceau P, Biron S, Marceau S. Impact of bariatric surgery on cardiac structure, function and clinical manifestations in morbid obesity. Expert Rev Cardiovasc Ther 2004;2:193-201.
20. Pascual M, Pascual D, Soria F et al. Effects of isolated obesity on systolic and diastolic left ventricular function. Heart 2003;89:1152-1156.
21. Poirier P, Giles TD, Bray GA et al. Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss. Arteriosclerosis, Thrombosis, and Vascular Biology 2006;26:968-976.
22. Poirier P, Eckel RH. Obesity and cardiovascular disease. Curr Atheroscler Rep 2002;4:448-53.
23. Yusuf S, Hawken S, Ôunpuu S et al. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. The Lancet 2005;366:1640-1649.
24. Nicklas BJ, Penninx BW, Cesari M et al. Association of Visceral Adipose Tissue with Incident Myocardial Infarction in Older Men and Women The Health, Aging and Body Composition Study. American journal of epidemiology 2004;160:741-749.
25. Adabag S, Huxley RR, Lopez FL et al. Obesity related risk of sudden cardiac death in the atherosclerosis risk in communities study. Heart 2014:heartjnl-2014-306238.
26. Chiuve SE, Sun Q, Sandhu RK et al. Adiposity throughout adulthood and risk of sudden cardiac death in women. JACC: Clinical Electrophysiology 2015;1:520-528.
27. Mitchell AB, Cole JW, McArdle PF et al. Obesity increases risk of ischemic stroke in young adults. Stroke 2015;46:1690-2.
28. Mulcahy D. "Circadian" variation in cardiovascular events and implications for therapy? J Cardiovasc Pharmacol 1999;34 Suppl 2:S3-8; discussion S29-31.
29. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. The American journal of cardiology 1987;60:801-806.
30. Willich SN, Goldberg RJ, Maclure M, Perriello L, Muller JE. Increased onset of sudden cardiac death in the first three hours after awakening. The American journal of cardiology 1992;70:65-68.
31. Muller JE, Stone PH, Turi ZG et al. Circadian variation in the frequency of onset of acute myocardial infarction. New England Journal of Medicine 1985;313:1315-1322.
32. Arntz H-R, Müller-Nordhorn J, Willich SN. Cold Monday mornings prove dangerous: epidemiology of sudden cardiac death. Current opinion in critical care 2001;7:139-144.
33. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738.

34. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111-9.
35. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. Annual review of neuroscience 2012;35:445-462.
36. Shea SA, Hilton MF, Muller JE. Day/Night Patterns of Myocardial Infarction and Sudden Cardiac Death: Interacting Roles of the Endogenous Circadian System and Behavioral Triggers. Blood Pressure Monitoring in Cardiovascular Medicine and Therapeutics: Humana Press, 2007:251-289.
37. Kleitman N. Sleep and Wakefulness as Alternating Phases in the Cycle of Existence. 1939.
38. Czeisler CA, Duffy JF, Shanahan TL et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 1999;284:2177-2181.
39. Scheer FA, Hu K, Evoniuk H et al. Impact of the human circadian system, exercise, and their interaction on cardiovascular function. Proceedings of the National Academy of Sciences 2010;107:20541-20546.
40. Hilton MF, Umali MU, Czeisler CA, Wyatt JK, Shea SA. Endogenous circadian control of the human autonomic nervous system. Comput Cardiol 2000;27:197-200.
41. Scheer FA, Michelson AD, Frelinger AL, 3rd et al. The Human Endogenous Circadian System Causes Greatest Platelet Activation during the Biological Morning Independent of Behaviors. PLoS One 2011;6:e24549.
42. Scheer FA, Shea SA. Human circadian system causes morning peak in pro-thrombotic plasminogen activator inhibitor-1 (PAI-1) independent of sleep/wake cycle. Blood 2013:blood-2013-07-517060.
43. Duffy JF, Cain SW, Chang A-M et al. Sex difference in the near-24-hour intrinsic period of the human circadian timing system. Proceedings of the National Academy of Sciences 2011;108:15602-15608.
44. Sadeh A, Hauri PJ, Kripke DF, Lavie P. The role of actigraphy in the evaluation of sleep disorders. Sleep 1995;18:288-302.
45. McCollough CH, Ulzheimer S, Halliburton SS, Shanneik K, White RD, Kalender WA. Coronary artery calcium: a multi-institutional, multimanufacturer international standard for quantification at cardiac CT. Radiology 2007;243:527-38.
46. Voros S, Rivera JJ, Berman DS et al. Guideline for minimizing radiation exposure during acquisition of coronary artery calcium scans with the use of multidetector computed tomography: a report by the Society for Atherosclerosis Imaging and Prevention Tomographic Imaging and Prevention Councils in collaboration with the Society of Cardiovascular Computed Tomography. J Cardiovasc Comput Tomogr 2011;5:75-83.
47. Herrero P, Markham J, Bergmann SR. Quantitation of Myocardial Blood Flow with H215O and Positron Emission Tomography: Assessment and Error Analysis of a Mathematical Approach. Journal of computer assisted tomography 1989;13:862-873.
48. Pollock ML, Gaesser GA, Butcher JD et al. ACSM position stand: the recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. Med Sci Sports Exerc 1998;30:975-991.
49. U.S. Department of Health and Human Services. Physical Activity and Health: A Report of the Surgeon General. In: Service PH, editor. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, 1996.
50. Jequier E, Acheson K, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187-208.
51. Association ATFotASD. EEG arousals: scoring rules and examples. Sleep 1992;15:174-184.

52. Shea SA, Hilton MF, Hu K, Scheer FA. Existence of an endogenous circadian blood pressure rhythm in humans that peaks in the evening. Circulation research 2011;108:980-984.
53. Peñáz J, Honzikova N, Fiser B. Spectral analysis of resting variability of some circulatory parameters in man. Physiologia Bohemoslovaca 1978;27:349.
54. Pickering TG, Hall JE, Appel LJ et al. Recommendations for blood pressure measurement in humans and experimental animals part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005;111:697-716.
55. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger A, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981;213:220-222.
56. Pomeranz B, Macaulay R, Caudill MA et al. Assessment of autonomic function in humans by heart rate spectral analysis. American Journal of Physiology-Heart and Circulatory Physiology 1985;248:H151-H153.
57. Camm A, Malik M, Bigger J et al. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93:1043-1065.
58. Welch P. The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. Audio and Electroacoustics, IEEE Transactions on 1967;15:70-73.
59. Thijssen DH, Black MA, Pyke KE et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. American Journal of Physiology-Heart and Circulatory Physiology 2011;300:H2-H12.
60. Thosar SS. BS, Wiggins CH.,Mather, KJ., Johnston JD., Wallace JP. Can breaking up sitting time alter femoral artery endothelial function. ACSM Annual Meeting 2014, 2014.
61. Thosar S, Bielko S, Wiggins C, Mather K, Klaunig J, Wallace J. Oral vitamin C prevents the attenuation in flow mediated dilation during prolonged sitting (546.3). The FASEB Journal 2014;28:546.3.
62. MP B, DA B, M R, SS T, FAJL S, SA S. Assessing the interacting challenges of sleep restriction and shift work. Military Health System Research Symposium. Fort Lauderdale, Florida, 2013.
63. Zhang C, Xu X, Potter BJ et al. TNF-α contributes to endothelial dysfunction in ischemia/reperfusion injury. Arteriosclerosis, thrombosis, and vascular biology 2006;26:475-480.
64. Rahman SA, Castanon-Cervantes O, Scheer FA et al. Endogenous circadian regulation of pro-inflammatory cytokines and chemokines in the presence of bacterial lipopolysaccharide in humans. Brain, Behavior, and Immunity 2014;47:4-13.
65. Dijk D-J, Czeisler CA. Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neuroscience letters 1994;166:63-68.
66. Dijk D-J, Duffy JF, Czeisler CA. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. Journal of sleep research 1992;1:112-117.
67. Nelson W, Tong YL, Lee J-K, Halberg F. Methods for cosinor-rhythmometry. Chronobiologia 1979;6:305.
68. van Leeuwen AP, Verhulst FC, Reijneveld SA, Vollebergh WA, Ormel J, Huizink AC. Can the gateway hypothesis, the common liability model and/or, the route of administration model predict initiation of cannabis use during adolescence? A survival analysis--the TRAILS study. J Adolesc Health 2011;48:73-8.

69. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827-32.
70. Butterworth E, Link J, Caldwell J. Automated left ventricular region of interest definition on cardiac PET images reduces operator time. Journal of Nuclear Medicine 1999;40:290P-290P.
71. Delforge J, Syrota A, Lançon J et al. Cardiac beta-adrenergic receptor density measured in vivo using PET, CGP 12177, and a new graphical method. Journal of Nuclear Medicine 1991;32:739-748.
72. Link JM, Stratton JR, Levy W et al. PET measures of pre-and post-synaptic cardiac beta adrenergic function. Nuclear medicine and biology 2003;30:795-803.
73. Otto ME, Svatikova A, de Mattos Barretto RB et al. Early morning attenuation of endothelial function in healthy humans. Circulation 2004;109:2507-2510.
74. Wong R, Howe P, Buckley J, Coates A, Kunz I, Berry N. Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutrition, Metabolism and Cardiovascular Diseases 2011;21:851-856.
75. Nerla R, Tarzia P, Sestito A et al. Effect of bariatric surgery on peripheral flow-mediated dilation and coronary microvascular function. Nutrition, Metabolism and Cardiovascular Diseases 2012;22:626-634.
76. Maruo T, Nakatani S, Kanzaki H et al. Circadian variation of endothelial function in idiopathic dilated cardiomyopathy. The American journal of cardiology 2006;97:699-702.
77. Tavernier G, Galitzky J, Bousquet-Melou A, Montastruc JL, Berlan M. The positive chronotropic effect induced by BRL 37344 and CGP 12177, two beta-3 adrenergic agonists, does not involve cardiac beta adrenoceptors but baroreflex mechanisms. J Pharmacol Exp Ther 1992;263:1083-90.
78. Link JM, Krohn KA, Clark JC. Production of [11C]CH3I by single pass reaction of [11C]CH4 with I2. Nucl Med Biol 1997;24:93-7.
79. Caldwell JH, Link JM, Levy WC, Poole JE, Stratton JR. Evidence for pre- to postsynaptic mismatch of the cardiac sympathetic nervous system in ischemic congestive heart failure. J Nucl Med 2008;49:234-41.
80. Caldwell JH, Kroll K, Li Z, Seymour K, Link JM, Krohn KA. Quantitation of presynaptic cardiac sympathetic function with carbon-11-meta-hydroxyephedrine. J Nucl Med 1998;39:1327-34.
81. Bernacki GM, Bahrainy S, Caldwell JH, Levy WC, Link JM, Stratton JR. Assessment of the Effects of Age, Gender, and Exercise Training on the Cardiac Sympathetic Nervous System Using Positron Emission Tomography Imaging. J Gerontol A Biol Sci Med Sci 2016;71:1195-201.
82. Merlet P, Delforge J, Syrota A et al. Positron emission tomography with 11C CGP-12177 to assess beta-adrenergic receptor concentration in idiopathic dilated cardiomyopathy. Circulation 1993;87:1169-78.
83. Qing F, Rahman SU, Rhodes CG et al. Pulmonary and cardiac beta-adrenoceptor density in vivo in asthmatic subjects. Am J Respir Crit Care Med 1997;155:1130-4.