Mechanisms for Sleep/Circadian Disruption-Induced Impairments in Bone Formation

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I. Hypotheses and Specific Aims:

This small intervention study will determine if and how sleep restriction, independent of circadian misalignment (e.g. shift work, jet lag), induces a decrease in the bone formation marker Procollagen I Intact N-Terminal Propeptide (PINP). Specifically, it will evaluate if sleep restriction itself is responsible for the decrease seen in PINP with cumulative sleep restriction and concurrent circadian misalignment. The specific aim is to evaluate the mechanistic underpinnings for the relationship between sleep restriction and suppression of bone formation through a small intervention study in healthy adult men. The hypothesis is that sleep restriction, independent of differences in posture, mechanical loading, or activity levels/patterns decreases bone formation and that the magnitude of the decrease will be greater in those with higher levels of bone turnover at baseline.

The work being proposed here for COMIRB consideration only covers the 3rd aim of my K23 award.

II. Background and Significance:

Osteoporosis is a prevalent disease with significant morbidity and mortality (1-3), yet no underlying cause is identified in ~50% of osteoporosis work-ups (4). Osteoporosis is traditionally associated with postmenopausal women, however, it is an under-recognized condition in men despite their higher mortality rate after hip fracture (5). Although the fracture burden of osteoporosis is predominantly seen later in life, peak bone mass is attained around 30 years of age and is a significant determinant of bone health and strength later in life. Skeletal insults prior to this age could limit attainment of optimal peak bone mass, resulting in an increased risk of osteoporosis and fracture later in life. Sleep disturbance occurs across the lifespan and may represent a novel cause of osteoporosis that could help explain the etiology of otherwise “idiopathic” osteoporosis. Identifying sleep disturbance as a novel cause of osteoporosis would provide an opportunity for appropriate intervention and potentially osteoporosis prevention if the sleep disturbance is identified and remedied prior to the attainment of peak bone mass or before bone loss occurs in older populations.

The literature on sleep duration and bone health in humans is very mixed with both long and short sleep duration associated with low bone mineral density (BMD) (6).

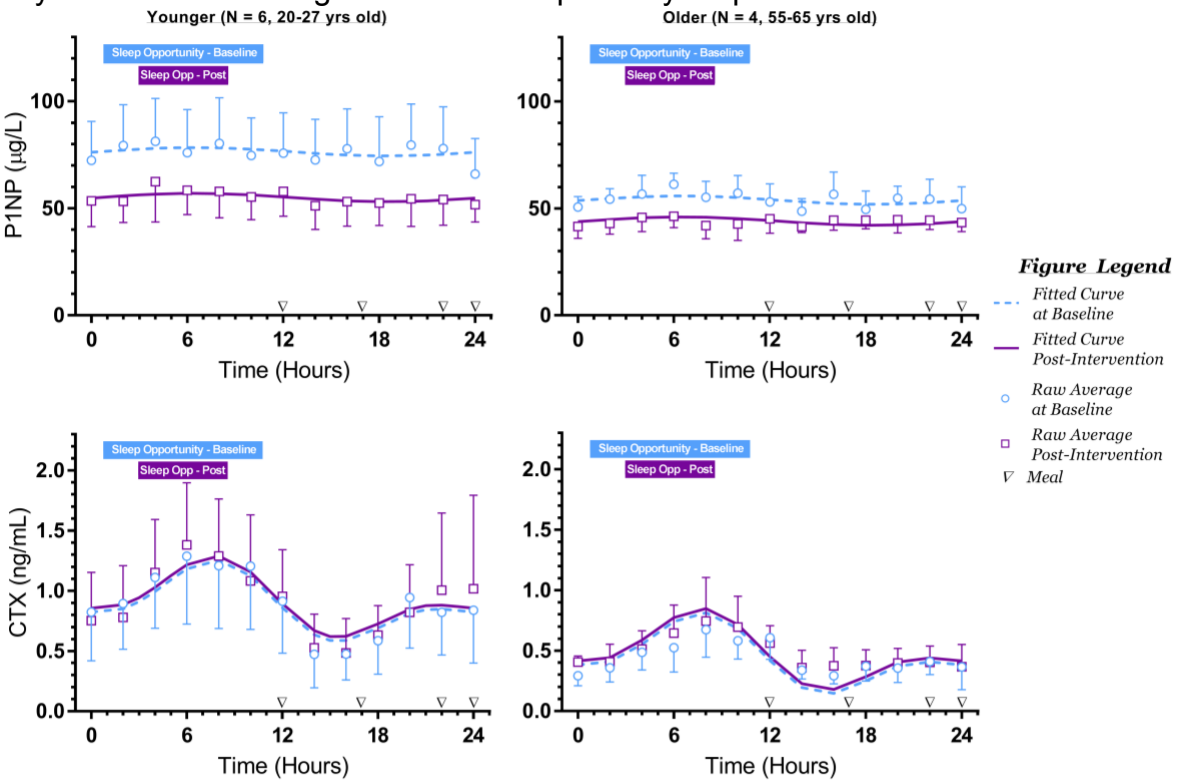
The inconsistent literature is due, in part, to cross-sectional studies utilizing subjective sleep duration and variable method/site for assessment of bone mineral density. There is a lack of pure sleep restriction studies that are designed to assess skeletal outcomes in humans but animal data suggest sleep restriction may be detrimental to bone health. Studies have shown impaired bone formation and subsequently lower BMD in chronically sleep deprived rats compared to controls (7, 8). Our data on the effects of cumulative sleep restriction and concurrent circadian misalignment in men closely parallel these animal data.

We were the first to describe lower levels of a bone formation marker (PINP) despite no change in a bone resorption marker (C-telopeptide of Type I Collagen - CTX) in ten healthy men after 3 weeks of cumulative sleep restriction with concurrent circadian disruption (akin to the stresses endured during rotating shift work) (9). In addition, these data showed that the magnitude of the decrease in PINP was greater in younger men (aged 20-27 years), who had higher levels of bone turnover at baseline, compared to older men (aged 55-65 years) (9). A significant decrease in bone formation without a concomitant decrease in bone resorption could lead to an unfavorable balance in bone turnover that, if sustained, could lead to bone loss, low BMD/osteoporosis and increased fracture risk. Furthermore, our preliminary data suggest that the magnitude of this insult may be magnified during skeletally vulnerable periods when bone turnover is high, such as early adulthood during attainment of peak bone mass. This implies that even if a sleep and/or circadian disruption is relatively transient, it may have lifelong implications for skeletal health if it compromises attainment of peak bone mass. The gap in knowledge is that it was not clear from our study if the decrease in PINP was due to the imposed sleep restriction or circadian misalignment (because they were imposed simultaneously) or other confounders. Since our original study was not specifically designed to investigate bone-related outcomes, it is important to evaluate possible confounders in this relationship and determine the mechanisms by which sleep and circadian disruption alter bone turnover in humans.

This study would fill this knowledge gap by determining if sleep restriction alone is capable of decreasing a marker of bone formation. In addition, this study will begin to dissect the mechanisms by which sleep restriction alters bone metabolism by controlling activity levels/patterns, dietary intake, light exposure, and posture. These data will be used to support and design a larger R01 application aimed at investigating all potential mechanisms for sleep/circadian-induced alterations in bone metabolism and the potential for reversal of these changes with normal sleep duration and timing (during the biological night). Currently, a sleep assessment is not performed during the routine evaluation of causes of secondary osteoporosis, but our research has the potential to change that. If our hypothesis is shown to be correct and sleep disruption is identified as a novel risk factor for osteoporosis then screening could lead to diagnosis and intervention for the sleep disturbance before osteoporosis is manifest or during osteoporosis evaluation. Furthermore, pharmacological interventions for low BMD may be avoided and/or their efficacy improved by prompt amelioration of the sleep abnormality. In addition, understanding the biological pathways by which sleep affects bone may help to identify a new causal pathway for low bone mass, fracture risk, and new therapeutic targets.

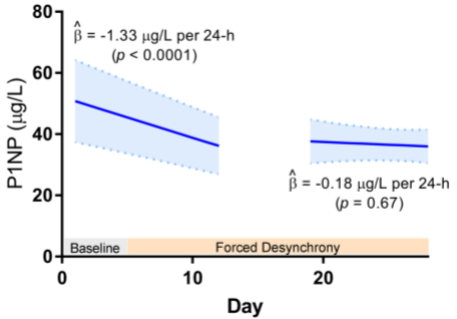
III. Preliminary Studies/Progress Report:

We have conducted similar work in this novel area and will be able to complete the proposed study. Our preliminary data (*Figure 1*) showed that in healthy men, three weeks of insufficient sleep (sleeping 5.6 hours/night) with a history of circadian misalignment was associated with a significant decrease in bone formation (PINP) while bone resorption (CTX) remained unchanged, creating a potential "bone loss window" (9). Our preliminary data also suggest that bone metabolism may be particularly susceptible to sleep disturbance during vulnerable periods when bone turnover is high (e.g. men 20-27 years old, at the end of peak modeling period) (9). These data indicate that sleep and circadian disturbance may be novel, modifiable risk factors for bone loss, low BMD/osteoporosis and increased fracture risk. To apply these data to clinical practice, more research is needed to determine whether it was the sleep restriction or history of circadian misalignment that was primarily responsible for the decrease in the



bone formation marker, PINP, and to investigate the mechanisms by which sleep restriction alters bone metabolism.

In my current research project, **we have determined that the decline in PINP is evident within the first 5 days of the imposed sleep restriction and concurrent circadian misalignment (*unpublished data – Figure 2*).** Therefore, we believe it is realistic to see appreciable changes in bone turnover markers within the proposed nine-day study period. We are also currently examining age and sex differences in the sleep bone relationship. Our preliminary data indicate that similar changes in bone turnover markers are not



seen in post-menopausal women with short (<6 hours/night) compared to normal (7-8 hours/night) sleep duration (*unpublished data*). Therefore, we will focus inclusion criteria for our current study to men.

IV. Research Methods

A. Outcome Measure(s):

- Primary Outcome: Change in Serum Procollagen I Intact N-Terminal Propeptide (PINP) – a marker of bone formation.
- Secondary Outcomes: Change in serum C-telopeptide of Type I Collagen (CTX) – a marker of bone resorption; change in sclerostin (a marker of osteocyte function and activity); resting energy expenditure.

Bone turnover markers (BTM) are well-established surrogate markers for estimating bone turnover because they usually correlate with direct measures of bone remodeling (i.e. histomorphometry obtained from bone biopsy) (10). We will be using the preferred (11) markers of bone formation (PINP) and resorption (CTX) as our primary and secondary outcomes, respectively. Serum CTX will be measured using the Immunodiagnostics ELISA assay (inter-assay CV 9.7%, intra-assay CV 1.7%) (12). PINP will be measured using the Orion RIA assay for intact (trimer only) PINP (inter-assay CV 5.5-9.5%, intra-assay CV 3.2-9.6%) (13). All will be run in duplicate and then averaged for the final result. Sclerostin will be measured using the Meso Scale Discovery ELISA assay (mean CV of 4%) (14).

B. Description of Population to be Enrolled:

- Inclusion Criteria:
    - Adult men aged 20-65 years old who habitually sleep 7-9 hours/night (15)
- Exclusion Criteria:
    - Regularly go to sleep after midnight;
    - Shift work 1 year prior to study;
    - Travel >1 time zone 4 weeks prior to study or need to travel during study;
    - More than moderate activity level (>3 days of exercise per week >30 min of exercise per session);
    - Current smokers (or within the previous year of study);
    - Positive drug test at screening or laboratory admission;
    - BMI > 30 kg/m^2;
    - Individuals who are concurrently participating in another research protocol that would influence their safe participation in this study. For example, participants involved in a study that requires blood draws or ingestion of experimental medication as this would increase the risk of participation in our study and/or compromise study results.
    - Any clinically significant unstable medical or surgical condition within the last year (treated or untreated), including history of a clinically significant abnormality of the neurological system (including cognitive disorders or significant head injury) or any history of seizure (including febrile seizure—sleep loss has been used clinically to induce seizures in patients with epilepsy). Given the wide range of illnesses that are encountered in medical practice, it would not be possible to provide a comprehensive list

of each and every disease that could serve as grounds for exclusion for the subject. However, the following is a list of illness categories that would certainly be grounds for exclusion: Connective Tissue and Joint Disorders; Neurologic/cognitive Disorders; Musculoskeletal Disorders; Immune Disorders; Chronobiologic Disorders; Cardiovascular Disorders; Respiratory Disorders; Kidney Disorders; Infectious Diseases; Hematopoietic Disorders; Neoplastic Diseases; and Endocrine and Metabolic Diseases.

- Self-reported or newly diagnosed medical condition that is still being investigated or is not under good control, including those identified on screening labs such as:
    - Out-of-range values measured on a fasting blood sample: glucose > 100 mg/dl, thyroid stimulating hormone <0.5 or >5.0 uU/ml, abnormal alkaline phosphatase <39 or >117 U/l, creatinine, or hemoglobin <14.3 g/dl men
- Any clinically significant psychiatric condition, as defined by DSM-V. Individuals with a history of most psychiatric illnesses or psychiatric disorders will be excluded, such as but not limited to depression, anxiety, alcoholism, drug dependency, schizophrenic disorders, and personality disorders (performed by medical history and physician interview). However, a personal history of limited prior counseling, psychotherapy (e.g., for adjustment reactions) will NOT be exclusionary. Evaluation of Psychiatric/Psychological Suitability:
    - Subjects must demonstrate a full understanding of the requirements and demands of the study.
    - Each subject will complete psychological screening questionnaires. Exclusionary: Center for Epidemiological Studies Depression (CES-D) ≥ 16;. Subject responses to the CES-D are reviewed immediately and appropriate referrals are made if necessary.
    - Individuals who are unaware of specific psychiatric diagnoses who have a history of having been treated with antidepressants, neuroleptic medications or major tranquilizers will be excluded from study.
    - Use of anti-depressants or any like therapeutics prescribed by a physician is exclusionary
- Individuals with any clinically significant sleep disorder; Diagnosis or symptoms of sleep disorders (history of significant parasomnia as an adult [night terrors, frequent sleep walking], insomnia, including but not limited to hypersomnias such as apnea, periodic limb movements, narcolepsy). Sleep disorders will be screened by self-report and physician interview including use of validated sleep questionnaires (PSQI, Epworth sleepiness scale, and Berlin sleep questionnaire for sleep apnea).
- Individuals on medications known to affect bone turnover (e.g. glucocorticoids, osteoporosis medications);

- Use of medications/supplements/drugs that impact sleep or bone metabolism (such as but not limited to sleep medications, marijuana etc.) within one month (participants can be studied at a later date).
- Dwelling below Denver altitude (1,600 m) 3 months prior to testing;
- Greater than moderate caffeine (>500 mg/day) or alcohol use (>14 standard drinks/week or >5 drinks in one sitting);
- Subjects with a history of heparin-induced thrombocytopenia (HIT) or an allergy to heparin;
- Inability to travel to the CU-AMC campus for study visits.
- Individuals with restrictive diets (e.g., vegan)
- Individuals with 25OHD < 20 ng/mL;
- Individuals with eGFR < 60 mL/min/1.73m^2 as this is known to affect CTX measurements;
- T-score ≤ -2.5 (men ≥50 years old) or Z-score < -2.0 (men <50 years old) for bone mineral density (BMD) at the L-spine, femoral neck, or total hip on baseline DXA as compared to the DXA machine's normative database;
- Symptoms of active illness (e.g., fever); note that subject can be studied at a later date.

C. Study Design and Research Methods

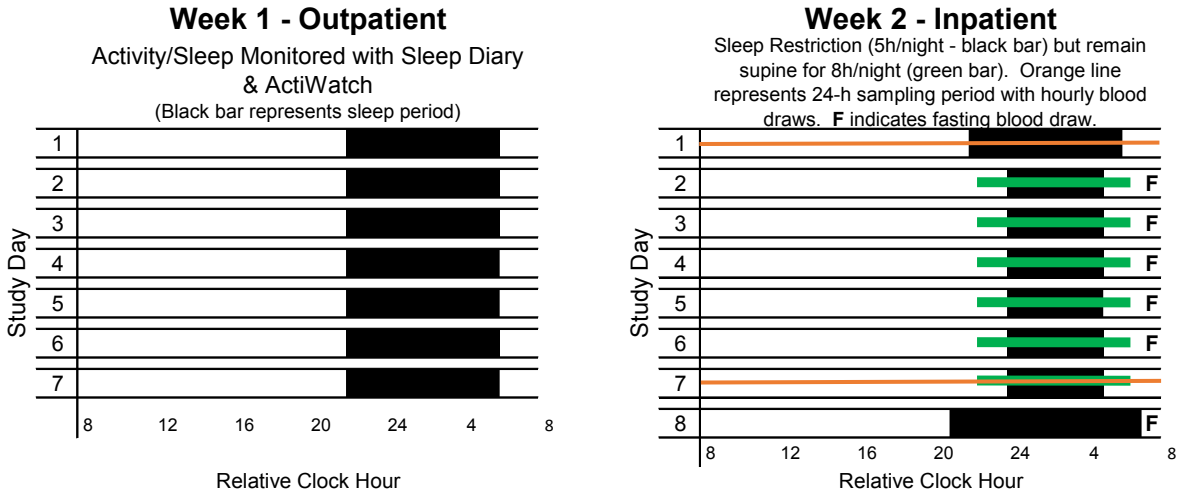
12 healthy adult men who habitually sleep 7-9 hours will be recruited and serve as their own control for an intervention (sleep restriction) study. Proposed study protocol is depicted in the figure below.

- Screening will be performed on the CU-AMC campus at the outpatient CTRC facility and will consist of:
  - Labs (CBC, CMP, TSH, 25OHD, urine drug screen)
  - WatchPAT to screen participants for concurrent sleep disorders meeting exclusion criteria (e.g. obstructive sleep apnea).
  - Completion of Pittsburgh Sleep Quality Index (PSQI) validated sleep assessment form
  - Baseline Dual-energy X-ray Absorptiometry (DXA) test to asses bone mineral density (BMD) will be performed at the hip and lumbar spine on all participants to characterize the study population and to retrospectively observe if an association exists between DXA parameters and any outliers of BTM change.
  - A general physical exam including psychological testing using the validated and standardized Center for Epidemiological Studies-Depression (CES-D) 20-item assessment.
  - Discussion regarding informed consent
- In Week 1, participants will sleep 8 hours/night at home during their habitual sleep time. Sleep will be monitored with ActiWATCH, a wrist actigraphy monitoring system, and verified with a concurrent sleep diary. Activity will be monitored with a FitBit. Participants will perform their usual daily routine and asked to complete three 20-minute standardized walking sessions per day with no other exercise. Meals on the last 3 days of the outpatient protocol will be

provided by CTRC Nutrition Service, and will be identical to the meals the participant will eat in Week 2.

- In Week 2, participants will be admitted to the inpatient calorimeter room (or equivalent space) at CU-AMC.
    - Participants will be at least 3 hours prior to habitual bedtime on Day 1 and will have hourly serum measurements and urine collected for 24 hours including an 8-hour habitual sleep period to represent BTMs during outpatient, regular conditions. The research unit has a special mechanism to collect blood without disturbing the participant's sleep
    - Polysomnography will be performed on night 1 to rule out any other sleep disorders
    - On Nights 2-7, participants will spend 8 hours supine in bed but will only be allowed to sleep for 5 hours/night. Sleep opportunities will be aligned to the midpoint of habitual sleep. Participants will be kept awake by study staff who will be present 24 hours a day, 7 days a week and who will also be present to ensure participant safety. Participants will be kept awake using conversation, games, electronics (TVs/computers/phones), and, if needed, gentle physical stimuli.
    - On the morning of Day 7 through the morning of Day 8, a second 24-hour serum profile (with urine) will be drawn.
    - Daily fasting serum will be obtained on Days 2-9.
    - Resting energy expenditure (REE): REE will be measured using standard indirect calorimetry38 (Parvo Medics TrueOne 2400, Salt Lake City, UT) before and after breakfast and dinner on days 2 and 8.
    - Night 8 will be a recovery sleep period (with ≥10 hours of sleep opportunity) with a final fasting serum draw on Day 9 prior to participant discharge. Recovery segment can be extended an additional 1-2 days depending on participant preference and assessment prior to discharge.
    - Throughout Week 2, participants will be encouraged to ambulate to match activity levels from Week 1. This will include repeating their standardized 20 minutes of walking three times a day. A treadmill will be provided in the room, if necessary, and participants will be allowed to ambulate outside with study staff. In addition to matching activity levels, efforts will be made to match the pattern of activity throughout the day to outpatient patterns. Changes in posture and mechanical loading are inherent in sleep restriction studies because patients are awake longer but are in a smaller space. Changes in mechanical loading are known to affect bone metabolism, largely through changes in sclerostin levels. Therefore, the actigraphy data will be used primarily to match activity levels and patterns during the outpatient and inpatient weeks to eliminate confounding due to changes in activity, we will verify this using FitBit. In addition, ActiWATCH will provide data on light exposure, which will be matched, to the best of our abilities, between outpatient and inpatient weeks, when not completing 24-hour sampling.
- In Weeks 3-5 participants will be monitored for an optional observational recovery phase. Participants will wear wrist monitoring systems (ActiWATCH

and FitBit) to quantify sleep and physical activity. In addition, they will maintain a sleep diary. No instructions will be given on sleep duration/ timing. Participants will return to the outpatient clinic 5 times after discharge from the inpatient unit to have fasting morning blood draws (each up to 7 mL).



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

Description of Study Procedures

Summary of Participant Activities and Burden During Outpatient and Inpatient Weeks:

Table 1

	Screening	Outpatient (Week 1)	Inpatient (Week 2)	Recovery (Weeks 3 -5)
Informed Consent, review inclusion/exclusion criteria	X			
Personal interview, medical history, medication use	X			
Vital signs	X			
Physical Exam	X			
CES-D Depression Assessment	X			
PSQI	X			
Urine drug screen	X		X (on admission)	
Screening Labs	X			
DXA	X			
Dispense CTRC Nutrition Meals		X	X	
ActiWATCH (wrist actigraphy)	X	X	X	X
FitBit (wrist monitor)		X	X	X
WatchPAT	X			
Polysomnography			X	
Resting Energy Expenditure			X	
Epworth Sleepiness Scale		X	X	
Sleep Diary		X		X
Karolinska Sleepiness Scale		X	X	
IV Placement			X	
Sleep Restriction			X	
Collect outcome measures (blood, urine)		X	X	X

Assess adverse experiences		X	X	X

Screening:

- Complete questionnaires, labs, DXA, physical exam and consent

Outpatient Phase (Week 1):

- Pick-up nutrition service meals from CU-AMC campus mid-week
- Maintain 7-9 hour sleep opportunity at habitual bedtime/wake time
- Wear wrist actigraph and complete sleep diary daily
- Complete three 20-minute standardized walking sessions per day
- Complete Epworth Sleepiness Scale at the beginning and end of the outpatient week
- Complete the Karolinska Sleepiness Scale three times per day

Inpatient Phase (Week 2):

- Complete Epworth Sleepiness Scale at the beginning and end of inpatient week
- Complete Karolinska Sleepiness Scale validated questionnaire three times per day.
- IV placement
- Resting energy expenditure (REE): REE will be measured using standard indirect calorimetry38 (Parvo Medics TrueOne 2400, Salt Lake City, UT) before and after breakfast and dinner on days 2 and 8.
- Sleep restriction (5 hours/night) on nights 2-7 verified by study staff and video monitoring throughout the protocol. Verified with PSG during the 24 hour collection intervals. Video will be recorded during PSG recording.
- Blood draws:
    - Hourly (via IV) for 24 hours at the beginning and again at the end of inpatient admission.
    - Daily fasting blood draw, including a fasting blood draw on the discharge day after recovery sleep.
    - 5, 1mL aliquots will be drawn at each time point for planned analyses and unspecified future use
    - IV can be removed in between the two, 24-hour collection periods if patient prefers to have daily venipuncture. Otherwise, IV will be changed every 72 hours.
    - A total of 56 blood draws will be performed throughout the inpatient phase (each up to 5mL). Over the course of the study the amount of blood drawn will be less than a typical blood donation (<1 pint).
- 24 hour urine collection during the two, 24-hour blood collection intervals
- Urine drug screen at admission
- Match activity level/patterns and light exposure to outpatient week including three 20-minute standardized walking sessions per day
- Night of recovery sleep (≥10 hours sleep opportunity) on night 8 prior to discharge on day 9. Recovery segment can be extended an additional 1-2 days depending on participant preference and assessment prior to discharge.

Outpatient Observational Recovery Phase (weeks 3-5):

- Participants will maintain a sleep diary and wear wrist actigraphy to document durations of recovery sleep and physical activity after discharge.
- No instructions on sleep duration or timing will be provided
- Fasting morning serum will collected on recovery days +1 (on the discharge day from the inpatient unit), +2, +3, +7, +14 and +21.
- Participants will return 1, 2, 6, 13 and 20 days after discharge to the outpatient clinic for these fasting blood draws (each up to 7 mL)

Risks & Justification of Study Procedures and Data Collection Tools

The risks of study procedures and data collection tools are minimal because they are not greater than what may be encountered in daily life.

- **Screening and study-related labs** are necessary to apply the necessary exclusion criteria, to characterize the study population and to analyze the primary and secondary outcomes. The risks will be minimized:
    - Excluding those with pre-existing anemia;
    - Experienced, trained personnel utilizing sterile technique for venipuncture and IV line placement will minimize the risks associated with blood draws;
    - Minimizing volume of blood drawn to what is needed for primary and secondary aims will minimize the risk associated with blood loss. Overall, the amount of blood drawn from screening to study completion will be less than a typical blood donation (<1 pint);
    - Referral will be offered to primary care physicians and/or specialists (including psychiatrist) if new diagnoses are made (or suspected) during screening.
        - Answers to psychiatric questionnaires will be reviewed in real-time and any time-sensitive referrals made for volunteers who indicate suicidality with a plan.
- **Catheterization** is necessary to perform hourly blood sampling on days 1 and 8 of week 2 of the study protocol. Risk will be minimized:
    - There is a potential risk of thrombophlebitis with the catheter placement but this risk is small. Local hematomas are also possible but infrequently observed. There is a slight risk of infection with the blood draw. 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 or swelling at the IV catheter site, dizziness or trouble breathing.
    - Experienced, trained CTRC personnel will place IV lines and nursing personnel will be present 24/7 to address any adverse reactions
    - IV lines will be changed every 72 hours, as per hospital policy
- **DXA** is needed to characterize the study population and apply the necessary exclusion criteria. The risks will be minimized:
    - The DXA procedures involve exposure to ionizing radiation to the whole body of approximately 2 mrem (0.2mSv). Overall radiation exposure from DXA is less than that from daily background radiation dose to the whole body and is <0.05% of the annual allowable exposure for radiation workers;

    - Trained technicians will administer the DXA exams, thereby reducing the likelihood of needing repeat assessments.
- **Sleep restriction** is necessary to test the study hypothesis, but can be physically and mentally challenging. Risks will be minimized:
    - Participants will be dropped off at the facility and picked up by a family member or provided a ride home
    - Degree of sleep restriction imposed (5h/night x 6 nights) is no greater than what may be encountered in normal daily life with occupational and social obligations;
    - Participants will undergo a screening evaluation and clearance for any mental health conditions that may preclude them from completing the protocol;
    - Excluding those with pre-existing sleep debt;
    - Excluding those with baseline metabolic derangements who may be more susceptible to sleep-induced metabolic disruption;
    - Participants will be supervised by study staff during waking hours and overnight only when study procedures are required. Nursing staff will be onsite 24/7 during the inpatient sleep restriction phase of the protocol. Participants will be allowed to stop the study at any time;
    - Participants will have an ad libitum recovery period with at least 10h sleep opportunity on the CTRC inpatient unit following completion of Week 2 on night 8 (prior to discharge) and will be picked up by a family member or offered a ride home. Recovery period can be extended an additional 1-2 days depending on participant preference and assessment prior to discharge.
    - Reaction time, cognitive function and drive simulator can be used, if necessary, to assess participants prior to discharge
    - Participants will be instructed to not perform any major tasks, drink alcohol, drive, or make any major decisions until they feel they have adequately recovered.
- **One week inpatient stay** is necessary to allow sufficient time to observe the predicted primary outcome. Risks will be minimized:
    - Screening evaluation will be used to exclude those with a mental health condition that may preclude them from completing the protocol;
    - Duration of study will be specifically addressed in consent form;
    - Participants will be allowed to ambulate outside of the study room during wake, non-supine, non-sleep hours to minimize claustrophobic triggers;
    - Participants will be allowed to stop the protocol at any time, at which a final blood draw will be performed.
- **Resting Energy Expenditure** is now included to improve our understanding of the impact of sleep restriction on fasting and postprandial nutrient metabolism. These tests are short and non-invasive. They do not add substantial participant burden and do not carry any significant risks. Risks will be minimized by:
    - Removing the clear plexiglass hood immediately if participant experiences claustrophobia.

- **Questionnaires, interviews, and collection of personal medical information** are necessary to screen participants, apply inclusion/exclusion criteria and characterize the study population. Risks related to confidentiality and privacy will be minimized:
    - No PHI will be included on data collection forms, whenever possible;
    - A private setting will be used when conducting interviews or collecting personal information.
    - Specimens processed in the CTRC lab will be labeled with the participant's name, medical record number, protocol number and study ID number, as required by the UCH clinical lab and CTRC Core Lab. Stored samples will be given indirect identifiers. Study personnel will have access to the code which is kept in an electronic database.

E. Potential Scientific Problems:

This study has significant potential to identify sleep disorders as a novel risk factor for osteoporosis and will begin to dissect the mechanisms by which sleep restriction alters bone metabolism. However, some potential scientific problems cannot be addressed with the limited scope and budget of this study:

- This study does not include a control population. However, participants will serve as self-controls. Larger future studies will include a control group to assess the effects of the inpatient study environment on the outcome.
- This study does not include an intervention to assess the effects of pure circadian misalignment (without sleep restriction). This project has a smaller scope with limited (K23 mentored career development award) budget that will provide pilot data for a larger, more comprehensive R01 application based on this study's findings that can explore the relationship between circadian misalignment and bone metabolism.
- Every effort will be made to match physical activity levels and patterns and light exposure between outpatient and inpatient weeks. We acknowledge that this is ambitious and that it may not be practical to achieve 100% accuracy but represents an attempt to control as much variability as possible.
- Recruitment of the proposed study population may be challenging, however, my mentors are experienced clinical scientists with expertise in this area. If necessary, I will screen individuals from their prior studies who have consented to be contacted in the future.
- Although we don't anticipate this short-duration protocol will have a long-lasting detrimental effect on the participants' bone health, the ability and degree to which bone turnover markers recover after a period of insufficient sleep is currently unknown. We will obtain a fasting sample the morning after the recovery night of sleep and if additional funding is secured we will propose further evaluation of bone turnover markers during a longer recovery phase, or address this in a subsequent, larger, more comprehensive study.

F. Data Analysis Plan:

- The percent change (%Δ) in each biomarker from the beginning of Week 2 (representing 8 hours of sleep/night in Week 1) to the end of Week 2

(representing 5 hours of sleep/night in Week 2) will be calculated for each participant and then averaged for the group. For each biomarker, the average %Δ will be tested using a 2-sided paired t-test controlling the type I error rate at 0.05. For each biomarker, the hypotheses tested will be H_0: %Δ = 0 versus H_1: %Δ ≠ 0. Based on our preliminary data, we estimate that PINP (our primary outcome) will change at least 10% from Week 1 to Week 2 and assume 11% variability in the %Δ and a correlation of 0.5 between the biomarker at the 2 time points. With 12 participants the t-test will have 81.7% power to detect a change of 10% or greater. If necessary, a mixed model may be used to analyze the data, with the help of Dr. Patrick J. Blatchford and Pamela Wolfe, MS (biostatisticians).

- Potential Confounders: activity level/pattern (matched between inpatient and outpatient weeks using a FitBit activity monitor); diet (identical meals provided during inpatient & last 3 days of outpatient week); light exposure (will attempt to match between inpatient and outpatient conditions using ActiWATCH); inherent external and internal circadian misalignment imposed by delaying sleep onset and advancing sleep wake; changes in inflammatory markers, hormones levels, sympathetic tone, metabolic parameters such as glucose/insulin (additional serum will be drawn and stored to measure these if additional funding is secured).
- Based on our prior experience recruiting for these kinds of studies we anticipated that we would need to screen 3-4 people for every 1 participant/completer. Therefore, we initially planned to consent 350% of our target, or 42 individuals to get 12 men to complete the entire protocol. Our screening:enrollment ratio has been closer to 10:1 over the last 9 months, therefore, we plan to consent up to 120 individuals to get 12 men to complete the entire protocol.

G. Summarize Knowledge to be Gained:

This study has significant potential to identify sleep disorders as a novel risk factor for osteoporosis and will begin to dissect the mechanisms by which sleep restriction alters bone metabolism. These data could impact patient care by altering how physicians screen for secondary causes of osteoporosis and may lead to additional treatment options for those with low bone density/osteoporosis and concurrent sleep disorders.

H. References:

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