**#PBRC 2020-033___**

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# Metabolic Effects of Metformin Therapy in Obstructive Sleep Apnea

# "MET-OSA"

**Funding from the Pennington Biomedical NORC**

**PROTOCOL**

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## 1. BACKGROUND AND SIGNIFICANCE

Obstructive sleep apnea (OSA) is a highly prevalent sleep disorder which is associated with insulin resistance (IR) and metabolic dysfunction.[1] As a consequence, OSA patients have a high risk for development of diabetes and cardiovascular disease independent of obesity.[2, 3] Positive airway pressure (PAP) therapy, utilized as standard of care for OSA, resolves pathological intermittent hypoxia (IH) but has poor compliance with modest effects on IR and metabolism.[4, 5] The limited efficacy of PAP therapy underscores the need to develop therapeutic augmentation strategies with the aim to reduce IR and improve metabolic profile to prevent diabetes and CVD in OSA patients. Metformin, with its insulin-sensitizing and pleotropic cellular effects, may be a good candidate.[6, 7]

Physiological links between OSA and metabolic dysfunction include sympathetic activation, altered hypothalamic-pituitary-adrenal axis, inflammation and oxidative stress.[1] While several of these mechanisms may readily resolve by mitigation of IH through PAP therapy, chronic IH exposure may have long-lasting effects on cellular physiology. Such cellular alterations resulting from chronic IH exposure may not readily reverse with elimination of IH and continue to persist to cause tissue dysfunction. We have shown that chronic IH induces senescence[11] and our preliminary in-vitro data suggest that metformin may attenuate IH induced senescence.

Enduring cellular senescence presents as a compelling mechanism for continued tissue dysfunction in OSA patients using PAP. First, recent studies link increased senescence (measured using biomarkers such as p16INK4a and γH2AX) to several age- and metabolism-related conditions such as obesity and diabetes.[8] Second, senescence is self-propagating. During senescence, cells undergo growth arrest along with profound pro-inflammatory secretome changes through which the neighboring cells also become senescent.[8] Third, increased senescence causes tissue dysfunction.[9] Senescent cells limit tissue regenerative capacity and the secretome changes impair cell and tissue function. Therefore, unless removal of senescent cells is targeted, tissue dysfunction would likely continue even after mitigation of IH by PAP therapy. Pharmaceuticals with anti-senescence effects, such as metformin, would be needed to disrupt the senescence cycle and thereby resolve tissue IR and restore metabolic profile in OSA patients.

Of note, there has been a renewed interest in the pleiotropic effects of metformin and initial proof-of-concept clinical trials are being undertaken to evaluate its benefits on aging and cancer treatment.[7] Moreover, while animal and in-vitro studies support the anti-senescence effects of metformin, these have not been investigated in humans.

We also propose using metformin as a first-line preventive treatment along with PAP in OSA patients to reduce IR for its well-established insulin-sensitizing properties[6] and clinical recommendations for diabetes prevention. Importantly, the therapeutic benefits of metformin in OSA patients have not been investigated.

## 2. STUDY AIMS

Our overarching hypothesis is that in addition to improving insulin sensitivity (IS) via modulating hepatic gluconeogenesis metformin therapy will counteract the elevated cellular senescence in OSA patients using PAP, and thereby reduce tissue IR and dysfunction. Specifically, we propose that cellular senescence continues to persist and propagate in tissue of OSA patients undergoing PAP treatment. Therefore, anti-senescent therapeutics such as metformin would benefit OSA patients by improving tissue function and consequent metabolic profile. Notably, the role of increased cellular senescence in obesity- and age-related metabolic disorders is being increasingly recognized. [8, 9]

To test our hypothesis, we will conduct a double-blind, placebo-controlled, randomized clinical trial in non-diabetic OSA patients (Apnea hypopnea index, AHI≥15 events/h, HbA1c<6.4%) (fig 1). All participants will be provided PAP therapy and adherence will be monitored via monthly compliance visits. In addition, participants will be randomized to receive either metformin (2g/day, n=10) or matching placebo (n=10) for 3 months. Study measures will be collected at 0 and 3 months to address the following specific aims:

**AIM 1: To determine the effects of metformin + PAP on whole body insulin sensitivity (IS).**

*Hypothesis:* Addition of metformin to PAP therapy in OSA patients will enhance reduction in IR. Measures derived from oral glucose tolerance test including Matsuda index (whole body-IS) and ISI-FAA (AT-IS) will be used to longitudinally examine changes in IS to inform about metabolic risk reduction in OSA patients.

**AIM 2: To evaluate the longitudinal changes in skeletal muscle (SM) and adipose tissue (AT) IS.**

*Hypothesis*: Metformin therapy will improve insulin actions in SM and AT. The effects on metformin/placebo therapy on cellular IS will be determined *ex-vivo* by examining insulin-mediated phosphorylation of AMPK and AKT along with insulin-dependent changes in glucose metabolism. These measures will evaluate tissue function.

**AIM 3: To examine the effects of metformin + PAP on the prevalence of AT and SM senescence.**

*Hypothesis*: Compared to placebo, metformin will reduce AT and SM senescence burden. Multiple biomarkers of senescence including $p16^{INK4A}$, γH2AX, phospho-p53, and senescence-associated β-galactosidase (SA β-gal)[8] activity will be measured to identify changes in prevalence of senescent cells. The outcome will inform about the progression of senescence in PAP treated OSA patients and its mitigation by metformin.

## 3. RESEARCH DESIGN

We will conduct a double-blind, placebo-controlled, randomized proof-of-concept RCT in 20 non-diabetic OSA participants (fig 1). After baseline measures are obtained, all participants will be asked to initiate and adhere to PAP therapy. Participants will be randomly assigned in a 1:1 ratio to receive placebo or metformin for 3 months.

All study visits will take place at Pennington Biomedical Research Center (PBRC). Qualifying sleep study will be conducted at PBRC and the data will be shared with our research collaborators (LSUHSC-NO, Dr. Mader) and ApneaMed to determine eligibility and obtain PAP devices. PAP devices will be provided at PBRC

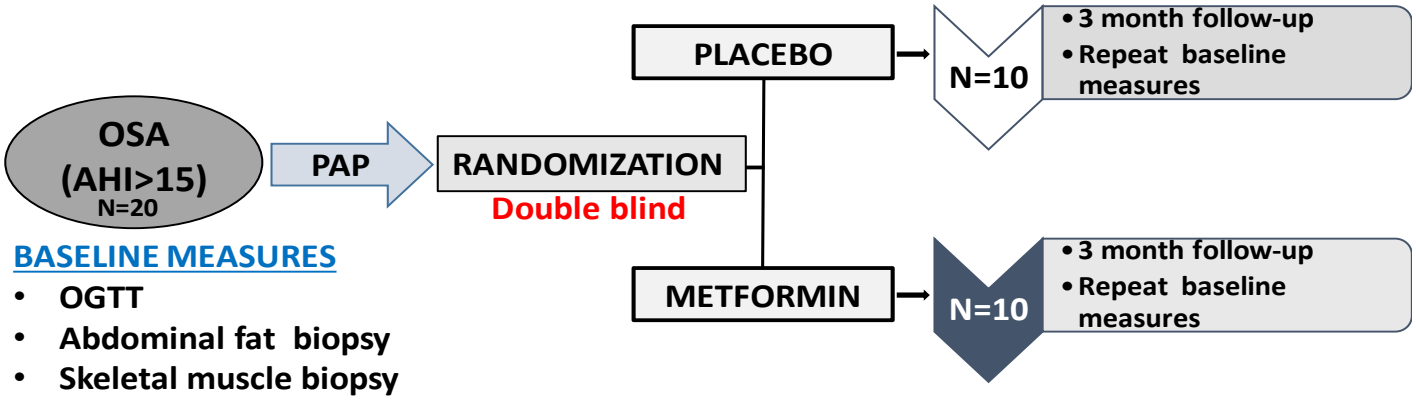


**Fig 1:** Schematic of the study design. Study measures will be obtained at baseline and at 3-month follow-up. Monthly compliance visits will monitor adherence to PAP and study drug. Participants will also undergo comprehensive phenotyping to assess metabolic risk.

and treatment will be administered online. Eligible participants will remain in the study for up to 4 months. The study will take place over a period of 1 year.

## 4. INVESTIGATIONAL DRUG (Metformin/Placebo)

### 4.1 Clinical Data to Date

Metformin is the most popular oral glucose-lowering drug worldwide. It has an excellent long-term safety profile with most professional guidelines recommending it as the first-line oral therapy for type 2 diabetes. Although not formally approved for prediabetes, it is commonly used for treatment of this condition. Importantly, even though OSA patients are at high risk for development of diabetes, specific recommendations related to use of metformin in OSA patients are lacking.

### 4.2 Risks

The most common adverse effects of metformin are diarrhea, abdominal discomfort and nausea, which are dose-related. Other adverse effects include a metallic taste and mild anorexia. These symptoms are usually mild, transient, and reversible after dose reduction or discontinuation of the drug. About 5% of patients cannot tolerate the gastrointestinal adverse effects of metformin. Malabsorption of vitamin B12 may lead to its deficiency during long-term metformin therapy although a precise estimate of the frequency of this adverse effect is not available. Metformin-associated lactic acidosis (MALA), a serious condition, is very rare, with an estimated incidence of 3-10 per 100,000 person-years. Risk for MALA is increased when renal clearance is decreased and drug levels rise very high. In 2016, FDA issued the following safety communication:

1. obtain the patient's estimated glomerular filtration rate (eGFR) prior to starting metformin.
2. metformin is contraindicated in patients with an eGFR below 30 mL/min/1.73 $m^2$.
3. starting metformin in patients with an eGFR between 30-45 is not recommended.
4. obtain eGFR at least annually.
5. assess benefits and risks of metformin if eGFR falls below 45.
6. discontinue metformin if eGFR falls below 30.
7. withhold metformin at the time of iodinated contrast imaging procedure in patients with eGFR between 30-60, in the setting of liver disease, alcoholism, or heart failure, or if intra-arterial contrast is used, with further recommendation that metformin may be restarted after 48 hours if renal function remains stable.

Participant selection criteria for our study are designed to minimize risks of metformin. To reduce the risk of gastrointestinal adverse effects, we will exclude participants with prior intolerance to metformin. This protocol allows for withholding or delaying dose escalation and dose reduction. At screening, to minimize the risk of MALA, we will assess eGFR and exclude participants with eGFR <60 mL/min/1.73$m^2$ (CKD-EPI method). Our eGFR exclusion criterion is more conservative than the FDA recommendation of not starting metformin in patients with eGFR <45. As noted above, the potential for lowering of B12 levels is only a concern during long-term metformin therapy. In the context of 3-month metformin treatment, monitoring for B12 levels is not warranted. The study will exclude participants who are pregnant or breastfeeding and those planning to become pregnant. Women of childbearing potential will be required to have a negative pregnancy test prior to starting the study drug.

### 4.3 Dose Rationale

Dosing of metformin XR in this study is in line with the drug's FDA-approved prescribing information.

### 4.4 Treatment Regimen

This RCT will compare metformin XR vs placebo. Each visually indistinguishable study drug capsule will contain metformin XR 500 mg or placebo. Metformin XR dosing will be 500 mg (1 x 500 mg) qPM during Week 1, 1000 mg (2 x 500 mg) qPM during Week 2, 1500 mg (3 x 500 mg) qPM during Week 3, and 2000 mg (4 x 500 mg) qPM from Week 4 onwards. Placebo dosing will be identical. For participants tolerating the study drug well, dosing will remain at 4 capsules qPM. Based on tolerability, scheduled dose increases may be delayed, or dose reduced to a tolerable level. Also, based on tolerability or subject preference, dosing schedule may be changed to BID (twice daily) rather than taking all 4 capsules qPM. Use of 4 capsules allows for this flexibility to match clinical practice. Participants who are unable to tolerate even one capsule (metformin 500 mg or placebo) may stop the study drug and continue to provide study assessments. Participants will be given detailed instructions at the time of dispensing and instructed to return unused medication. The capsules dispensed and returned will be recorded on a log-sheet. Expired or returned study drug will be destroyed on-site per PBRC policy by the Research Pharmacy. Destructions are also documented in log-sheets.

### 4.5 Study drug preparation and masking

The study will be conducted in a double-blind manner. The study statistician and research pharmacist will be the only personnel who will have information about randomization and treatment assignment. PBRC research pharmacist will procure metformin, repackage the active drugs and placebo in identically appearing capsules, and dispense at monthly visits. Investigators and all other study personnel and the study participants will be blinded to the treatment assignment until participants have completed all visits and all data have been entered and the data file has been locked to prevent changes.

### 4.6 Prior and Concomitant Therapy

Individuals taking certain prescription medications including antihistamines (cetirizine, fexofenadine, desloratadine, loratadine, etc.), oral contraceptive pills, antacids, proton pump inhibitors, antidepressants, diuretics, or intrauterine devices will be included in the study and allowed to continue with the treatment. Participants will be instructed to not make changes to their medications including over-the-counter medicines, vitamins, and supplements. Any changes in medications will be monitored. The PI and MI will decide if the changes in medication will allow continuation in the study. Participants will be encouraged to initiate and strictly adhere to standard PAP therapy for OSA treatment. PAP therapy would be initiated via collaborating DME. Participants who are unable to tolerate CPAP may discontinue CPAP and continue in the study to provide study assessments. They will be advised to consult with their primary care physician regarding alternative treatments for OSA after completing the study. Since OSA is a chronic condition that does not pose an imminent threat to a person's health, given the short duration of the study, we believe that a short delay in receiving alternative therapy is reasonable.

## 5. STUDY POPULATION

### 5.1 Subject Population

We will enroll up to 20 untreated OSA patients. The study may need to screen up to 60 participants to enroll 20 participants in the RCT.

### 5.2 Inclusion Criteria

Eligibility criteria include:

- Age 35-65 years (inclusive).
- Body mass index (BMI) ≥ 30-50 kg/m$^2$ (inclusive).

- Apnea-Hypopnea Index ≥ 15 events/h.
- Must be able to provide written informed consent.
- Willing to participate and adhere to study procedures (video recorded in-lab sleep studies, PAP treatment, take study drug, have adipose tissue and skeletal muscle biopsies).
- Women of child-bearing potential must agree to use appropriate contraception to avoid pregnancy throughout the study.
- Willing to have blood, as well as adipose and muscle tissue stored for future use.

### 5.3 Exclusion Criteria

Study volunteers are ineligible to participate (or will be excluded from participating in this study) if they meet any of the following criteria:

- HbA1c > 6.4%.
- Severe or uncontrolled hypertension defined as systolic BP ≥180 mmHg and/or diastolic BP ≥110 mmHg on the average of three seated measurements after being at rest for at least 5 minutes.
- Significant cardiovascular, hepatic, renal, neurologic, or psychiatric disease as determined by the study physician.
- Pregnancy, breast feeding or planning pregnancy in the coming 4 months.
- Impaired renal function defined as eGFR <60ml/min/1.73m$^2$ (estimated with CKD-EPI method).
- Known hypersensitivity to metformin.
- Currently taking a glucose lowering or weight loss medications.
- Current PAP use or use of PAP in the past 6 months.
- Currently taking antihypertensive and lipid-lowering medications known to affect adipose tissue and skeletal muscle metabolism. For example, statins and drugs targeting renin-angiotensin system will not be allowed. However, use of diuretics, beta-blockers, alpha-blockers and calcium channel blockers may be allowed provided the participant is on a stable dose for at least 3 months prior to the study visit.
- Oxygen desaturation index <15 events/h of sleep.
- Any medication or condition that, in the opinion of the medical investigator, could interfere with the study outcomes or put the subject at risk by participating in the study.

## 6. RECRUITMENT

Community advertisements, including targeted and passive outreach (e.g., web, Listserv, community events, etc.), and promotional materials will be used. Eligible OSA patients and patients with high risk for OSA may also be referred through local Clinics (Sleep Foundation, Baton Rouge General, Our Lady of the Lake etc.). Study staff will explain the study and procedures to interested individuals and may provide them with a copy of the consent form for further explanation. Following determination of initial eligibility (e.g., BMI, age, willingness to undertake study procedures), a fasting screening visit will be scheduled.

## 7. ASSESSMENT SCHEDULE AND PROCEDURES

An overview of all clinic visits and assessments is presented in **Table 1**. Eligibility of the study participants will be determined during two screening visits. Study procedures will require 4 visits. Among the study visits, Visit 3 and Visit 6 will last ~ 4.5 h each during which baseline and 3-month follow-up measures will be obtained. Visit 4 and Visit 5 will comprise of short (~ 45 min) visits during which compliance to study drug and PAP would be monitored and study drug will be dispensed.

**Table 1:** Clinic Visits and Assessments

| Assessment | Screening Visit 1 | Screening Visit 2 | Test Period | | | |
|---|---|---|---|---|---|---|
| | | | Visit 3 Baseline | Visit 4 Month 1 | Visit 5 Month 2 | Visit 6 Month 3 |
| **Informed consent** | X | | | | | |
| **Inclusion/exclusion criteria** | X | | | | | |
| **Medical History (Health Questionnaire)** | X | | | | | |
| **Physical examination** | X | | | | | |
| **Concomitant medications** | X | | | | | |
| **Vital Signs (BP, pulse)** | X | | | | | |
| **Body weight** | X | | X | | | X |
| **Height and BMI** | X | | | | | |
| **Oximetry** | X | | | | | |
| **Blood draw (fasting)** | X | | X | | | X |
| **Overnight Sleep study** | | X | | | | |
| **Waist, hip, neck, circumference** | | | X | | | X |
| **OGTT** | | | X | | | X |
| **Urine Pregnancy testing*** | | | X | | | X |
| **Questionnaires** | | | X | | | X |
| **Dietary assessment (food record)** | | | X | | | X |
| **Whole body DXA** | | | X | | | X |
| **Abdominal fat biopsy** | | | X | | | X |
| **Muscle biopsy** | | | X | | | X |
| **ABPM -24 hour** | | | X | | | X |
| **PAP provided** | | | X | | | |
| **Randomization** | | | X | | | |
| **Drug Dispense** | | | X | X | X | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Adverse events** | | | | X | X | X |
| **Monitoring adherence (PAP and Drug)** | | | | X | X | X |

*urine pregnancy testing is only for women of childbearing potential. BP: blood pressure; BMI: body mass index, OGTT: oral glucose tolerance test; DXA: dual energy X-ray absorptiometry; ABPM: ambulatory blood pressure monitoring; PAP: positive airway pressure.

## 8. SCREENING

### 8.1 Screening Visit 1

Study volunteers will arrive at PBRC for an early morning fasting visit. During this visit, participants will provide informed consent prior to the initiation of any study procedures. The informed consent process will be conducted primarily by the study coordinator, but also on occasion by the study PI (Dr. Prachi Singh) or by a trained clinic staff. Potential enrollees will be given ample time to read the informed consent and ask questions about the study. After signing the consent form, physical examination will be conducted to ensure inclusion criterion is being met. Height, weight, and vital signs (blood pressure, heart rate) will be measured. Medical history will be reviewed via screening health questionnaire and concomitant medication determined for exclusionary criteria. In addition, participants will have blood drawn for measurement of chem15 panel (includes creatinine to determine eGFR via CKD-EPI method, and other electrolytes and hepatic enzymes), and HbA1c. If all immediately available eligibility criteria are satisfied, eligible volunteers will be provided oximeters to measure changes in blood oxygen saturation during sleep at home.

### 8.2 Screening Visit 2

Participants with oxygen desaturation index (ODI) >15 events/h of sleep will proceed to screening visit 2. During this visit an overnight sleep study (including video recording) will be conducted in PI's lab. The sleep study data will be read and interpreted by Sleep Board Certified Physician (Dr. Mader – LSUHSC-NO) and/or ApneaMed. Required PAP prescription will be provided to ApneaMed for procurement of PAP devices.

## 9. METHOD FOR ASSIGNING PARTICIPANTS TO TREATMENT GROUPS (RANDOMIZATION)

After confirmation of eligibility criteria, participants will be randomized to receive either placebo or metformin in a 1:1 ratio. Robbie Beyl, PhD, the study statistician, will perform the randomization, and the study drug assignment will be implemented by Claire Hazlett, investigational pharmacist. Randomization will be done using a dynamic allocation based on age and OSA severity determined by Apnea Hypopnea Index (AHI).

## 10. STUDY VISITS

### 10.1 Visit 3 (Baseline)

Participants will arrive to PBRC for an early morning fasting visit. Body weight will be measured along with measurement of waist, hip, arm and neck circumference. Blood sample will be drawn, questionnaires will be administered, and if needed, urine pregnancy test will be conducted. Participants will be given a standard glucose drink (75g glucose) to consume while timed blood samples will be collected every 30 min for 2 hours to measure glucose, insulin and free fatty acids (FFA). An abdominal adipose tissue biopsy and a skeletal muscle biopsy will be performed. Participants will also undergo whole-body DXA to determine body composition. At the end of this visit, participants will be provided a monitor to measure 24-h ambulatory blood

pressure and study drug will be dispensed with appropriate dose and safety instructions. Lunch will be provided prior to discharging the participants.

Following this visit, we will provide the study participants with PAP therapy through standard on-line resources (ApneaMD). PAP devices will be given to the participants at PBRC. PI will obtain equipment serial number to monitor adherence. For the first 4-weeks, study personal will monitor PAP compliance weekly (on-line data). Thereafter, we will monitor compliance on a monthly basis. Appropriate PAP use will be encouraged via additional consults and/or mask replacement as needed.

### 10.2 Visit 4 (Month 1) and Visit 5 (Month 2)

During this visit, participants will be asked to bring remaining study drug to determine compliance. Participants will also be questioned for any possible side-effects and adverse events.

Non-compliant participants (<80% drug taken as determined by pill count[10]) will be interviewed to determine factors that resulted in poor compliance. If needed, guidance will be provided to improve management of minor side-effects which may improve adherence such as bloating etc. Patients will continue in the study but will be counselled on the importance of taking their medication as prescribed by the study protocol.

Compliance to PAP therapy for OSA will also be monitored through cloud-based technology. Non-compliant participants (using PAP <4hours each night) will be interviewed to determine factors resulting in poor adherence. If needed, participants may be referred to consulting DME (ApneaMed) /physician (Dr. Mader) to help improve CPAP adherence.

All participants will be reminded to continue /improve adherence for both PAP and study drug.

Study drug for the next ~30 days will be dispensed.

### 10.3 Visit 6 (Month 3/ End-of-Study)

All procedures from Visit 3 will be repeated and any remaining study drug will be collected and disposed. Additionally, information related to potential adverse events will also be collected.

## 11. MEASUREMENTS AND OUTCOME ASSESSMENTS

### 11.1 Questionnaires

Participants will complete a Screening Health Questionnaire at Screening Visit 1 only. Participants will be asked to complete other questionnaires including the Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, and International Physical Activity Questionnaire during Visit 3 and Visit 6.

- ***Screening Health Questionnaire:*** The Screening Health Questionnaire is a 25-item questionnaire that assesses general health and medical history (e.g., medical conditions, diet and exercise habits, reproductive health).
- ***Epworth Sleepiness Scale (ESS):*** The ESS is an 8-item questionnaire intended to measure daytime sleepiness. The questionnaire asks individuals to rate the probability of falling asleep on a scale of increasing probability (from 0 to 3) for 8 different situations during their daily lives, though not necessarily every day. The scores for the eight questions are added together to obtain a single total score. A total score of 11-15 indicates excessive daytime sleepiness, while a score of ≥16 indicates the possibility of severe sleep apnea or narcolepsy.[5]

- ***Pittsburgh Sleep Quality Index (PSQI):*** The PSQI is used to assess sleep quality over a 1-month time period. The measure consists of 19 individual items, creating 7 components that produce one global PSQI score. The 7 component scores consist of: 1) subjective sleep quality; 2) sleep latency (i.e., how long it takes to fall asleep); 3) sleep duration; 4) habitual sleep efficiency (i.e., the percentage of time in bed that one is asleep); 5) sleep disturbances; 6) use of sleeping medication; and 7) daytime dysfunction. Each item is weighted on a 0-3 interval scale. The global PSQI score is then calculated by totaling the 7 component scores, providing an overall score ranging from 0-21, where lower scores denote a healthier sleep quality.[6]
- ***International Physical Activity Questionnaire IPAQ:*** The IPAQ is a well validated instrument used to estimate physical activity across a comprehensive set of domains including leisure time physical activity, domestic and gardening activities, work-related physical activity and transport-related physical activity.

### 11.2 Fasting Blood Draw

A fasting blood sample will be taken at Screening Visit 1 to assess inclusion criteria and overall health (HbA1c, Chem15 including ALT, creatinine, lipid panel and glucose), as well as during Visit 3 and Visit 6 to measure insulin, glucose, and free fatty acids (FFA). HbA1c and measures of Chem15 panel will also be obtained at Visit 6. Fasting blood will also be archived at Visit 3 and Visit 6.

### 11.3 Overnight oximetry

Pulse oximeters (Nonin WristOX2) will be provided to the participants during Screening Visit 1 to determine risk for sleep apnea. Participants will be asked to use the oximeter for one night of sleep. Determination of nocturnal oxygen desaturations to objectively asses risk for sleep apnea will be important to reduce screen failures. Only participants with oxygen desaturation index (ODI) >15 events/h of sleep will be asked to participate in the overnight sleep study. Participants may be asked to repeat oximetry if incomplete data are obtained.

### 11.4 Overnight sleep study (Polysomnography, PSG)

Overnight video PSG will be performed in individuals with ODI >15 events/h at PI's lab at Screening Visit 2. PSG will include electroencephalography, electrooculography, electromyography, electrocardiography, pulse oximetry, nasal pressure transducer, oronasal thermocouple, calibrated respiratory inductance plethysmography. Sleep staging and events will be scored by registered PSG technologists according to AASM established criteria and report will be finalized by a Sleep Board Certified Physician (Dr. Mader).[11] PAP prescription will be generated for the eligible participants and provided to ApneaMed to procure appropriate PAP device.

### 11.5 Body Composition (by Dual-Energy X-ray Absorptiometry)

Dual-energy X-ray absorptiometry (DXA) scans will be performed at the Imaging Facility of PBRC at Visit 3 and Visit 6. Total adiposity and regional fat mass will be assessed with DXA using a whole-body scanner (Lunar iDXA; General Electric, Milwaukee, WI). The DXA protocol requires that participant lie on a table wearing a hospital gown and with no metal objects on them while both legs will be placed together using two Velcro straps. The scanner emitting low energy X-rays and a detector passes along the body. The scan takes ~10 minutes and the radiation dose is less than 1 mrem, equal to about 12-h of background radiation. The scans will be analyzed with the latest software. The software version is enCORE 13.6. We will run quality control scans on a daily basis, and GE has indicated that accuracy of the data is confirmed with these daily QC scans. If needed,

a pregnancy test will be given before DXA scans to confirm absence of pregnancy. Fat mass will be calculated by taking the metabolic weight (i.e., total body weight minus gown weight) multiplied by the regional percent body fat given by the DXA.

### 11.6 Three-day Dietary Records

Participants will be given instruction and a form to complete dietary record for 3 non-consecutive days which would include 2 weekdays and 1 weekend during Visit 3 and Visit 6. This would provide rough estimation of the participants' normal daily intake and any changes that may be mediated by PAP and/or metformin treatment.

### 11.7 Oral Glucose Tolerance Test

Glucose tolerance will be assessed using an oral 75 g oral glucose tolerance test (OGTT). Participants will be studied after an overnight fast. An intravenous line will be placed and one baseline sample will be drawn at -5 minutes. The participants will then consume a 75 g glucose beverage within 5 minutes. Blood samples will be collected at 30, 60, 90, and 120 to measure serum glucose, insulin and free fatty acid concentrations. Whole body insulin sensitivity will be calculated using Matsuda index (10,000/ √[fasting glucose X fasting insulin] X [mean OGTT glucose X mean OGTT insulin]) and other indices related to whole body glucose homeostasis. Adipose tissue insulin sensitivity will be calculated using ISI- FFA [2/(Insulin AUC X FFA AUC) +1].[12]

### 11.8 Ambulatory Blood Pressure Monitoring

Ambulatory 24-hour blood pressure (BP) and heart rate will be continuously collected via an Ambulatory BP Monitor (or ABPM) starting in the afternoons of Visit 3 and Visit 6. The system will be programmed to record blood pressure and heart rate every 30 minutes during the day and night. The blood pressure cuff will be placed on the *non-dominant* arm. Both written and oral instructions pertinent to the use of ABPM will be provided to all participants. Volunteers will be instructed to avoid caffeine, alcohol, moderate-to-vigorous exercise, and daytime naps during this period and asked to maintain an Activity Log (bedtimes, mealtime, physical activity, showering, etc.). Participants taking antihypertensive medications will be instructed to adhere to their prescribed dosing. The ABPM records key aspects of blood pressure including 24-hour, daytime and nighttime systolic BP, diastolic BP, mean BP, and heart rate. Participants may be asked to repeat the blood pressure monitoring if incomplete data are obtained.

### 11.9 Biopsies

On the mornings of Visit 3 and Visit 6, skeletal muscle and adipose tissue biopsies will be performed in the PBRC Inpatient Unit on the *non-dominant* side of the body.

#### *11.9.1 Skeletal Muscle Biopsy*

A *vastus lateralis* muscle biopsy will be performed using a Bergstrom needle in the PBRC Inpatient Unit. After cleansing the skin on the *non-dominant side* of the leg with povidone-iodine solution and placement of a sterile drape, topical anesthesia will be administered using a 50%/50% mixture of bupivacaine 0.5% and lidocaine 2%. The skin will be incised (0.75 cm) with a #11 scalpel. The fascia fibers are separated with the blunt edge of the scalpel and the Bergstrom needle inserted into the *vastus lateralis*. After suction is applied, tissue will be cut and removed. Several passes will be used to obtain *up to* 500 mg of muscle tissue. Pressure will then be applied and the skin closed with sterile tape. After cleaning the sample, tissue will be processed immediately to develop primary myotubes, while the remaining tissue will be snap frozen in liquid nitrogen and stored for subsequent analyses.

#### *11.9.2 Adipose Tissue Biopsy*

A subcutaneous abdominal adipose tissue (two-thirds from the umbilicus to the anterior superior iliac spine) will be performed in the PBRC Inpatient Unit. After cleansing the skin on the *non-dominant side* of the abdomen with povidone-iodine solution and placement of a sterile drape, topical anesthesia will be administered using a 50%/50% mixture of bupivacaine 0.5% and lidocaine 2%. The skin will be incised (0.75 cm) with a #11 scalpel. The fascia fibers are separated with the blunt edge of the scalpel and a Bergstrom needle and Mercedes lipoaspirate technique will be applied. After aspiration is applied, ~1.5-2 grams of adipose tissue will be extracted. Pressure will be applied and the skin closed with sterile tape.

#### *11.9.3 Tissue biopsy analysis*

Tissue samples obtained from the biopsy will be used to address the hypothesis stated in Aim 2 and Aim 3. As shown in **Table 2**, skeletal muscle and adipose tissue samples will be used specifically for: 1) ex-vivo determination of insulin signaling; 2) determination of insulin-stimulated GLUT4 translocation determined by confocal imaging; 3) determination of the prevalence of senescence biomarkers ($p16^{INK4A}$, γH2AX, phospho-p53, and SA β-gal) and SASP using confocal imaging, Western blot, and mRNA quantification; 4) establishment of primary cultures to measure basal and insulin stimulated glycogen synthesis and lipogenesis using [U-$^{14}$C]glucose[13, 14]; 5) measurement of basal and insulin stimulated glucose uptake using [$^{3}$H]-2-deoxyglucose; and 5) a tissue archive for future analyses. These ex-vivo studies will be performed in Dr. Singh's and Dr. Noland's lab.

**Table 2: Planned tissue analysis.**

| Tissue Type | Total | Aim 2 | | | Aim 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Primary Culture | Insulin signaling | GLUT4 location | Confocal | RNA | Western blots | Secretome | β-gal staining |
| SM (mg) | 500 | 150 | 50 | 80 | 25 | 25 | 50 | - | - |
| AT (mg) | 1500 | 300 | 100 | 200 | 50 | 50 | 100 | 75 | 75 |

SM: Skeletal muscle, AT: adipose tissue.

### 11.10 Compliance procedures

- ***Medication reconciliation***: Adherence to study drug regimen will be determined by examining the medication possession ratio and the proportion of days covered on a monthly basis. Participants taking study drug ≥80% will be considered compliant.
- ***PAP adherence****: Study participants will be provided with standard PAP therapy.* PAP usage will be monitored using real-time data acquisition by cloud-based software on a weekly basis during the initial weeks and later on a monthly basis. If low compliance is observed, participant will be contacted to improve adherence and referred for further PAP adjustment, if needed.

## 12 COMPENSATION

Participants will receive *up to* $300 for completion the study ($100 for Visit 3, $25 each for Visit 4 and Visit 5, and $150 for Visit 6/ End-of-Study).

Participants will not be compensated for screening visits. Payment will be received via check requested from the LSU payroll department.

All eligible participants will be provided PAP equipment for the study duration and allowed to keep the equipment for continued OSA treatment after the study is completed. Participants would be advised to follow-up with a Sleep-board certified physician for clinical care.

## 13 PATIENT SAFETY AND CONFIDENTIALITY

### 13.3 Consent

Consent will be obtained upon screening before initiating study specific procedures. A quiet, comfortable, and private setting for the informed consent process will be provided. The consent process will be explained to the subject, time will be provided to consider all options and when possible, the subject will be provided a copy of the consent form well in advance for review at home. Study staff will consider the subject's likely reading abilities and provide an impartial witness in the informed consent process if necessary. All questions will be answered. Coercion or other undue influences will be avoided through the use of a quiet, comfortable, and private setting will be used for the informed consent process. The subject will be told that informed consent can be revisited at any time and consent can be withdrawn at any time without affecting the subject's medical care. The informed consent process will continue throughout the subject's participation in the study and consent will be informally verified on a continuing basis. Significant new information will be given to the subject as it is available.

### 13.4 Risks to Participants

This Human Subjects Research meets the definition of a Clinical Trial. This study does not involve major risk to participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research.

The known risks, inconveniences, or side effects from the proposed procedures in the project are included here (in alphabetical order):

| **Potential Risks and Efforts to Minimize the Risks** | | |
|---|---|---|
| **Procedure** | **Potential Risks** | **Efforts to Minimize the Risks** |
| Adipose Tissue Biopsies | Pain, infection, scaring, bleeding, and loss of sensation to the skin (usually temporary but in some cases permanent) around biopsy sites are possible risks. | Trained nurses and personnel will be performing the biopsies using sterile technique after local anesthesia to minimize risks. |
| Ambulatory Blood Pressure Monitoring | There are no known risks associated with the blood pressure monitor. Some discomfort having the cuff inflate every 30 minutes around the clock may be experienced. | The device can easily be removed should the subject become uncomfortable. |
| Blood Draws | Bruising, bleeding, pain, and infection pose minimal risks. | Trained phlebotomists and personnel will use sterile technique. |

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|---|---|---|
| Confidentiality of Data | Taking part in this research may involve providing information that one considers confidential or private. There is a slight risk that data could be revealed inappropriately or accidentally | Study researchers and staff will take steps to protect data that is collected. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to access research records, will be made to keep the data safe. |
| Dual-Energy X-Ray Absorptiometry (DXA) | DXA measures the amount of bone, muscle, and fat in the body. The expected dose of radiation is minimal, equivalent to less than 0.0004mSv or ~12 h of exposure to the sun. | The amount of radiation used for this procedure is very small. The radiation dose for a DXA scan is equivalent to the radiation an individual is naturally exposed to in the environment in less than one day. Scans will not be performed on any subject who is pregnant. A pregnancy test will be performed within 72 hours before the scan on females of child-bearing-potential.<br><br>Lifetime radiation exposure: We are exposed to radiation in the environment on a daily basis; however, some scientists have suggested that humans have a lifetime maximum exposure limit. Exposure to radiation is not without risk, but it is difficult to quantify the exact amount someone is exposed to. By participating in this study, individuals will be exposed to radiation that will add to this lifetime maximum exposure limit. If participants believe that they have been exposed to a significant amount of radiation as part of your occupation or due to treatment for a specific medical condition, they would be asked to notify the study team to discuss whether this study would be appropriate for them. |
| Fasting for 10-h | Nausea. | Light snacks will be available to eat once fasting procedures completed. |

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| Oral Glucose Tolerance Test (OGTT) | There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. The drink may cause nausea, vomiting, abdominal bloating or a headache. | Trained nurses and personnel minimize the risk associated with IV. Participant may choose to drink the glucose solution more slowly or stop drinking completely. |
| Oximetry | There are no significant risks related to overnight use of oximeter other than temporary discomfort while wearing the device. | The device can easily be removed should the subject become uncomfortable. |
| Questionnaires | Uncomfortable answering questions. | Participants may skip any questions. |
| Skeletal Muscle Biopsies | Pain, infection, scaring, bleeding, and loss of sensation to the skin (usually temporary but in some cases permanent) around biopsy sites are possible risks. | Trained nurses and personnel will be performing the biopsies using sterile technique after local anesthesia to minimize risks. |
| Sleep Study | There are no significant risks related to sleep study (polysomnography). However, to obtain the polysomnographic measurements, it may be necessary to glue many small surface electrodes on the participant's scalp and face. The adhesive used to attach these wires has a strong smell, and the subject may feel a scratching sensation when the wires are applied. | Trained personnel will place the electrodes using adhesives approved for sensitive skin. |

| | | |
|---|---|---|
| Study drug (metformin) | Common side-effects associated with metformin use include diarrhea, gas (flatulence), upset stomach (throwing up) and feeling tired or weak. Uncommon or rare side effect might include metformin-associated lactic acidosis. This extremely rare side effect may be severe enough to cause death, hypothermia, hypotension, and resistant bradyarrhythmias. | The GI associated side-effects will be minimized by using the extended release formulation and following an individualized dose escalation schedule. We will also minimize the risk of lactic acidosis by asking study participants to stop taking the study medicine and contacting the study investigators within 24 hours if they develop symptoms including malaise, myalgias, respiratory distress, somnolence and abdominal pain. Importantly, individuals with a higher risk for metformin-associated lactic acidosis include renal impairment, concomitant use of certain drugs, old age (≥80 years), and hepatic impairment will be excluded from study participation. Furthermore, individuals in whom metformin should be used with caution, including pregnant or nursing women are not included in the study. |

In addition to the risks listed above, participants may experience a previously unknown risk or side effect.

## 13.5 Safety Monitoring

### 13.5.1 Personnel responsible for the safety review and its frequency

The Principal Investigator (Dr. Prachi Singh, PI) and the Medical Investigator (Dr. Kishore Gadde, MI) will be responsible for monitoring the data, assuring protocol compliance and conducting the safety reviews on a quarterly basis (including at the time of continuing review). During the review process, the Principal Investigator will evaluate whether the study should continue unchanged, requires modification/amendment or should close to enrollment. Findings will be reported at the time of continuing review. The IRB, PI or regulatory body (e.g., FDA, OHRP) has the authority to stop or suspend the study or require modifications.

### 13.5.2 Determination of study risk

The risks associated with the current study are deemed moderate for the following reasons:

- We do not view the risks associated with the study procedures including metformin use, fat biopsy and muscle biopsy as minimal.
- Given the established safety and validity of metformin and biopsy procedures in our prior work, we do not view the proposed study as high-risk.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to

predict with certainty the absolute risk in any given individual or in advance of firsthand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

#### 13.5.2.1 Adverse events

In this study, an **adverse event or experience** is defined as any health-related unfavorable or unintended medical occurrence that happens throughout study participation. Examples of adverse events include but are not limited to the following:

- A clinically significant laboratory or clinical test result.
- An event that results in missing a study visit.
- An event that requires a visit to a physician.
- An event that occurs as a result of a study procedure.
- Unanticipated or untoward medical events that may be study related.

#### 13.5.2.2 Attribution of adverse events

Adverse events for each subject participating in the study will be monitored and attributed to the study procedures/design by the Principal Investigator (PI) (Prachi Singh, PhD) and/or Medical Investigator (MI) (Kishore Gadde, MD) according to the following categories:

- Definite: Adverse event is clearly related to investigational procedure(s)/agent(s).
- Probable: Adverse event is likely related to investigational procedure(s)/agent(s).
- Possible: Adverse event may be related to investigational procedure(s)/agent(s).
- Unlikely: Adverse event is likely not related to the investigational procedure(s)/agent(s).
- Unrelated: Adverse event is clearly not related to investigational procedure(s)/agent(s).

#### 13.5.2.3 Plan for grading adverse events

The following scale will be used in grading the severity of adverse events noted during the study:

- Mild adverse event
- Moderate adverse event
- Severe or medically significant

#### 13.5.2.4 Plan for determining seriousness of adverse events

In addition to grading the adverse event, the PI and MI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if ANY of the following apply:

- It is life-threatening.
- It results in inpatient hospitalization or prolongation of existing hospitalization.
- It results in persistent or significant disability or incapacity.

- It results in a congenital anomaly or birth defect.
- It results in death.
- Based upon appropriate medical judgment, it may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.
- It adversely affects the risk/benefit ratio of the study.

An adverse event may be graded as severe but still not meet the criteria for a SAE. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its seriousness when determining whether reporting to the IRB is necessary.

#### 13.5.2.5 Plan for reporting adverse events

- ***Reporting adverse events to the IRB***

The investigator will report the following types of adverse events to the IRB:

- Serious AND unanticipated AND possibly, probably or definitely related events.
- Anticipated adverse events occurring with a greater frequency than expected.
- Other unanticipated problems involving risks to participants or others.

These adverse events or unanticipated problems involving risks to participants or others will be reported to the IRB within 10 working days of becoming known to the investigator, using the appropriate forms found in IRB Manager.

- ***Reporting adverse events to co-investigators***

The following individuals and funding and/or regulatory agencies will be notified of adverse events*:*

- All co-investigators listed on the protocol.
- Study sponsor(s) – Pennington NORC.
- National Institutes of Health.

The Principal Investigator (Prachi Singh) and/or Medical Investigator (MI) (Kishore Gadde) will conduct a review of all adverse events upon completion of every study subject. The Principal Investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

#### 13.5.2.6 Surveillance Procedures

Adverse events will be documented during the scheduled visits or during phone calls during off-weeks. For each sign, symptom or adverse event, the following information will be recorded:

- A brief descriptor of the adverse event
- Start and stop dates
- Intensity (mild / moderate / severe)
- Whether the AE was "serious" or not (as defined below)

- Causal association with the intervention assigned (unrelated/ unlikely/ possible/ probable/ definite)
- Outcome (resolved / resolved with sequelae / improving / still present and unchanged / death)
- Action taken with respect to the intervention (none / intervention temporarily discontinued / medical therapy required / intervention permanently discontinued / other).

Adverse event data will be collected from the date of consent until the final visit. Adverse event data will be analyzed quarterly, but serious or life-threatening adverse events may require immediate reporting and follow-up. We anticipate most adverse events will be mild and the subject will be able to resume activities within a day or two of reporting the event.

### 13.6 Withdrawal of Participants

There is no risk associated with withdrawal from the study. Participants may be withdrawn from the study if either the PI or MI feels that their continued participation would jeopardize the participant's health. In case of withdrawal, early termination procedures would be followed including documentation of adverse events, drug side effects, and reason for withdrawal. Any remaining study drug would also be collected at this time.

### 13.7 Stopping Rules

This study does not involve major risk for participating. Nevertheless, in addition to monitoring recruitment, we also will monitor the rates of adverse events in our participants. The study investigators will alert the IRB, if a larger than reasonably expected adverse event rate occurs in our participants.

### 13.8 Data Collection, Storage, and Confidentiality

#### 13.8.1 Protection of Stored Data

Protection of subject privacy will be accomplished by a variety of stringent security measures. All medical records will be stored in locked areas, and access to these areas is limited to the clinic support staff, the Director of the clinical facilities, PIs at PBRC, and authorized designees of the PI. Volunteer medical records will be filed according to an assigned volunteer ID number. All forms on the chart, with the exception of the consent form, will display only the ID number, with no subject-identifiable information. All of these will be kept separate from records with names and other personal information. The only people who will know that these patients are research participants are members of the research team (including research collaborators). However, participant information will be shared with ApneaMed via prescription to obtain required PAP devices. No information about them, or provided by them during the research, will be disclosed to others without their written permission, except if it is necessary to protect their rights or welfare (for example, in case of injury or emergency care), or if it is required by law. When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of the individuals. Participants will be identified by codes when the data gathered in this procedure is presented or published. Authorized representatives of the NIH may need to review records of individual participants. As a result, they may see their name; but they are bound by rules of confidentiality not to reveal the participants' identity to others. Electronic data storage is secured with password protection and similarly restricted with only the PI and authorized persons having access to databases containing confidential clinical records (i.e. those containing name, social security number, or other identifying information). Electronic communication will involve only unidentifiable information.

### 13.8.2 Confidentiality

PBRC complies with the federal 1996 Health Insurance Portability and Accountability Act (HIPAA). Specifically, PBRC protects the privacy and confidentiality of medical records and information contained in medical records of persons who are participants of research projects, including all protected health information (PHI) as defined by the HIPAA privacy Regulations. PHI of research participants and the use or disclosure of such information is governed by PBRC research policies as well as Common Rule, FDA regulations, and other applicable laws.

PBRC and study PI (the person chiefly responsible for the record) protect the privacy of research participants and their PHI collected during a research project. PBRC will not use or disclose existing PHI or PHI created during a research project, unless the:

- Subject signs both (a) a HIPAA Authorization for use and disclosure of PHI using an approved Authorization Form or other form containing all the elements of legally effective HIPAA authorization; and (b) the informed consent to participate in research form approved by IRB; or
- IRB grants a waiver to the requirement of obtaining a signed HIPAA authorization Form, or
- IRB approved protocol uses properly de-identified PHI

All volunteers are assured of their anonymity and confidentiality both verbally and in the informed consent.

### 13.8.3 ClinicalTrials.gov Requirements

The clinical study is registered on *ClinicalTrials.gov*, in accordance with NIH recommendations. The unique NCT identifier (NCT04530747) will be included in all future Progress Reports and publications.

## 14 DATA MANAGEMENT AND ANALYSIS

All questionnaire data will be stored using REDCap. Other participant data will be stored on secured servers at Pennington Biomedical.

### 14.3 Data management

The data management will be conducted by the Pennington Biomedical Research Computing Group. There will be limited access to all data including locked cabinets for paper files and password protected computers for electronic data.

**REDCap**, a web-based application used to build and manage surveys and databases, will be used for questionnaire collection in this study. REDCap (Research Electronic Data Capture) is a secure, web-based application that is flexible enough to be used for a variety of types of research. REDCap provides an intuitive user interface that streamlines project development and improves data entry through real-time validation rules (with automated data type and range checks). REDCap also provides easy data manipulation (with audit trails for reporting, monitoring and querying patient records) and an automated export mechanism to common statistical packages (JMP, SPSS, SAS, Stata, R/S-Plus).

### 14.4 Statistical Plan:

Statistical analyses will be performed by Dr. Robbie Beyl with support from the LA CaTS Center (U54-GM104940) in accordance with a pre-specified statistical analysis plan.

### 14.4.1 Sample Size Considerations

Although a formal power analysis was not feasible in this randomized, crossover pilot trial, we can expect significant improvements to be detectable with up to 18 completers for whole-body insulin sensitivity determined by Matsuda Index. Importantly, the proposed trial will be used to generate first-of-its-kind preliminary data for our investigative team to establish effect sizes and, therefore, develop power calculations as part of a future R01-level grant application.

### 14.4.2 Statistical Analyses

The primary outcome is insulin sensitivity via Matsuda Index. We will use mixed effect linear models, accounting for correlation over time and treatment to assess outcome difference in metformin and placebo treated groups. Primary analysis will be conducted on all study participants who have taken one dose of the study drug and used CPAP for at least one night. Sensitivity analyses will be conducted on per-protocol sample of subjects adherent to the study interventions. Publications will acknowledge the limitation of small sample and any other weaknesses.

## 14.5 Data Sharing

- **With Collaborators**: There are no plans for any data sharing. If needed, data sharing agreements will be put into place with the collaborators prior to any data transfer. All shared data will be de-identified and occur over a secure, encrypted network.
- **Sharing of Results with Participants:** Clinically relevant incidental findings from questionnaires (excessive daytime sleepiness), overnight oximetry (risk for sleep apnea) and overnight sleep study will be shared with participants as determined by study MI.

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