COMIRB Protocol

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 18-0369
Project Title: Effects of Exercise on Sleep Quality and Nocturnal Fat Oxidation in Individuals with Metabolic Syndrome
Principal Investigator: Jennifer M. Blankenship, PhD
Coinvestigator: Edward Melanson, PhD
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I. Hypotheses and Specific Aims:

Our long-term goal is to understand how physical activity and sleep interact to influence cardiovascular disease and diabetes risk, and use these findings to tailor interventions for individuals prone to sleep disorders (e.g., individuals with metabolic syndrome, older adults). This pilot study will generate key preliminary data to inform an in-depth mechanistic study to investigate factors that may modulate the relationship between exercise and sleep and their subsequent metabolic impact (e.g. dietary fat oxidation, insulin sensitivity). Specifically, **to determine the effects of daytime exercise on sleep quality and nocturnal metabolism, and to explore potential mechanisms by which exercise-induced changes in nocturnal metabolism and sleep quality occur. The specific aims are:**

Aim 1: Determine the effects of daytime exercise on nocturnal metabolism and sleep quality. The primary outcome is nocturnal FFA concentrations. Secondary outcomes are nocturnal fat oxidation, nocturnal glucose and insulin concentrations, and sleep quality (e.g. percent time in slow wave sleep [SWS], sleep latency, and wake after sleep onset [WASO]). To capture the dynamic changes in nocturnal hormones and metabolites, I will perform overnight hourly blood sampling. Fat oxidation will be measured using whole-room indirect calorimetry. Sleep quality will be assessed using gold standard polysomnography. I hypothesize that exercise will increase nocturnal FFA concentrations and fat oxidation, and reduce nocturnal glucose and insulin concentrations. I further hypothesize that exercise will increase percent time in SWS and decrease sleep latency and WASO.

Aim 2: Determine whether exercise-induced changes in nocturnal fat and glucose metabolism and sleep quality are associated with the known improvements in next day insulin sensitivity. Insulin sensitivity will be measured by intravenous glucose tolerance test in the morning upon waking. I hypothesize exercise-induced changes in nocturnal metabolism and sleep quality will be associated with improved next day insulin sensitivity.

Exploratory Aim: Explore mechanisms that contribute to exercise-induced changes in nocturnal fat and glucose metabolism and sleep quality. Outcomes are distal-proximal skin temperature gradients and melatonin offset. Data generated from this exploratory aim will provide preliminary data to power a more mechanistically-driven studies of the impact of daytime exercise on sleep quality and nocturnal metabolism.

II. Background and Significance

1.1 Exercise and metabolic syndrome (MetS) risk: Exercise reduces the risk of acquiring the MetS (a clustering of several metabolic disorders) and is the cornerstone of behavioral lifestyle interventions (23). The presence of MetS, increases the risk of cardiovascular disease and diabetes more than when any of these disorders is presented alone (2, 30). While the health benefits effects of exercise are well documented (e.g. improved insulin sensitivity and increased post-exercise fat oxidation), the impact of exercise on metabolism during sleep, a time frame that occupies approximately a third of the human lifespan, are largely unknown.

1.2 Sleep quality, nocturnal fat metabolism and risk of MetS: A strong body of evidence has accumulated indicating that impaired sleep contributes to the development of components of the MetS (20, 29, 34, 38, 39). Interestingly, the nocturnal period is full of dynamic changes in many hormones (e.g. insulin, growth hormone) and metabolites (free fatty acids, glucose) (10, 11, 14). Several studies have shown that daytime behaviors (e.g. overfeeding) or disruptions in normal sleep patterns cause changes in nocturnal metabolism which, in turn, impact metabolic outcomes such as insulin sensitivity. Studies by my mentors have shown that experimental sleep restriction in humans causes increases in nocturnal free fatty acid (FFA) concentrations and impairments in insulin sensitivity (10). Additional studies by my mentor, Dr. Broussard, have shown that elevated nocturnal FFA concentrations may be an early signal that induces compensatory hyperinsulinemia in response to insulin resistance induced by a high

fat diet (10, 11). Work by another one of my mentors, Dr. Rynders, has shown that an inability to increase nocturnal fat oxidation after periods of overfeeding was associated with subsequent weight gain 5 years later (37). Interestingly, 24-hour fat oxidation in this study was not associated with future weight gain. Collectively, these data indicate that **nocturnal fat metabolism is related to clinically relevant health outcomes and represents an important, and understudied, component of metabolic health.**

1.3 The influence of exercise on sleep quality: Both acute and chronic exercise improve sleep quality (9, 17, 18, 42). However, most studies investigating the effects of exercise on sleep have been performed in healthy adult populations without any sleep disturbances. The effect of exercise on people who commonly have sleep problems (e.g. individuals with MetS) have not been studied and remains an important area of investigation. Understanding both whether exercise positively influences sleep in these populations at-risk to have sleep problems is critical to reduce the risk of cardiovascular disease and diabetes.

Changes in body temperature may be a potential mechanism responsible for the impact of exercise on sleep. Body temperature is a key physiological signal that is associated with the initiation of sleep (43). Exercise causes an increase in core body temperature, which induces thermoregulatory responses to promote dissipation. This can result in an imbalance in body temperatures between the proximal (core) and distal regions. This temperature gradient has been referred to as the distal-proximal gradient (DPG). A wider DPG (i.e. higher temperatures in the distal regions compared to proximal regions), is associated with the onset of sleep, and a wider DPG at sleep onset is associated with improved sleep quality (36). Thus, the heat loss promoted by exercise may, in fact, result in improvements in sleep quality.

1.4 Effects of exercise on nocturnal metabolism: While it is well established that exercise has numerous health benefits, the impact of exercise on nocturnal fat metabolism and sleep quality has not been systematically investigated. Dr. Melanson has spent nearly two decades studying the effect of lifestyle behaviors (e.g., exercise, diet, sleep) on fat oxidation (25-28). His studies have shown that exercise performed in a state of energy balance (i.e. adding back the calories expended from an exercise bout) increases the fat oxidation capacity of the muscle but does not increase 24-hour fat oxidation (27). While we have not investigated the effects of exercise on the nocturnal period exclusively, this remains an area of interest for our lab group.

1.5 Interaction between exercise, sleep, and nocturnal metabolism: There is clearly a dynamic relationship between exercise and sleep quality (13, 16), however the mechanisms responsible for this interplay have not been systematically investigated. Understanding how health enhancing behaviors (e.g. exercise), influence nocturnal fat metabolism and sleep quality would provide novel information that would be useful in developing effective and targeted behavioral interventions to prevent chronic disease in at-risk populations. Given that exercise improves sleep quality, exercise may also influence nocturnal fat metabolism through an indirect effect of improved sleep quality. By understanding the dynamic interplay between exercise and sleep quality, we will be better able to utilize exercise as a method to improve multiple domains of health related to chronic disease risk. **In this study, we will conduct a controlled inpatient clinical study to determine the effect of exercise on nocturnal metabolism and sleep quality in individuals with MetS.**

1.6 Innovation: This study is innovative in several ways. **Firstly**, individuals with MetS will be studied for 24-hours in a whole room calorimeter; by tightly controlling energy balance, I will be able to isolate the effects of exercise on nocturnal metabolism. **Secondly**, I will simultaneously measure sleep quality using gold standard techniques (PSG) to generate novel data on how sleep quality impacts nocturnal metabolism. **Thirdly,** I will probe potential mechanisms involved in the effects of exercise on sleep (e.g. body temperature regulation and catecholamines). **Finally,** I will compare the effects of morning versus evening exercise to determine whether exercise timing has a significant influence on sleep quality or nocturnal metabolism. There have been very few studies that have systematically tested the effects of exercise timing on these measures, therefore, very little is known. Data generated from this proposal will inform future larger studies.

III. Preliminary Studies/Progress Report:

Work from my master's and dissertation sought to determine the impact of light physical activity bouts on glycemic control in sedentary individuals and individuals with type 2 diabetes. To assess glycemic control, we used continuous glucose monitoring, which allowed for the assessment of nocturnal glycemia. In both the free-living environment and a controlled laboratory setting, short light bouts of physical activity performed in the afternoon and early evening resulted in lower nocturnal glycemia (7, 15). Very little is known about how exercise influences nocturnal fat metabolism.

Both of my co-mentors, Dr. Rynders and Dr. Broussard, have implemented frequent nocturnal blood sampling in their inpatient studies to measure hormones and metabolites during sleep in humans (11). Specifically, Dr. Rynders has preliminary data demonstrating that exercise performed in the morning and evening results in lower nocturnal insulin concentrations, increased FFA concentrations and increased fat oxidation (Figure 1, unpublished data). At night, concentrations of the antilipolytic hormone insulin are low (8) while concentrations of hormones promoting lipolysis (e.g. growth hormone, and glucagon) are high (33, 40, 41). This nocturnal hormonal milieu results in increased FFA availability and increased fat oxidation (3, 24, 32). It seems plausible that exercise potentiates this latter effect. These preliminary data from Dr. Rynders demonstrate that exercise can influence the nocturnal metabolic environment. However, because energy expended during exercise was not replaced in the diet, it is unclear from this study whether the observed effects are due to exercise or energy deficit. Further, the mechanisms driving alterations to nocturnal FFA and fat oxidation in response to exercise are not known and deserve investigation.

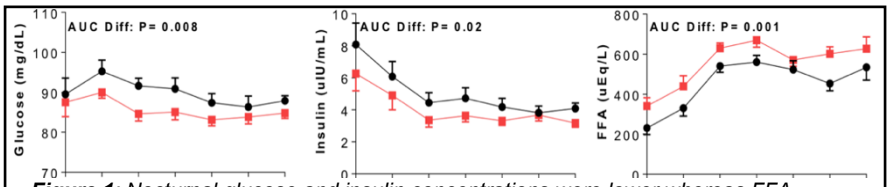


***Figure 1**: Nocturnal glucose and insulin concentrations were lower whereas FFA concentrations were higher after 2 bouts of exercise (red line) compared to a non-exercise control condition (black line).*

IV. Research Methods

A. Outcome Measure(s):

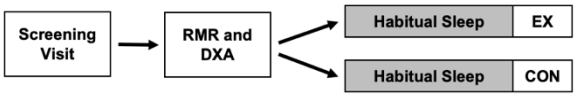
The primary outcome is nocturnal FFA concentrations. Secondary outcomes are nocturnal fat oxidation, nocturnal glucose and insulin concentrations, and sleep quality (e.g. percent time in slow wave sleep [SWS], sleep latency, and wake after sleep onset [WASO]). An exploratory outcome is the distal-proximal skin temperature gradient (DPG). The methods that will be used to measure these outcomes are described below in section C (Study Design and Research Methods).

B. Description of Population to be Enrolled:

Physically inactive men and women (N=18, [9 men, 9 women]; 19-45 years) will be recruited from the local Denver area. Individuals must have overweight or obesity and central adiposity as defined by waist circumference >40 inches (men) or >35 inches (women). Additional inclusion and exclusion criteria are:

- Inclusion criteria: not currently meeting physical activity guidelines (<150 minutes moderate to vigorous physical activity per week); weight stable (<5% change in weight over the last 6 months); habitual sleep duration of 7-8 hours and consistent sleep/wake schedule (<1 hour variation night-to-night).
- Exclusion criteria: body mass index (BMI) ≥35.0 kg/m^2; pregnancy; post-menopausal status in women, confirmed by elevated follicular stimulating hormone concentrations (>50 mIU/mL); hormonal contraception in premenopausal women; self-reported diabetes, cardiovascular disease, untreated hyper/hypothyroidism, or cancer; inability to perform moderate to vigorous treadmill exercise; a clinical diagnosis of a sleep disorder (e.g. sleep apnea); working night shifts or traveling across more than 2 time zones within 1 month of and throughout the study; currently taking medications for glucose management, thyroid conditions or blood pressure control. Anyone who has experienced a serious illness within the last 6 months (per discretion of PI and study MD). Anyone with a confirmed positive COVID test within the last 6 months (per discretion of PI and study MD)

C. Study Design and Research Methods



D. ***3.1 Overview of Study Design (Figure 2):*** Participants will perform 4 study visits over 2-3 months. Eligibility will be determined during the 1st visit (screening visit). During the 2nd visit, resting metabolic rate (RMR) and body composition will be measured. Habitual sleep/wake patterns will then be measured for 1 week. Participants will then perform each of the 3 study conditions (visits 3-4) in randomized order with at least a 1-week washout period between conditions.

Visit 1, Screening Procedures: After preliminary telephone screening, participants will attend a virtual screening visit to determine eligibility. Participants will provide written informed consent (via RedCap or by sending a scanned copy of the signed informed consent to the study coordinator email). Participants will then complete a medical history questionnaire and a sleep quality questionnaire (Pittsburgh Sleep Quality Index, PSQI (12)). All questionnaires will be completed on RedCap.

***Visit 2, Baseline Assessments*:** Eligible participants will return to the laboratory and provide a blood sample for screening blood tests (fasting glucose, HDL cholesterol, and triglyceride concentration). Fasting estrogen and progesterone will be assayed during the screening visit and during each of the inpatient study visits. Researchers will also measure anthropometrics (height, weight, waist circumference, and blood pressure). Participants will then have their RMR measured following an overnight fast (≥ 10 hours). Participants will arrive in the Clinical and Translational Research Center (CTRC) between 7 and 10 AM. They will rest quietly for 30 minutes, after which RMR will be measured for 20 minutes using indirect calorimetry with ventilated hood method (Parvo Medics Trueone 2400 Metabolic Cart, Sandy, UT). Participants will then perform a 45-minute submaximal exercise test at moderate intensity (65% age predicted maximum heart rate) on a motorized treadmill. We will measure energy expenditure (EE) with indirect calorimetry to determine expected exercise EE during the EX condition. Speed will be recorded during the exercise bout for participants to replicate during EX conditions. Body composition (i.e. fat mass and free fat mass) will be measured using dual-energy x-ray absorptiometry (DXA). Prior to continuing with the study, the study M.D. will review medical history forms and blood sample results to confirm eligibility.

Pre-study Controls (Diet, Sleep, and Physical Activity): Participants will be provided a 1-day diet to ensure participants are in energy balance prior to beginning the study. Energy intake (kcal/d) will be estimated to meet total daily energy needs (by multiplying RMR by an activity factor of 1.5-1.6 (1)). The macronutrient composition of meals will be representative of a traditional western diet (55% CHO, 30% fat, 15% protein).

To ensure regular sleep schedules during the week before each study condition, participants will be instructed to stay in bed for 8 hours each night and maintain a consistent sleep and wake time. Sleep and wake times will be monitored continuously using self-reported and objective measures (described in *section D*).

During the week prior to the EX condition, participants will be asked to perform 2 habituation bouts of moderate intensity exercise (45 minutes in duration). Each bout will be separated by at least 1 day. The last habituation bout of exercise will occur at least 2 days before the EX in-patient study condition. This exercise bout will be unsupervised and performed at the participant's home environment.

Visit 3-4, In-patient Study Conditions (EX and CON):

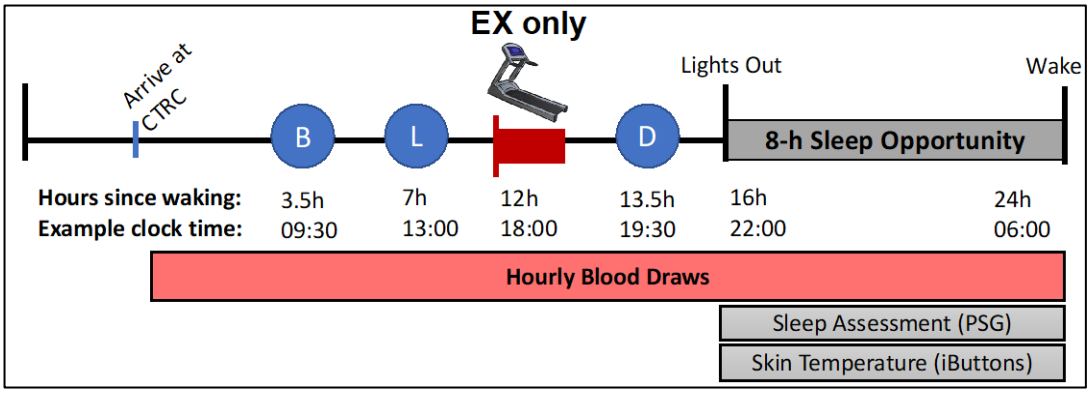


Figure 3: Timing of in-patient study conditions

Each experimental condition (EX and CON) will last 24-hours in the whole room calorimeter. Timing of meals (blue circles), exercise (treadmill image) and study measurements (lower red and grey boxes) are shown for both conditions (EX, and CON) relative to participant's habitual wake time. The absolute time of day for an example participant who normally wakes up at 06:00 is listed below relative times in italics (see example clock time).

Participants will arrive in the CTRC in the morning within 2 hours of their habitual wake time. Participants will enter the whole room calorimeter approximately 1 hour after arriving at the CTRC. Participants will be permitted to perform activities of daily living (e.g. watching TV, reading, computer work), but bed times will be standardized to each participant's habitual bedtime. At the time of bed, participants will be instructed to turn off the lights, lay down in bed

and to refrain from using electronic devices. Participants will be woken up the following morning at their habitual wake time. For subsequent in-patient study conditions, the timing of events during the study (e.g. CTRC arrival, bedtime) will be identical. During the EX condition, participants will perform 45-minutes of moderate intensity continuous exercise on a motorized treadmill 12 hours their habitual wake time in the evening (Figure 3). Prior to the exercise bout, participants will warm-up by walking on the treadmill for 5 minutes at 2.0-2.5 mph. Participants will wear a Zephyr heart rate monitor (Medtronic) to continuously track and record heart rate on a minute-by-minute basis using Bluetooth technology. Research staff will confirm start and stop time of exercise bouts to ensure participant compliance to the exercise bout. Participants will replicate the exercise bout performed during the submaximal exercise test during the baseline assessment (described in section D) and maintain heart rate at 65% of age-predicted maximum heart rate.

Energy intake will be standardized during each condition and will be provided as 3 discrete meals increasing in size over the day (breakfast: 25%, lunch: 35%, dinner: 40%) to simulate usual eating patterns of western adults (55). To compensate for anticipated lower physical activity levels while in the whole room calorimeter, energy intake (kcal/d) energy intake will be adjusted by multiplying RMR by an activity factor of 1.4. To ensure energy balance is maintained during the EX conditions, we will add back the calories expended during exercise evenly across breakfast, lunch and dinner (i.e. 300 kcal expended during exercise, 100 kcal will be added to each meal). We will use the measured EE during the baseline submaximal exercise test as an estimate of energy expended during exercise in the whole room calorimeter. To measure dietary fat trafficking, we will administer a D31 palmitate stable isotope tracer orally during the breakfast meal. Urine samples will be collected throughout the calorimeter stay to measure assess dietary fat oxidation.

Upon waking, participants will be given light blocking glasses to wear for the measurement of melatonin. Saliva samples will be collected hourly for 6 hours after waking to assess melatonin offset. Simultaneously, an intravenous glucose tolerance test (IVGTT) will be performed to assess insulin sensitivity.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Description and Justification of Procedures

Anthropometrics (visit 2): Body weight will be measured using a digital scale accurate to $\pm$ 0.1 kg and height will be measured to the nearest 1mm with a stadiometer. Waist circumference will be measured using a tape measure just over the iliac crests.

Questionnaires (visit 1, 3, 4): Participants will be asked to complete the Pittsburgh Sleep Quality Index questionnaire (12) during their screening visit and the Post Sleep Questionnaire (31) upon waking during each of their in patient stays. Additional questionnaires will be delivered to ask about participant's sleep, anxiety, and depression levels during the screening visit. During the in-patient study conditions (visit 3 and 4), participants will complete questionnaires about their sleep quality/duration and appetite.

Resting Metabolic Rate (RMR, visit 2): After lying quietly for 30 minutes, RMR will be measured for 20 minutes by indirect calorimetry using the ventilated hood method (Parvo Medics Trueone 2400 Metabolic Cart, Sandy, UT). RMR will be assessed in the morning, after an overnight fast; participants will be asked to refrain from strenuous physical activity during the 48 hours prior to the test.

Body Composition (visit 2): Body composition (i.e. fat mass and free fat mass) will be measured using dual-energy x-ray absorptiometry (DXA, Hologic Discovery W version 12.6; Hologic Inc., Bedford MA).

Habitual Sleep Schedule (prior to visit 3-4): Based on their usual bedtimes, researchers will give participants a target sleep and wake time to maintain during the week. Participants will be asked to keep a digitally time-stamped sleep/wake time log by sending a text message to a google voice account upon going to bed and waking up daily. Researchers will monitor sleep and wake times daily to ensure participants maintain their habitual sleep/wake schedule. If participants deviate from their habitual sleep/wake times by more than 1 hour (i.e. wake up and/or go to bed 30 minutes earlier or 30 minutes later than the target time instructed by researchers) or are sleep restricted (<7 hours sleep during the previous 2 nights of sleep), participants will be rescheduled for another day. We will also objectively monitor habitual sleep/wake times using an ActiWatch accelerometer (Philips Actiwatch Spectrum).

Sleep quality (visit 3-4, secondary outcome): Sleep quality will be measured objectively using PSG (Siesta Systems digital sleep recorders, Compumedics USA Ltd). PSG electrodes will be placed after the completion of the dinner meal. Researchers will temporarily enter the calorimeter and pause the data recording. Upon exiting the calorimeter, data recording will resume. Dr. Rynders has experience in processing calorimeter data that has been interrupted

and will advise me in the analysis of the calorimeter data. Sleep stages (REM and non-REM sleep: stages 1, 2 and 3) will be visually scored in 30-epochs windows using standardized guidelines (6). Measures of sleep quality including, sleep latency, wake after sleep onset (WASO), number of nocturnal awakenings, and percent time in SWS will be quantified using PSG data (22). Subjective sleep quality will be measured within 10 minutes of when the participant is awoken using the Post Sleep Questionnaire (31).

Dietary fat oxidation: 15 mg/kg body weight of D31 palmitic acid will be added to a meal replacement beverage to be ingested orally with breakfast. Spot urine samples will be taken prior to the ingestion of the tracer and at hourly intervals throughout the day to measure elimination rates of D31 (35).

Nocturnal fat oxidation (secondary outcome): Nocturnal fat oxidation will be measured using whole-room indirect calorimetry. Differences in O_2 and CO_2 concentrations between the air entering and exiting the calorimeter are continuously measured. Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), EE and fat oxidation will be calculated using previously published equations (21).

Nocturnal metabolites and hormones (primary and secondary outcomes): During each calorimeter study, we will obtain hourly blood samples. Blood will be drawn using 12-foot extension tubing connected to an intravenous line, which will be inserted upon arrival in the CTRC (daytime hourly samples will be stored for future analysis). The tubing will be kept patent with a low dose heparinized saline drip. The tubing is fed through a porthole to allow for frequent blood draws without disturbing the participant during sleep. Blood samples will be used to measure metabolites (free fatty acids [FFA] and glucose) and lipolytic and antilipolytic hormones (insulin, growth hormone, glucagon norepinephrine and epinephrine). CTRC core lab services will be used to analyze all blood samples. EDTA vacutainers containing aprotinin will be used to collect blood samples for glucagon analysis. Radioimmunoassay (Millipore) will be used to quantify glucagon concentrations. Nocturnal area under the curve (AUC) will be calculated for all metabolites and hormones using time to bed and wake time. Drs. Broussard and Dr. Rynders (co-mentors on this project) have performed several in-patient research studies that incorporate frequent blood sampling overnight to assess nocturnal metabolism (10, 11).

Distal/proximal temperature (exploratory outcome): Skin temperature will be measured at the distal and proximal regions during each study condition using digital skin thermometers (iButton, Maxim Integrated) (19) placed on 8 different sites (distal: hands and feet; proximal: chest, thigh, abdomen, and forehead) (36). Minute by minute data from the distal and proximal regions will be averaged to yield a single continuous temperature variable for the distal and proximal region that will be used to calculate the DPG (ratio of distal to proximal temperatures).

Next day insulin sensitivity: Insulin sensitivity will be assessed the morning after study conditions with a frequently sampled IVGTT. A glucose bolus (300 mg/kg) will be injected into an intravenous line within 60 seconds. Blood will be drawn at 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 75, 90, 100, 120, 140, and 180 minutes to measure plasma glucose, insulin and C-peptide. We will estimate total body insulin sensitivity with Bergman's Minimal Model (5).

Dim light melatonin offset (DLMOff): Upon waking, participants will wear light blocking glasses to maintain dim light conditions (<30 lux) for the measurement of DLMOff. Hourly saliva samples will be taken for 6 hours after wake. DLMOff will be the time at which salivary melatonin concentrations are less than 3 pg/mL (4).

Risks of Procedures

Acute exercise bouts: The discomforts of performing unaccustomed exercise and testing will be explained to the participants (e.g. fatigue, possible muscle soreness). The acute exercise bout will be of submaximal intensity. To minimize the potential risks of acute exercise, we have taken the following precautions:

1. Subjects will undergo a physical examination by the study physician prior to participation in any exercise
2. In the event that a serious cardiac event occurs while at the Exercise Research Laboratory, CPR will be initiated and an AED will be available for use by certified staff until emergency medical personnel arrive to take over care
3. Every exercise bout will be supervised by qualified study personnel who are versed in exercise training for clinical populations and BLS-certified. Exercise intensity will be monitored by study personnel to decrease likelihood of an acute cardiac event, and participants will be monitored for symptoms including chest pain, acute shortness of breath at rest, or dizziness
4. If a participant reports any contraindications to exercise after initiating this study, the participant will be instructed to suspend their exercise and will required to obtain additional clearance from the study physician prior to re-engaging in exercise

Radiation/DXA scan: Participants will be exposed to ionizing radiation during the DXA (0.3 mrems per total body scan). Total radiation exposure (0.6 mrems) is less than 1% of annual allowable exposure permitted for radiation workers by federal regulations. The risk of excess radiation exposure is minimized by having trained technicians administer the DXA, reducing the likelihood of needing repeat assessments.

Venipuncture: There is a small risk of local hematoma or infection associated with blood sampling. These risks are minimized by having trained CTRC clinical personnel perform the procedures using sterile techniques.

Heparin: A small amount of heparin will be used to keep the blood draw catheter patent. Allergic reactions and side effects to heparin are rare but possible. Symptoms of an allergic reaction to heparin may include rash itching swelling at the IV catheter site, dizziness or trouble breathing. To mitigate this risk, the study physician will ask the subjects about known allergies to heparin or allergies to pork products at the time of screening medical history and physical exam. CTRC nurses will monitor for signs of an allergic reaction to heparin (e.g. rash itching swelling at the IV catheter site, dizziness, or trouble breathing, etc.)

Whole room calorimeter: Claustrophobia or noisiness in the calorimeter may disturb volunteers. However, the new room calorimeter at CU-AMC is large (12' x 12') with a large picture window, and claustrophobia has never been reported. Furthermore, potential volunteers will be shown the calorimeter during the screening to familiarize them with the calorimeter.

Economic risks: Possibility of finding a previously undiagnosed medical condition during screening and not having insurance coverage for further evaluation and treatment, as well as time lost from work or studies. This risk is minimized by ensuring subjects are in an appropriate economic position to participate. Participants will be asked if they are able to comply with study conditions and attend scheduled visits.

Psychological risks: may involve the stress of identifying a previous unknown medical condition during the pre-screening selection.

Confidentiality and privacy: The use of questionnaires and collection of personal medical information poses a risk to confidentiality and privacy and may cause embarrassment. These risks will be minimized by not including personal identifying information on the forms, when possible, and by conducting interviews and collection of personal information in a private setting.

E. Potential Scientific Problems:

The proposed study is feasible and well designed to make meaningful scientific contributions. The following limitations were considered for this proposal:

1. *Exercise Time of Day:* My mentors and I have discussed extensively whether the acute exercise bout should be performed in the morning, afternoon, or evening. After considering the strengths and limitations, we decided to time the exercise bout in the early evening, prior to dinner. Our decision was guided by the observation that this mimics a typical daily pattern for many individuals, and the assumption that evening exercise would have the most pronounced effect on the nocturnal hormonal milieu and thus nocturnal metabolism.
2. *Energy Balance:* We elected to perform these studies in energy balance (e.g. replace the calories expended in exercise) which permits us to eliminate the potential confounding effect of negative energy balance on nocturnal metabolism. Based on our preliminary data, we are confident that performing this study in energy balance will still have pronounced effect on nocturnal metabolism.
3. *Dietary Control*: We will only control diet for 1 day prior to in-patient study conditions. If we are successful in obtaining additional funding, we will include 3 days of controlled feeding prior to each study condition.
4. *Menstrual Cycle*: We will be enrolling pre-menopausal women who will have fluctuating hormones over the course of 1 month. Ideally, we would test women during the same phase of their menstrual cycle; however, this would extend the testing duration from a maximum of 5 months to 6 months, which is not feasible from both a research scheduling and participant burden perspective. Thus, we will record the date each woman's last period to record the approximate phase of their menstrual cycle. We will also measure morning, fasted estrogen and progesterone concentrations and include these as covariates in the final analysis.

F. Data Analysis Plan:

Sample size estimate: The goal of this pilot study is to obtain preliminary data to inform an in-depth mechanistic study (K01 application) investigating factors that may modulate the relationship between exercise and sleep (e.g.

exercise timing) and their subsequent metabolic impact (e.g. dietary fat oxidation, insulin sensitivity). Conservatively assuming 15% attrition, we propose to recruit a total 18 participants with at least 15 participants completing all 3 conditions.

Statistical Analysis Plan: All statistical analyses will be performed using the R statistics and computing package. We will employ conventional approaches to the interpretation and reporting of results from statistical analyses (i.e. 2-sided p=values and 95% confidence intervals, and significance levels of 0.05 for all hypotheses tests). To accomplish **aim 1 and 2**, we will compare the effect of EX vs. CON our primary and secondary outcomes (described in section 3.1) using repeated measures linear mixed models with planned contrasts. To accomplish the **exploratory aim**, we will compare the effect of EX and CON on the distal/proximal gradient and melatonin responses and explore whether those differences are associated with changes in sleep quality and nocturnal metabolism. Finally, we will explore whether there are differences in any of our outcomes by sex.

G. Summarize Knowledge to be Gained:

Metabolic syndrome is a major risk factor for many chronic diseases, including diabetes and cardiovascular disease. Poor sleep quality is related to numerous poor health outcomes and is common among individuals with metabolic syndrome. Impairments in nocturnal fat metabolism are associated with insulin resistance and a strong predictor of subsequent weight gain. Exercise is a potential therapeutic target which may improve both sleep quality and nocturnal fat metabolism. Findings from the proposed study will identify how exercise influences novel contributors to metabolic syndrome (sleep quality and nocturnal metabolism) and elucidate potential mechanisms to explain the variability in exercise responses.

H. References:

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