Propranolol for Sleep Apnea Treatment (PROSAT)

NCT03049306

August 17, 2021

JHM IRB - eForm A – Protocol

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Obstructive sleep apnea (OSA) is a common disorder affecting 24% of men and 9% of women in the United States. OSA causes repetitive upper airway closure during sleep, resulting in hypoxemia, hypercapnia, and arousals. OSA induces cardiometabolic dysfunction including insulin resistance, dyslipidemia, and endothelial dysfunction, culminating in increased rates of diabetes and cardiovascular mortality. We have accumulated evidence that OSA acutely increases levels of glucose and free fatty acids (FFA) during sleep, reflecting insulin resistance and excessive adipose tissue lipolysis. Repetitive nocturnal increases in glucose and FFA may eventually lead to diabetes and cardiovascular disease[1]. In animal studies, we have shown that intermittent hypoxia simulating OSA causes adipose tissue lipolysis, which is attenuated by beta blockade with propranolol [2]. **Thus, *we hypothesize that acute metabolic changes in OSA are mediated by sympathetic activation, and are preventable by beta blockade*.** This challenges the prevailing view that OSA causes metabolic dysfunction through hypoxia or re-oxygenation injury [3]. We will test this hypothesis in a randomized double blind placebo controlled, cross-over study of propranolol taken before sleep in patients with OSA.

2. Objectives (include all primary and secondary objectives)

Our primary objective is to test whether propranolol lowers glucose and FFA levels during sleep in OSA, and preserves vascular function (EndoPAT) versus placebo. Our secondary objective is to test whether propranolol influences sleep quality, architecture, and hemodynamics in OSA. Additional outcomes of interest are effects of OSA, CPAP, and propranolol on lipolysis and fatty acid oxidation, as measured with stable isotopes; and circulating genetic/epigenetic changes.

We also will collaborate with the National Institutes of Health (NIH) on an ancillary study of energy expenditure under each study condition, to be performed in a whole-body calorimeter, in the laboratory of Dr. Kong Chen (NIDDK). The procedures are briefly described here, but the participants will have a separate written consent process. The extent of what is to be performed in the PROSAT study is consenting participants to being contacted by the NIH for potential enrollment in their study.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

20 million adults in the United States[4] and >100 million adults worldwide [5] have OSA, and the prevalence continues to rise with obesity[6]. These figures are especially alarming as OSA is associated with diabetes [7-9], cardiovascular disease (CVD) [10-13], and mortality [14, 15]. However, mechanisms linking OSA to these outcomes are unclear. As a result of this knowledge gap, fundamental questions such as what features of OSA are most harmful, and which patients can safely decline treatment, cannot be answered. Moreover, therapies for OSA such as CPAP are poorly tolerated, leaving non-adherent

patients susceptible to CVD. Equally problematic, OSA patients who might not be at increased risk for CVD may be burdened with CPAP or invasive (and often ineffective) surgical procedures [16, 17].

We have observed that OSA acutely and dynamically elevates levels of FFA and glucose during sleep (fig 1). These changes, if recurring nightly, may foster progression towards diabetes and CVD. In mice, we showed that inhibiting the sympathetic nervous system (SNS) by surgical and pharmacologic methods during exposures to simulated OSA prevented these metabolic changes. Thus, we hypothesize that catecholamines lead to excessive FFA and glucose mobilization during sleep which can in turn lead to insulin resistance, hyperlipidemia, and vascular dysfunction. In this project we will examine whether these metabolic changes can be prevented by beta blockade, and we will use stable isotopes to quantify FFA metabolism including rates of lipolysis (glycerol and palmitate infusions) and fatty acid oxidation (palmitate ingestion).

We are also interested in interactions between OSA and genetics. Recently, our group discovered epigenetic and transcriptional changes associated with sleep apnea. In a cohort of Andean highlanders from the CRONICAS cohort study in Puno, Peru (3825m above sea level) we showed that sleep apnea was associated with differential methylation of protein tyrosine phosphatase, receptor type N2 (PTPRN2), which plays a role in the secretion of insulin and neurotransmitters. We also identified differentially methylated regions in pathways that regulate energy utilization, including mitogen activated protein kinase (MAPK), a regulator of insulin signaling ($p=10^{-4}$) and Peroxisome Proliferator-Activated Receptor (PPAR) ($p<0.01$). These findings are not limited to high altitude conditions; intermittent hypoxia exposure in healthy volunteers up-regulated pro-inflammatory gene toll receptor 2 (TLR2) in peripheral blood mononuclear cells (Polotsky et al, PLoS One 2015). These findings collectively show that sleep apnea at high altitude, and intermittent hypoxemia at sea level are associated with genetic changes that predispose to diabetes and cardiovascular disease. It is not yet known whether OSA at sea level leads to a similar epigenetic and inflammatory signature. Therefore we will also examine epigenetic and transcriptional impacts of exposure to OSA, focusing on the methylated regions identified in the cross-sectional analysis above, and on the transcription of TLR2.

In a preliminary study described below, we illustrate our discovery that OSA (elicited by CPAP withdrawal in study NA_00086830) leads to nocturnal metabolic dysfunction.

Preliminary Studies:
Methods: OSA patients (AHI>20, age 20-65) acclimated to CPAP were studied while sleeping with CPAP, or on the third night after CPAP withdrawal. During sleep, from 10:40 PM to 6:40 AM, we measured serum FFA, glycerol, insulin, glucose, and triglycerides every 20 minutes via an indwelling IV. Upon awakening, we performed an oral glucose tolerance test (OGTT) and peripheral artery tonometry (EndoPAT) to assess reactive hyperemia index (RHI). We compared effects of OSA versus CPAP in the same subjects on two different nights, one to 4 weeks apart. *Results*: 18 patients (13 men, 5 women) completed most aspects of the study to date. CPAP withdrawal led to re-emergence of OSA (CPAP AHI: 4.9±0.9, *vs.* OSA AHI: 66.7±7.5; mean ± SEM, $p<0.001$). Fig. 1

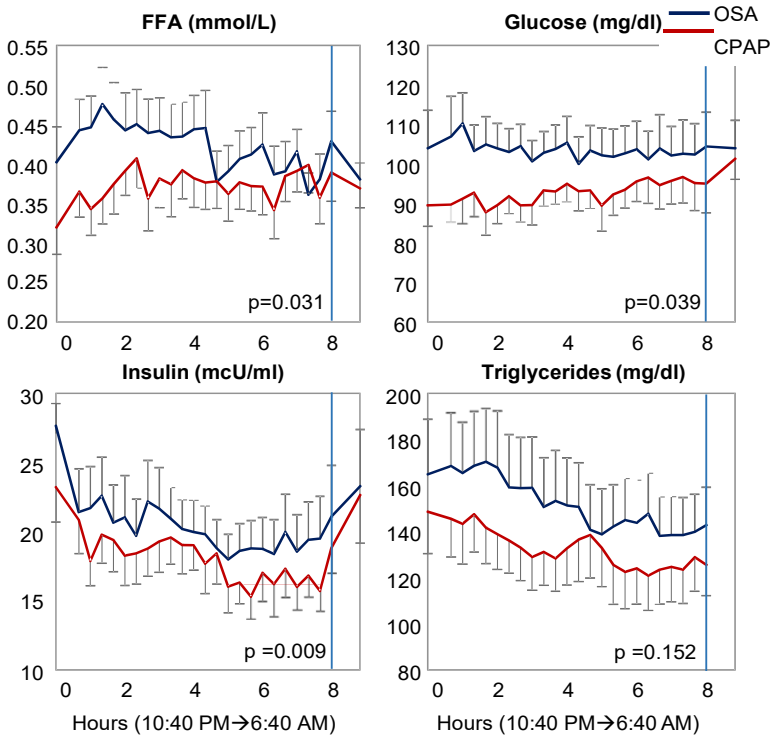


Figure 1. OSA increases nocturnal FFA, glucose, and insulin. Blood was sampled at 20 min intervals beginning at 10:40 PM (lights out) until 6:40 AM (lights on, dashed line). The same 18 patients were studied asleep wearing CPAP (red) or with OSA (on the third night after stopping CPAP, blue). Lines depict mean ± SEM.

demonstrates that OSA also increased nocturnal FFA, glucose, insulin, and triglycerides (trend) compared to CPAP. Glycerol measured in 7 subjects was also elevated (CPAP: 0.17±0.06; OSA: 0.29±0.11 mmol, p=0.04) confirming adipose tissue lipolysis during sleep. While RHI did not differ between CPAP/OSA, the extent of FFA elevation correlated with the decrease in RHI (r=0.51, p<0.05).

Mixed model analysis demonstrated that the nocturnal FFA dynamically changed with heart rate, but not with apnea hypopnea index (AHI), β=0.0043 mmol per 1 BPM increase (CI: 0.001-0.0067; p=0.008) after adjustment for AHI and BMI. This suggests that SNS activation may be the cause of lipolysis and hyperglycemia. In mice, we showed that exposure to intermittent hypoxia caused lipolysis, hyperglycemia, hyperlipidemia, and glucose intolerance, and many of these findings were abolished by propranolol [2]. Hence, the autonomic response to OSA, not a high AHI or low oxygen level *per se*, elicits excessive lipolysis, insulin resistance, and hyperglycemia during sleep. This physiology is consistent with the effects of OSA on blood pressure [18, 19] and the role of catecholamines in lipolysis, insulin resistance, and hyperglycemia in other forms of physical or emotional stress [20, 21].

Our overarching hypothesis, diagrammed in fig 2, is that SNS activation in OSA causes lipolysis, hyperglycemia, insulin resistance, vascular dysfunction and that beta blockade will abolish these consequences. This challenges the prevailing theory that intermittent hypoxia directly mediates metabolic pathology in OSA, through a mechanism comparable to ischemia-reperfusion injury [3]. If SNS activation mediates metabolic dysfunction in OSA, then beta blockade could become a cornerstone therapy for control of OSA consequences. Perhaps, the limited benefit of CPAP for secondary prevention of cardiovascular disease in those with established coronary artery disease is related to nearly ubiquitous use of beta blockers in this patient population [22, 23]. Dedicated study of beta blockade in OSA for metabolic health is therefore justified and of high clinical yield.

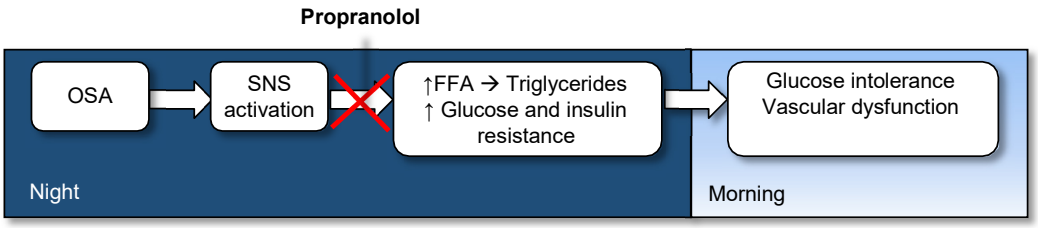


Figure 2. Hypothesis of this proposal – SNS activation during sleep causes glucose and lipid elevations and hyperglycemia, which in turn causes morning glucose intolerance vascular dysfunction.

The safety of this project is reinforced by the fact that beta blockers have been shown to attenuate OSA-induced hypertension [24, 25] without bradycardia or aggravating apnea-induced heart rate decelerations [26]. Beta blockade may similarly prevent stress-related changes in metabolism. We selected propranolol because it (1) is a non-selective beta antagonist that broadly inhibits norepinephrine-simulated lipolysis; (2) is lipophilic and crosses the blood-brain barrier, which may be desirable in mitigating OSA-related distress; (3) is well tolerated in non-hypertensive subjects [27]; (4) is effective in preventing hyperglycemia and/or lipolysis in other acute stresses including burn trauma [21], surgery [28], high altitude hypoxia [29] and animal models of autonomic stress [30] [31]; (5) and is mechanistically revealing, as it does not block alpha adrenergic receptors which are less likely to be involved in the stress hyperglycemic effect in humans [28].

4. Study Procedures

a. Study design, including the sequence and timing of study procedures

There are two primary interventions in this study: CPAP withdrawal and propranolol administration which are described below:

CPAP withdrawal: By manipulating CPAP, OSA can literally be switched “on” or “off” in the same individual, once they are accustomed to CPAP use[32]. This affords a straightforward means to study the isolated metabolic impact of OSA, using each patient as his or her own control. Based on CPAP withdrawal [33-36], or acute CPAP depressurization studies in our laboratory [32], OSA may not fully re-emerge until after a few days of cessation. Our preliminary data demonstrate that 3 nights of CPAP

withdrawal leads to a full recurrence of OSA and increases nocturnal FFA in a manner similar to that of treatment naïve patients [37]. CPAP withdrawal also induces the same sympathomimetic, neurocognitive, and vascular consequences of OSA as in treatment naïve subjects [33-36] indicating that findings of such studies are relevant to the untreated OSA population. Our 3 night CPAP withdrawal protocol has been approved by the Johns Hopkins IRB "Metabolic Impact of Intermittent CPAP" (8/5/2013). In this study, we use CPAP withdrawal to induce OSA, to provide a setting for testing a pharmacologic intervention designed to mitigate OSA consequences. To facilitate participant blinding of intervention, we will administer a nasal dilator strip (NDS) as a placebo during all CPAP withdrawal periods. NDS have been used in other studies as a CPAP placebo and have no impact on OSA severity [38].

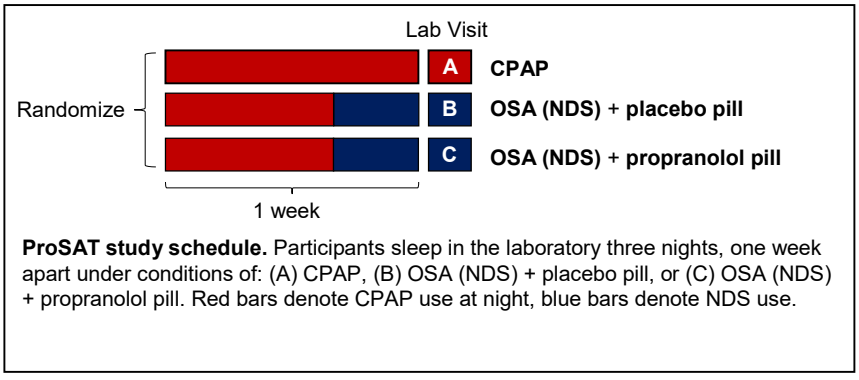
Propranolol administration: Long acting propranolol LA (Inderal ® LA) 80 mg or placebo will be given before sleep to suppress SNS responses to OSA. Propranolol LA has a 24-hour duration of effect which makes it suitable for this study, eliminating the need for repeated oral dosing.

Study overview: There are a total of 4 study visits. The first visit is a consent, screening, and randomization visit. After consent, a screening EKG will be performed to exclude rhythm or conduction abnormalities and a urine pregnancy test to exclude pregnancy in potentially pregnant females. (If the participant has had a clinical EKG or EKG report in the last year that does not indicate any conduction disorders listed in the exclusion criteria, the screening EKG may be deferred.) A point of care screening test for hemoglobin will also be performed to exclude pre-existing anemia. Patients with a hemoglobin < 10 g/dL are ineligible for this study. They will be provided with ClearPassage® NDS to use at home during CPAP withdrawal nights.

Thereafter, there are 3 overnight visits to the CRU in random order. This protocol compares nocturnal metabolism in treated OSA (CPAP), untreated OSA (NDS), and beta-blocked OSA (NDS) in the same subject. OSA nights are performed in the lab after using NDS instead of CPAP for 2 nights at home.

Covid-19 screening: We will screen participants before their arrival for their each sleep study with a Covid-19 test. A negative screen within 72 hours of the CPAP visit will be needed prior to admission for the CPAP study visit.

During each OSA visit, propranolol or placebo is taken in the evening, in random order. Frequent blood sampling is performed throughout the visit. In the morning, DEXA (only 1 DEXA needed for entire study) is performed. We are targeting 1 week between visits, but will allow flexibility in scheduling patients unable to schedule 3 consecutive visits exactly 1 week apart; in this scenario we will allow a duration of ≥ 4days or ≤ 3 weeks between visits. Each visit we will also administer stable isotopes to measure detailed lipid metabolism.



ProSAT study schedule. Participants sleep in the laboratory three nights, one week apart under conditions of: (A) CPAP, (B) OSA (NDS) + placebo pill, or (C) OSA (NDS) + propranolol pill. Red bars denote CPAP use at night, blue bars denote NDS use.

Methods to minimize in-person contact:
In order to reduce the need for / length of research-only in-person visits, telemedicine visits will be substituted for in-person informed consent whenever logistically possible (However, we are aware that this will depend on the participants' familiarity, access to, and willingness to use telemedicine). Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine

visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

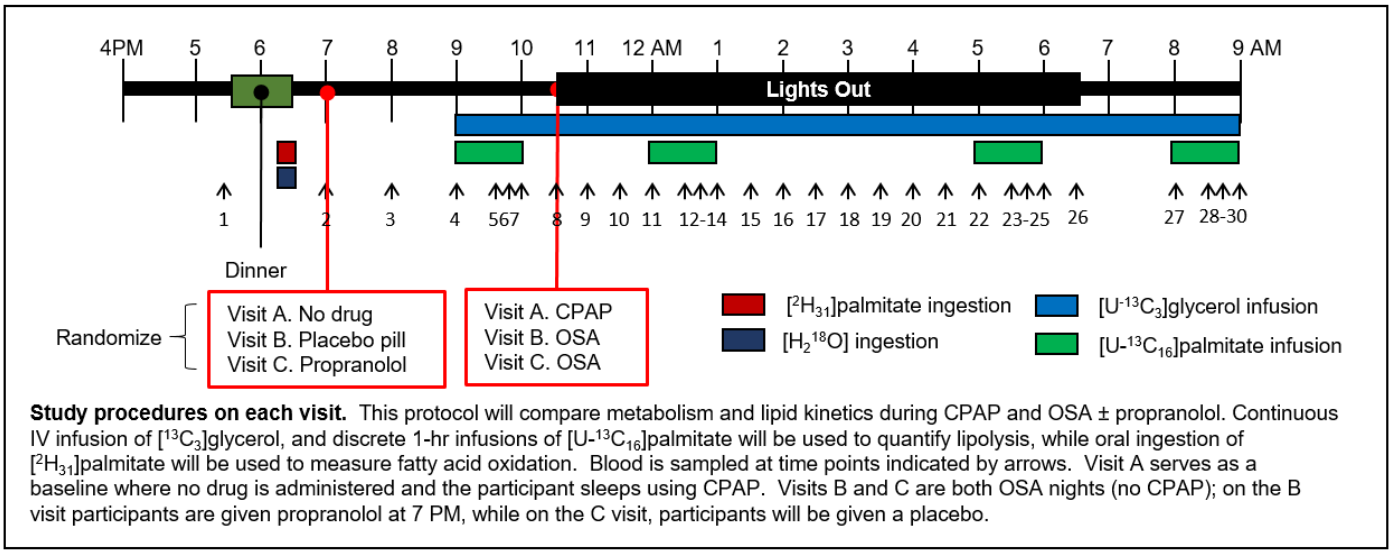
Potential subjects will be provided with a copy of the Informed Consent form prior to the remote consent meeting either via email, fax, mail or previously provided during an in person visit. Potential subjects will then be contacted by phone by a consent designee to review the consent form in detail. Potential subjects will be given adequate time to consider the research study and ask questions prior to signing the consent form. The potential subject will then sign and date/time the informed consent document and it will be mailed, emailed or faxed to the consent designee, who will also sign/date/time. A copy of the fully signed CF will be returned to the volunteer

Each sleep study night will consist of the following procedures:

Pre-sleep procedures. Subjects will undergo a history and physical examination. They will report to the CRU at 4:00 PM and undergo anthropometric assessment including weight, height, neck, and waist circumference. At 5:30 PM, they will eat a standard dinner containing 30% fat, 50% carbohydrate, and 20% protein with kcal based upon the Mifflin-St Jeor formula. Two peripheral IVs (18 to 20 gauge) will be placed. All samples are obtained through the IV without disturbing the subject during sleep. Blood pressure will be checked at 6:30 PM, before administration of study drug. If systolic blood pressure is <90 or the heart rate is <50 then no drug will be administered. (In the event that this occurs, the study will still proceed so that CPAP and OSA nights can be compared and analyzed as a "no drug" group.) If blood pressure and heart rate are acceptable, propranolol LA 80 mg, or placebo (random order, crossover on study night 2 and 3) will be given. Participants and the investigators will be blinded to drug assignment.

PSG setup. On each study night from 10:30 PM until 6:30 AM, a PSG will be performed. The sleep study will include electroencephalography, electrooculography, oxygenation, respiratory effort, CPAP airflow, and transcutaneous CO_2 monitoring. Respiratory effort will be measured by thoraco-abdominal movement assessed by mercury strain gauges. Surface electrodes will be placed at C_3A_2 and C_3O_1, a submental electrode, and left and right electro-oculogram will be used to stage sleep. Electrocardiograph (EKG) tracings will be recorded from three chest electrodes. Continuous measurement of oxygen saturation will be recorded using an ear oximeter (model No 472-1A, Hewlett Packard, Waltham, Mass). Transcutaneous CO_2 will be monitored using a Radiometer TCM-4 device. Signals from the electroencephalograph, EKG, electromyogram, electrooculogram, respiratory strain gauge, ear oximeter and thermistors will be recorded on a computer with RemLogic software.

Respiratory interventions. During the CPAP night, patients will sleep using CPAP set to their prescribed home CPAP settings. Airflow will be recorded with a pneumotachograph in the CPAP circuit. CPAP will be titrated if necessary to prevent obstructive hypopneas and apneas. During NDS nights, ClearPassage® strips will be applied at bedtime. Flow will be measured using a nasal cannula and oral thermistor. Sleep and respiratory events will be scored according to AASM criteria. Apneas will be considered present when there is no air flow for >10 seconds. Obstructive apneas will be considered present when apneas are associated with positive strain gauge deflections indicating thoracic movement. Apneas will be considered central in origin when cessation of air flow is not accompanied by thoracic movement as measured by the strain gauge. Mixed apneas will be defined by episodes of no air movement resulting from central apnea followed by obstruction. Hypopneas will be scored for reductions in airflow of 30% lasting >10 seconds accompanied by either an arousal or fall in oxyhemoglobin saturation of 3% or greater. Blood pressure is measured at 7 PM, 10:30 PM, and upon awakening after the morning blood sample at 6:30 AM.



Study procedures on each visit. This protocol will compare metabolism and lipid kinetics during CPAP and OSA ± propranolol. Continuous IV infusion of [$^{13}C_3$]glycerol, and discrete 1-hr infusions of [U-$^{13}C_{16}$]palmitate will be used to quantify lipolysis, while oral ingestion of [$^{2}H_{31}$]palmitate will be used to measure fatty acid oxidation. Blood is sampled at time points indicated by arrows. Visit A serves as a baseline where no drug is administered and the participant sleeps using CPAP. Visits B and C are both OSA nights (no CPAP); on the B visit participants are given propranolol at 7 PM, while on the C visit, participants will be given a placebo.

Stable isotope ingestion and infusions. Stable isotopes are being used in this project is to quantify rates of adipose tissue lipolysis (infusions of [U-$^{13}C_3$]glycerol and [U-$^{13}C_{16}$]palmitate) and rates of fatty acid oxidation (FAO, ingestion of [$^{2}H_{31}$]palmitate). Ingested $^{2}H_2$ molecules on palmitate are incorporated into plasma $^{2}H_2O$ during FAO and detected by mass spectrometry. This technique permits FAO measurement without needing to capture exhaled CO_2, measure VCO_2, or administer acetate to adjust for CO_2 sequestration. By adopting this approach, FAO can be assessed without interrupting sleep, respiratory monitoring, or CPAP. Catheters: Before dinner, an IV will be inserted into a vein of each arm; one for blood sampling, and the other for infusion of labeled glycerol and palmitate. Patency of all catheters will be maintained by saline infusion. Infusions of tracers: On each visit, after obtaining a blood sample to determine background enrichment, a constant infusion of [U-$^{13}C_3$]glycerol (0.1 μmol·kg^{-1}·min^{-1}; Sigma-Aldrich, St. Louis, MO) will be administered. Glycerol and palmitate stable isotopes are obtained as a sterile powder from Sigma-Aldrich, and compounded and dispensed by the Johns Hopkins research pharmacy. The product is initially tested for sterility and pyrogenicity by Sigma-Aldrich. In the Johns Hopkins research pharmacy, the powders will be solubilized and aliquots tested for sterility and pyrogenicity. Infusions are given at a "tracer" dose detectable only by gas chromatography/mass spectroscopy (GC/MS). All tracers in this protocol are tested for microbiological contamination, and are exempt from FDA IND status (see correspondence with FDA # PIND 132066).

Blood samples will be collected at the intervals depicted (every 30-60 min, with additional samples towards end of each palmitate infusion period to verify isotopic steady-state) and analyzed for isotope enrichment by gas chromatography-mass spectrometry (GC/MS). Infusion of [$^{13}C_3$]glycerol and quantification its enrichment in plasma of will be used to calculate lipolysis (R_a glycerol) throughout the night. Infusion of [U-$^{13}C_{16}$]palmitate (0.04 μmol·kg^{-1}·min^{-1}; Sigma-Aldrich, St. Louis, MO) will be infused for 1-hour intervals at 9-10 PM (pre-sleep), 12-1 AM (early night), 5-6 AM (late night), and 8-9 AM (awake). Palmitate isotope will be bound to human albumin prior to infusion to facilitate steady-state infusion rates. Plasma enrichment of [$^{13}C_{16}$]palmitate will be measured by GC/MS and used to calculate FFA turnover. R_a glycerol and R_a palmitate will be calculated from non-steady state Steele equations. R_a FFA will be derived from R_a palmitate based on the fractional contribution of palmitate to the total FFA concentration, as determined by GC. R_a FFA will be determined for each of the four infusion periods based on the average plasma [$^{13}C_{16}$]palmitate enrichment during the last 3 samples of each infusion period.

Ingestion of [$^{2}H_{31}$]palmitate: Conventional FAO assessment requires breath collection (e.g. enrichment of $^{13}CO_2$ from infused [^{13}C]palmitate), and calorimetry which would disrupt sleep, respiratory monitoring,

and CPAP airflow. Therefore, we use a validated technique to quantify differences in FAO between studies by providing an oral liquid dose of 15 mg/kg body mass of [$^2H_{31}$]palmitate taken with dinner. The isotope is dissolved in a warm (60 °C) liquid shake (such as Ensure®). The isotope is non-radioactive and synthetic (Cambridge Isotope Laboratories DLM-215-MPT). FAO will be quantified thereafter by hourly assessment of deuterium incorporation into plasma ^2H_2O using an isotope ratio MS (IR/MS). Subjects will also ingest 0.4 g/kg body mass of $H_2{}^{18}O$ (10% enriched; CIL OLM-240-10-0, Andover MA) to calculate total body water (TBW) by isotope dilution. The FAO rate will be determined by excess plasma ^2H times TBW, while percent recovery of deuterium will be determined by dividing by dose of ^2H administered. All tracers in this protocol are tested for microbiological contamination, and are exempt from FDA IND status (see email correspondence with FDA # PIND 132066). The isotopes of $H_2^{18}O$ and [$^2H_{31}$]palmitate are stored in the PI's laboratory. Their required amount is prepared for each participant and brought to the CRU. The [$^2H_{31}$]palmitate is dissolved in an Ensure shake and heated in the kitchen by nursing staff or dietician in CRU.

Blood sampling and morning urine sample collection. The first blood sample is taken at 5:30 PM, just before dinner and serves as a pre-medication, pre-isotope baseline. Subsequent samples are drawn hourly while the subject is awake. At 10:30 PM, when the sleep study starts, blood is sampled more frequently, at 30 minute intervals in order to capture dynamic changes in metabolism during sleep. Additional samples will be drawn towards the end of each palmitate infusion period, to ensure a plateau of isotope enrichment. Each blood sample will be 4 cc in volume and placed into lavender top tubes (EDTA) for centrifugation to obtain plasma. Cells are removed from plasma by centrifugation for 10 minutes at 1500 x using a refrigerated centrifuge. Following centrifugation, plasma will be transferred to cryovials for storage. In patients that have signed the optional consent for isolation of genetic material, we will collect an additional 16 cc (4 tubes) at 6:30 AM upon awakening since this volume is required for isolation of peripheral mononuclear cells. The final sample is obtained at 9:00 AM. .

In total, the blood collected will be approximately 30 samples x 4 mL = 120 ml. For patients that consent to isolation of genetic material, the total will be 136 cc. This quantity is less than half the volume of blood given during a donation (350 mL). A morning urine sample will be collected for measurement of catecholamines. The final blood sample will be drawn at 9 AM, after which the IV will be removed, and the subject will be discharged from the CRU. Samples will be frozen at -80°C for research pertaining to metabolic effects of OSA, with the consent of participants.

Morning procedures:

EndoPAT. The stable isotope infusion is stopped, the 09:00 AM blood draw is obtained, the IV is discontinued. The subject will report to the EndoPAT in the CRU at about 09:15 AM, and will rest awake in the supine position. A blood pressure cuff is placed on the non-dominant arm while the contralateral arm is used for control comparisons. Baseline measurements will be taken for 5 minutes, during 5-min occlusion of the brachial artery by inflation of the automated cuff 50 mmHg above systolic blood pressure, and for 5 minutes upon release of the cuff. Endothelium-mediated vasodilation is assessed using a finger pulse wave amplifier. The RHI and the augmentation index will be derived by software and used as metrics of endothelial function and arterial stiffness respectively. After the test is finished, breakfast will be served and the patient is discharged from the CRU.

This portion of the study is completed: [OGTT. The subject will report to the nurse's station after EndoPAT is completed. From the indwelling IV, blood samples will be obtained. The patient will be given a 75 g glucose solution to drink within 5 minutes. Blood samples of insulin and glucose are then collected in EDTA tubes at 30, 60, 90, and 120 minutes after drinking the glucose solution, and centrifuged to obtain plasma. They will be asked, if possible, to remain awake during the OGTT. After OGTT, breakfast is served and the participant is discharged from the CRU.]

Dual-energy X-ray absorptiometry (DEXA). Each subject will undergo a DEXA scan to estimate fat mass, which will be one of the variables analyzed as a predictor of FFA elevation. Each participant only needs 1 DEXA scan for the study and this can be performed on any one of the visits. If necessary for staffing reasons, the DEXA can be performed at a different time such as the afternoon or evening before sleep.

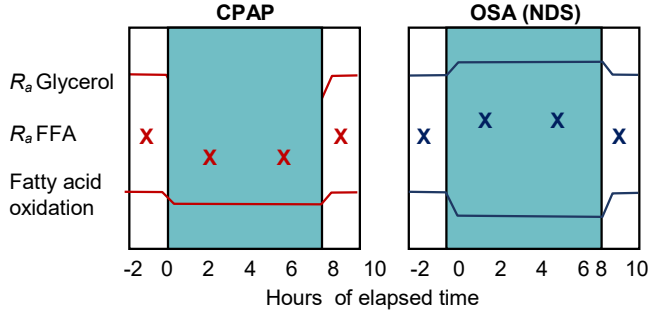
Post-test procedures: To minimize the impact of sleep loss on driving safety, subjects are asked to either (1) arrange for an alternate means of transportation home (this is mentioned on the consent or to (2) sleep as needed for up to 4 additional hours before discharge. Repeat sleep tests may be requested from the subject under the following circumstances: (1) A technical problem with data acquisition including PSG lead failure/artifacts, incomplete blood collection from IV insertion or maintenance problems. (2) Poor sleep efficiency <50% of time in bed. (3) inadequate resumption of OSA (AHI>15) or its effective treatment with CPAP (AHI<10). If sleep studies meet these criteria and a repeat test is deemed necessary by the PI, then the repeat testing will be scheduled at least 1 week following the inadequate study.

Ancillary Study at NIH:
During consent for this study, participants will be informed an optional study that will be performed at the NIH to measure an additional outcome of total energy expenditure using a whole room indirect calorimetry (WRIC). WRIC allows detailed assessment of energy and nutrient balance. Measurements are conducted at stable interior (room) temperature, humidity and barometric pressure, which are continuously measured. The NIH will have its own written consent and IRB procedures performed on its Bethesda campus. For participants that choose to participate in the NIH study, their WRIC data will be uploaded into a REDCAP database and transferred to our encypted database for integration with other study outcomes.

Data Analysis and Anticipated Outcomes:

The primary outcomes are nocturnal glucose and FFA levels. The main independent variable is experimental condition: [CPAP], [OSA+placebo], or [OSA+propranolol]. Since glucose and FFA are measured multiple times per subject per night, we will perform mixed linear model analysis using condition as a fixed factor and subject ID as a random factor. We will also examine whether there are independent or interacting effects of time, or time x condition on trajectories of FFA and glucose. We hypothesize that OSA increases glucose and FFA levels during [OSA + placebo] nights, compared to [CPAP], or [OSA + placebo] nights. We further hypothesize that propranolol will suppress nocturnal FFA and glucose to levels similar to that of CPAP. Sleep physiology that is measured on a continuous basis will be condensed into 30 minute bins to create a parallel data structure with metabolic parameters. We will then examine whether sleep physiology (e.g. AHI, arousals, heart rate) is dynamically associated with glucose or FFA, and whether urine catecholamine levels are associated with increases in FFA or glucose. For variables measured once (RHI, morning lipid profile) or averaged together (e.g. nocturnal glucose level) we will perform one-way ANOVA for comparisons. We will also compare sleep physiology between the 3 nights, examining variables such as apnea hypopnea index, metrics of hypoxia, and sleep architecture/duration. A questionnaire will be administered each morning to assess subjective sleep quality from the night prior, and to screen for any symptoms related to study medication. These responses will used to determine how symptomatic a subject's OSA is, whether subjective sleep quality correlates with extent of metabolic change, and whether any side effects of beta blockade were perceived.

Regarding tracer data, we predict that R_a FFA and R_a glycerol will exhibit a ~3:1 ratio reflecting typical patterns of lipolysis without significant intra-cellular recycling. Based on studies of normal sleep [39, 40], we predict that on CPAP, sleep reduces lipolysis and FAO compared to wakefulness. During CPAP withdrawal, sleep will increase lipolysis (or at least mitigate sleep-induced suppression) compared to wakefulness. We hypothesize that FAO will be reduced or unchanged by OSA. This pattern of increased lipolysis without increased FAO may lead to



Predicted qualitative changes in lipolysis and FAO. We hypothesize that normal sleep (CPAP) reduces lipolysis and FAO. OSA stimulates lipolysis without affecting FAO, a scenario that could lead to ectopic lipid deposition.

ectopic lipid deposition and extracellular TG cycling (see figure).

b. Study duration and number of study visits required of research participants.

Each subject will undergo 3 sleep studies spaced one week apart. Including the baseline consent/screening/randomization visit, there are a total of 4 study visits. Since visits are 1 week apart, the duration of involvement will be ~3 weeks. In the event that participants are not available to make visits exactly 1 week apart, we will allow visits to be spaced apart by ≥ 4 days and ≤ 3 weeks. Therefore, the minimum duration in the study is about 2 weeks, and the maximum time is 6 weeks.

If participants agree to being contacted for the ancillary calorimetry experiments, the time frame will be at least 1 week following completion of the PROSAT protocol. Participants will undergo 2 visits to the NIH (1 week apart) and under conditions of CPAP or NDS (CPAP withdrawal).

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

The subject and investigator will be blinded to whether they receive placebo or propranolol. This will control for the potential placebo effect of taking any medication before sleep. The investigator will unblind the drug assignment after the first 6 completed participants, so that adjustments to propranolol timing or dosage can be performed depending upon hemodynamic and metabolic effects. At that point, if the average OSA-induced FFA level is not reduced by at least 10%, the dosage of Inderal LA will be increased from 80 to 120 mg. The dosage will be reduced if there is any evidence of excessive bradycardia (>15% reduction in heart rate) or hypotension (systolic BP<90). In addition, if the FFA nadir does not occur during the main sleep period, the timing of propranolol administration will be advanced or delayed as necessary. These adjustments will be made in collaboration with the research pharmacy. Regardless of whether the PI is blinded or unblinded, all data collection (sleep study, EndoPAT etc) will be performed in a blinded manner. For CPAP withdrawal nights, NDS will be used as a CPAP placebo. This will control for unintended effects of stopping CPAP such as anxiety before sleep.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

CPAP will be discontinued for a short period of time to elicit OSA. CPAP withdrawal induces the same sympathomimetic, neurocognitive, and vascular consequences of OSA as in treatment naïve subjects [33-36] indicating that findings from CPAP withdrawal studies are relevant to the untreated OSA population. Outcomes of this study – acute changes in lipid and glucose metabolism - are unlikely to irreversibly change with antecedent CPAP use. Another advantage is that retention in the study will be higher than enrolling treatment naïve patients, many of whom will be unwilling or unable to defer, to start, or to maintain CPAP therapy. Thus, a transient change in physiology in a small group of patients is being elicited with CPAP withdrawal to reveal mechanisms that may prevent refractory pathology in millions of others with OSA. There is no other deviation from routine care in this study.

e. Justification for inclusion of a placebo or non-treatment group.

In terms of NDS as a CPAP placebo during withdrawal nights, we wish to minimize confounding that might be introduced by other factors such as anxiety about stopping CPAP. Participants will be told that NDS are not as effective as CPAP for treatment of OSA. The written consent will state that NDS may be effective for nasal congestion but that long-term data have not shown an effect on OSA severity. In terms of the drug intervention, participants will be informed that we are studying the metabolic and vascular consequences of OSA and whether a medication taken before sleep can prevent these consequences. Propranolol is not being utilized as a therapeutic agent but as a mechanistic tool. Placebo is used to control for subconscious effects of taking a medication before sleep.

Definition of treatment failure or participant removal criteria.

Participants will be removed from the study if they do not meet eligibility criteria. Otherwise, this study is expected to induce OSA and its consequences, and the drug is being used as a tool to test mechanisms rather than treat a specific disorder. Patients may be removed from the study if they elect to withdrawal at any time.

f. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Propranolol is given only one time per participant and is not considered therapy, but a mechanistic intervention. After each study visit, CPAP is resumed. Sleep study results will be discussed with the patient upon completion of the study. When clinically appropriate, referral for treatment will be provided. Patients may request results of their sleep study before completion of the study.

5. Inclusion/Exclusion Criteria

This study is similar to "MIIC: Metabolic Impact of Intermittent CPAP" (NA_00086830) in terms of CPAP withdrawal procedures. Thus, inclusion/exclusion criteria are similar but are expanded to exclude patients with contraindications to taking beta blockers. In addition, we are attempting to enrich the cohort with participants who exhibit high FFA or glucose levels during CPAP withdrawal. Participants who competed their involvement in MIIC, who have resumed CPAP for at least 1 month, may enroll in this study. They must have exhibited a FFA or glucose elevation of ≥10% during their CPAP withdrawal study.

Inclusion Criteria:

1. Men and women of all races ≥ 20 and ≤ 70 years old
2. History of OSA (AHI>20, >50% events obstructive)
3. Accustomed to CPAP use, and willing to discontinue CPAP temporarily for the study and switch to NDS
4. If the participant has already completed "Metabolic Impact of Intermittent CPAP" (NA_00086830, they must have exhibited a >10% increase in nocturnal FFA or glucose during CPAP.

Exclusion Criteria:

1. Cardiovascular risks
    a. Decompensated congestive heart failure
    b. Atrial fibrillation, sick sinus syndrome, 2nd or 3rd degree heart block, pacemaker implantation, Wolff-Parkinson-White Syndrome (if not known, will check on a screening EKG)
    c. Uncontrolled hypertension > 170/110
    d. History of postural hypotension.
    e. Resting systolic pressure <90 or heart rate < 50 on screening visit

2. Drug interactions – currently taking any of the following drugs Subjects on these medications are excluded from participation and will not have the drug in question discontinued for the purposes of participation in the study.
    a. Calcium channel blockers that reduce heart rate (diltiazem, verapamil, fendiline, gallopamil)
    b. Sympatholytic drugs: any other beta blocker; clonidine, terazosin or doxazosin; reserpine
    c. Anti-arrhythmic drugs: (e.g. amiodarone, sotalol, digoxin, quinidine, lidocaine, propafenone)
    d. Coumadin (propranolol may prolong INR)
    e. Drugs that Inhibit CYP2D6, CYP1A2, or CYP2C19: amiodarone, ciprofloxacin, cimetidine, delavudin, fluconazole，fluoxetine, fluvoxamine, imipramine, isoniazid, paroxetine, quinidine, ritonavir, rizatriptan, tenioposide, theophylline, tolbutamide, zileuton, zolmitriptan
    f. Drugs that increase hepatic metabolism of propranolol: rifampin, ethanol, phenytoin, and phenobarbital
    g. Neuroleptics/anxiolytics: (thioridazine, chlorpromazine – may increase propranolol level), haloperidol, valium
    h. Illicit drugs such as cocaine or amphetamines.

3. Other medical conditions
    a. Sleep disorder other than OSA, including: restless leg syndrome, parasomnia, or narcolepsy.
    b. Shift work or circadian rhythm disorder that is expected to prevent good sleep as scheduled in the protocol
    c. Insulin-dependent diabetes mellitus
    d. Myasthenia gravis
    e. Pheochromocytoma
    f. Uncontrolled bronchospastic lung disease such as COPD, mild persistent or worse asthma, as well as those requiring maintenance therapy.
    g. Current smoking
    h. Chronic renal or liver failure
    i. Known pregnancy, by urine testing in women of child-bearing age; nursing mothers
    j. Known hypersensitivity to any beta blocker
    k. Hemoglobin < 10 g/dL on point of care screening

4. Patients with a history of falling asleep while driving, involved in a near-miss accident while driving, and those in high-risk occupations such as commercial drivers or pilots.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

FFA and glucose elevations occur during the night in OSA patients (fig 1), and these elevations may be responsible for adverse cardio-metabolic consequences of OSA. The SNS is well known to mediate adipose tissue lipolysis as well as “stress hyperglycemia”. Thus, we may be able to mitigate these nocturnal metabolic changes with beta adrenergic blockade. We selected propranolol because it (1) is a non-selective beta antagonist that broadly inhibits norepinephrine-simulated lipolysis; (2) is lipophilic and crosses the blood-brain barrier, which may be desirable in mitigating OSA-related distress; (3) is well tolerated in non-hypertensive subjects [27]; (4) is effective in preventing hyperglycemia and/or lipolysis in other acute stresses including burn trauma [21], surgery [28], high altitude hypoxia [29] and animal models of autonomic stress [30] [31]; (5) and is mechanistically revealing, as it does not block alpha adrenergic receptors which are less likely to be involved in the stress hyperglycemic effect in humans [28].

Propranolol is rapidly metabolized with a plasma elimination half-life of 2 to 4 hours, requiring up to 4 doses daily for management of hypertension or angina. In our study, patients will require beta blockade for > 12 hours, which is why long acting propranolol has been selected. The areas under the propranolol plasma concentration-time curve (AUCs) for sustained release propranolol are lower than for comparable divided daily doses of propranolol [41] [42].

In this study, we will administer a single dose of long-acting (or “sustained release”) propranolol (Inderal® LA) 80 mg at 7:00 PM. This is the standard clinical starting dose for hypertension, and less than the nighttime dose of propranolol LA 120 mg used in a study of patients with bruxism[43]. The drug is coated with a semipermeable membrane to allow gradual absorption over a 12 hour period and a therapeutic window lasting ~24 hr. In a pharmacokinetic study, 160 mg of sustained release propranolol demonstrated gradual increase in plasma concentration to a peak ~6 hours after administration, then a gradual decline. By comparison, propranolol XL (Innopran XL) has a later onset of absorption, and a higher plasma peak and AUC than Inderal LA [44] which make it less desirable for this study. Propranolol LA 80 mg is less than the equivalent dosing of propranolol 40 T.I.D, the starting clinical dose for a variety of indications. For example, propranolol was used in the Beta Blocker Heart Attack Trial (BHAT, n=3,837), which administered 40 mg T.I.D, and later titrated up to 80 mg T.I.D [45]. In migraine headache, typical dosing is up to 160 mg propranolol daily in divided doses [46]. Propranolol LA at 160 mg daily is the target clinical dose in studies of angina pectoris, hypertension, and hyperthyroidism [42].

After interim analysis of the first 6 participants, we may adjust the dose and/or timing of propranolol based on its safety and efficacy, if necessary. For safety, the criterion is that on the propranolol night, the median heart rate during sleep should decrease by no more than 15% of basal heart rate as measured during the

placebo night. In addition, systolic blood pressure on the propranolol night should remain ≥ 90 mmHg. For efficacy, the decrease in overnight FFA levels by propranolol needs to be at least 10% as compared to the placebo night.

During CPAP withdrawal nights (both at home and in the lab) we will use NDS as a CPAP placebo. NDS may improve snoring but have no treatment value for OSA [38]. By including NDS as a placebo, we will avoid potential confounding such as anxiety caused by stopping CPAP.

Stable isotope (non-radioactive) tracers are being used in this project is to quantify rates of adipose tissue lipolysis (infusions of [U-$^{13}C_3$]glycerol and [U-$^{13}C_{16}$]palmitate) and rates of fatty acid oxidation (FAO, ingestion of [$^2H_{31}$]palmitate and $H_2{}^{18}O$). These tracers have been used safely in several metabolic research studies [47-50]. The breakdown of stored triglycerides (lipolysis) results in the release of fatty acids and glycerol from fat cells. Thus, the rates of release of fatty acids and glycerol into the bloodstream reflect rate of lipolysis, which are usually measured by the constant-infusion-isotope-dilution technique [51]. In this approach, labelled fatty acid and glycerol are infused intravenously in tracer amounts at a constant rate. The *R*a of endogenous unlabeled fatty acids and glycerol into the bloodstream can be determined by calculating the dilution of the infused isotope [51]. Labelled palmitate is usually used as the tracer since it is the typical of other fatty acids and reflects whole-body free fatty acid kinetics [51]. The palmitate tracer will be bound to albumin for infusion, since it is not soluble in water [51]. We chose [U-$^{13}C_{16}$]palmitate and [U-$^{13}C_3$]glycerol for infusion as they are uniformly carbon-labeled stable isotopes with high detection sensitivity which require lower dose and infusion rate as compared to single carbon labeled tracers [52]. The infusion is given at a "tracer" dose detectable only by gas chromatography/mass spectroscopy (GC/MS). The dose and infusion rate of the isotopes will depend upon the subject's body weight. Specifically, we will administer a constant infusion of [U-$^{13}C_3$]glycerol 0.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (x 12 hours) = 72 µmol/kg of total per night (approximately 8.64 mmol = 2.68g for a 120kg person, Sigma-Aldrich 660701), and [U-$^{13}C_{16}$]palmitate will be infused 0.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for 1-hour intervals (x 4) = 9.6 µmol/kg of total per night (approximately 1.152 mmol = 0.36g for a 120kg person, Sigma-Aldrich 687871).

Stable isotopes [$^2H_{31}$]palmitate and $H_2{}^{18}O$ will be used to follow the fate of ingested fat (palmitate), and to quantify the plasma water pool size to permit calculation of tracer oxidation rate. This is a validated technique to quantify FAO by providing an oral liquid dose of [$^2H_{31}$] palmitate taken with dinner [53]. We will administer [$^2H_{31}$] palmitate orally with dinner in a liquid emulsion (such as an Ensure® shake) at a dose of 15 mg/kg body weight (approximately 1.7 g of fat for a 120 kg person, Cambridge Isotope Laboratories DLM-215-MPT). In addition, we will administer a stable isotope of water, [$H_2{}^{18}O$] 10% orally at a dose of 0.4 g/kg body weight (about 48 mL for a 120-kg person, Cambridge Isotope Laboratories OLM-240-10-0). When [$^2H_{31}$]palmitate is oxidized, the ^2H is released into the plasma ^2H_2O and is quantified by mass spectroscopy.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

We will be administering unmodified, marketed product via the typical oral route at a standard clinical dose. This proposal was presented the FDA and qualified as exempt from IND application (see separate documentation). Propranolol is FDA approved for indications including hypertension, migraine, supraventricular tachycardia, and essential tremor and lawfully marketed in the United States. This research is not intended to support any change in the advertising or labeling of the drug. A conventional starting dose of drug is being administered, and the drug will be administered one time during the study. The investigator will not represent the drug in any promotional context, distribute, test-market, or charge for the drug.

Propranolol is a non-selective (beta-1 and beta-2 receptor), non-vasodilating beta adrenergic receptor antagonist. It has been used for decades for hypertension and angina as well as "off label" for other

indications such as anxiety. It has been used in research settings to inhibit SNS responses to a variety of environmental stressors. Beta blockers such as atenolol [24] and nebivolol [25] have been safely studied in OSA-related hypertension. Clinical and chronic beta blocker use does not aggravate apnea-induced heart rate decelerations [26]. In an animal study of experimental OSA, propranolol blunted arousal-induced tachycardia and hypertension without exacerbating bradycardia during apneas [54]. Propranolol LA 120 mg has been given as a single dose before sleep and reduces heart rate without lowering blood pressure [43]. Propranolol has been used since the 1970's for anxiety in non-hypertensive patients [27]. In a pharmacokinetic study of propranolol in normotensive men, a single dose of Inderal LA 160 mg caused a mean peak reduction of systolic blood pressure of 11.6 mmHg and 8 BPM reduction in heart rate[44].

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Fat is stored in adipose tissue as triglycerides, which are comprised of glycerol and fatty acids. Measurement of glycerol and fatty acid levels in plasma does not provide information about the turnover rate or flux of these substances through the body. To assess these dynamic parameters requires administration of isotopes. Stable isotopes have been used safely for decades of metabolic research. Stable isotopes are not radioactive and occur in nature. A small percentage of the body's glycerol, fatty acids, and water already exist as stable isotopes. In this study we will temporarily increase the amount of these isotopes to tracer levels that can only be detected by a mass spectrometer.

In terms of safety, our infusates will be prepared with the Johns Hopkins Research Pharmacy under sterile conditions. The FDA's policy on stable isotopes states that if substance would not otherwise require submission of an IND, then that substance enriched with a stable isotope will not require an IND. If the isotope enrichment is for metabolic tracer studies employing drugs or non-pharmacological substances, such as glucose and individual amino acids, for which there are adequate data demonstrating their safety and if the substances are used within the limits for which the data are adequate, no IND will be required. The exemption information has been separately uploaded in the IRB application.

7. Study Statistics

a. Primary outcome variable: Nocturnal FFA and glucose profile
b. Secondary outcome variables: Morning EndoPAT, lipid panel. Sleep study parameters (e.g. total sleep time, latency, wake after sleep onset, sleep stages, AHI, oximetry, heart rate). Lipid flux data (rate of lipolysis and FFA turnover using stable isotope data; rate of fatty acid oxidation), DNA methylation profile.
c. Statistical plan including sample size justification and interim data analysis.

Comparisons between CPAP, [NDS + propranolol], and [NDS + Placebo]: Primary outcome is comparison of metabolism and blood pressure for [NDS + propranolol] versus [NDS + placebo]. For metabolic parameters that are repeatedly measured during sleep (e.g. FFA, glucose) we will use mixed linear model analysis with experimental group as a fixed factor and subject ID as a random factor. Sleep physiology that is measured on a continuous basis (e.g. heart rate, AHI, SpO2) will be condensed into 30 minute bins to create a parallel data structure with metabolic parameters. We will then examine whether these sleep physiological metrics are dynamically associated with glucose/FFA level. For those parameters that are measured once per visit (e.g. RHI, lipid panel), we will compare using paired T-tests for within-subject comparisons between nights. A secondary outcome is whether CPAP and [NDS + propranolol] are similar, and this will be performed using a similar pairwise comparison. In addition, one way ANOVA will be used to ascertain whether there is a detectable difference across all three groups. Multiple regression will be used to examine associations between FFA, glucose, CPAP status, and drug adjusting for potential confounders such as diabetes, BMI, body fat (DEXA) and age. As an exploratory analysis, Pearson correlation matrices will be used to probe the interrelatedness of glucose, FFA, insulin, lipids, and sleep outcomes, followed by targeted multivariable regression. We plan an analysis of each subject's pulse rate and blood pressure after their PSG to determine whether beta blocker dosage or timing requires adjustment, which will expedite the ability to tailor the therapy if necessary.

An exploratory aim of this study is to examine effects of CPAP withdrawal on peripheral blood mononuclear cell (PBMC) gene expression. We will utilize real-time polymerase chain reaction (RT-PCR) arrays (Qiagen MAP Kinase RT2 Profiler and Human Toll-Like Receptor (TLR) Signaling Pathway RT2 Profiler PCR Arrays, Qiagen, Valencia, CA) to characterize gene changes that result from nocturnal hypoxemia.

Sample size: In our preliminary data from 3 patients who have completed their study visits, FFA levels increased with OSA (CPAP withdrawal), and propranolol abolished this response. The mean nocturnal FFA level on CPAP, [OSA + placebo], and [OSA + propranolol] nights was 0.413±0.107, 0.447±0.084 and 0.313±0.067 mmol/L respectively. We base our sample size on the ability to detect an effect of propranolol on FFA levels during sleep. The mean difference of FFA between [OSA + placebo] and [OSA + propranolol] nights was 0.133 ± 0.076 mmol/L (mean ± standard deviation). This yields a large effect size (Cohen's *d* = 1.673). However, we anticipate a smaller effect size with ongoing enrollment. In addition, our pilot data consisted only of men, and we do not have data from which to estimate an effect size for women. We estimate a more modest difference in means of 0.06. This yields a Cohen's *d* = 0.79. To detect a difference at 90% power at a significance level of 0.05 requires a sample size of 15 subjects. We will compare both sexes, so we will target an enrollment of 30 subjects. To account for 20% data loss from technical problems (IV failure, hemolysis, poor sleep efficiency) we will consent 36 subjects.

d. Early stopping rules.

At any point in the study, the subject may decline to participate. Indications for stopping are the development of severe adverse events. The IRB will be informed of any adverse events. Any serious adverse events will result in study discontinuation.

If propranolol has not yet been administered, and systolic BP <90 or HR <50 the study will continue without giving propranolol. The P.I. should be notified of this occurrence. If propranolol has been administered, and systolic BP <90 or HR<50 the study will be stopped early. The subject will be awoken from sleep to assess symptoms, hemodynamics, and orthostasis. The P.I. is to be notified for severe nocturnal hypoxemia with SpO2 consistently <85% for >5 min, or unstable cardiac arrhythmia on EKG during sleep.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

- Withdrawal of CPAP: During abstinence from CPAP over 3 days, subjects may experience relatively unrefreshing sleep. During the night, they may experience arousals from sleep, morning headache. During the daytime, they may experience subjective and/or objective daytime sleepiness. The risk of sleepiness contributing to an accident such as work-related injury or motor vehicle collision is also increased. Chronically, OSA is associated with increased risks of hypertension, diabetes, stroke, and ischemic heart disease. In a recent study on the effects of 2-weeks of CPAP withdrawal, there were modest and gradual increases in daytime blood pressure and heart rate [35, 36]. Risks of stopping CPAP up to 2 weeks is therefore a low-risk, high yield research method. In this study, withholding CPAP for a few days is associated with even fewer risks.

- Propranolol: The main risk of propranolol is an overdose leading to bradycardia and hypotension, but not this is not anticipated at this low clinical starting dose. Common side effects are dizziness, fatigue, constipation. In the aforementioned BHAT trial [45] in which 1,915 persons received propranolol and 1,921 placebo, the following adverse effects occurred that were statistically significant between propranolol and placebo ($p<0.05$) resulting in study medical withdrawal: hypotension (1.2% versus 0.3%); reduced sexual activity (2% vs.0%). In terms of patient complaints, those effects that were statistically significant ($p<0.05$) between propranolol and placebo were: bronchospasm (31% vs. 27%), cold hands or feet (10% vs. 7.7%), tiredness (66.8% vs. 62.1%), diarrhea (5.5% vs. 3.8%). Notably this was a 25-month study and our study only administers a single dose of drug. Serious but rare hypersensitivity reactions have been reported including anaphylaxis and cutaneous reactions such as Stevens-Johnson syndrome, toxic epidermal necrolysis, dry eyes, exfoliative dermatitis, erythema

multiforme, urticaria, alopecia, SLE-like reactions, and psoriaform rashes[41]. Beta blockers, as a class, can cause bronchospasm in those with significant underlying asthma.

- Isotope infusion and ingestion: Stable isotopes are naturally occurring, non-radioactive compounds normally present in the body at low levels. Infusion or ingestion of isotopes at very low tracer doses transiently increases the level of these molecules by <10% and is not associated with any adverse effects. As is the case with any infusion, there is a small chance of infection.

- DEXA scan: DEXA scans are considered safe and deliver minimal radiation.

- Venipuncture: May be associated with discomfort and bruising.

  Phlebotomy: In total, the blood collected will be approximately 116 cc (as compared to approximately 350 mL during a donation). Theoretically, if too much blood is lost, this could lead to low blood pressure and cardiovascular stress or dizziness.

- Nasal dilator strips: These are non-latex, adhesive tape applied to the nose designed to mitigate snoring and nasal congestion. There may be discomfort using NDS and they are likely to be inferior to CPAP for treating OSA.

- Confidentiality of genetic material: There is the risk that genetic data is revealed outside the study. Information can also lead to unwanted psychological or financial consequences if it were released to a third party.

b. Steps taken to minimize the risks.

- Withdrawal of CPAP: The period of withdrawal is kept relatively short, at 3 nights. Subjects will be advised to arrange transportation home so that they do not need to drive. They will be advised to increase their total sleep time, if necessary, during CPAP abstinence to minimize sleep deprivation off CPAP. On the day of the non-CPAP sleep study, if they plan to drive home themselves, they will be provided with an additional 3-4 hours of time in the lab to sleep before discharge. Patients at increased cardiovascular risk (uncontrolled hypertension, heart failure) are excluded. Participants with high-risk occupations requiring vigilance, such as commercial drivers and pilots, will be excluded. Participants who have ever been in a near-miss or motor vehicle accident due to sleepiness will be excluded. When communicating with patients about their off-CPAP visits, patients will be asked in the 1-2 days before their visit if they have experienced any near-miss events or motor vehicle accidents. If they have, then they will be excluded from the study, advised to resume CPAP immediately, and to avoid driving or other high risk activities until they have used CPAP for 3 nights.

- Propranolol: Risks are minimized by providing only a single dose of drug at a low clinical dose. We will exclude patients with contraindications to the drug or those who are taking medications that affect (or are affected by) propranolol metabolism. We will exclude elderly (>65 years old) in whom propranolol LA has not been well studied. We will monitor blood pressure and heart rate during the study. We perform a screening EKG to exclude those with underlying conduction or rhythm disorders. The EKG will be reviewed by Dr. Jonathan Jun, Luu Pham, or Vsevolod Polotsky. All physicians are board certified in pulmonary and critical care medicine. If a conduction or rhythm abnormality is detected (see exclusion criteria) the participant will be informed of the finding. With the participant's consent, we will also provide a copy of the EKG to the patient, the patient's primary care provider or cardiologist, and provide them with contact information for the Johns Hopkins cardiology clinic for further evaluation. Any unstable arrhythmia such as sustained wide complex tachycardia, atrial fibrillation with rapid ventricular response, or complete heart block will be dealt with as a medical emergency with transfer to the emergency room. In terms of bronchospasm, patients with a history of asthma (no worse than mild intermittent as per NHLBI 2007 guidelines) including those on regular controller medications will be excluded.

- Isotope infusion and ingestion: To mitigate any chance of infection from infused isotopes, they will be compounded in a sterile facility by a registered pharmacist. The infusion is also filtered through a 22 micron (Millipore) filter during infusion. The patient will be monitored for any infection or allergic reaction to the infusion, and the infusion stopped in the event of the development of symptoms such as urticaria, flushing, nausea, fever chills.

- Venipuncture: Discomfort, bruise formation, and infection will be minimized by use of sterile supplies and aseptic technique by trained personnel.

- Phlebotomy: The volume of each blood draw is minimized to balance safety with the need to obtain a useful amount of plasma after centrifugation. The risk of inducing hemodynamic or cardiovascular stress is minimized by excluding those with significant baseline anemia (hemoglobin <10 mg/dl). Those found to have anemia will be informed of the results and advised to seek medical evaluation as appriopriate.

- DEXA scan: DEXA uses a beam of very low radiation, delivering <10% radiation as during a clinical chest X-ray. Each participant will only require 1 DEXA scan.

- NDS: This is only being used for a few nights as a CPAP placebo.

- Confidentiality of genetic material: Blood is de-identified using the study IDs. When DNA is purified, a second number will be assigned to the DNA material. We will maintain a separate "key" linking this DNA sample to the study ID. This "key" will be stored on an encrypted server, and access to this server will be limited to only certain members of the study team. Participants may opt out from the genetic analysis.

c. Plan for reporting unanticipated problems or study deviations.

The plan for collection, description, monitoring and analysis of adverse events is presented in accordance with guidelines for adverse event reporting to the IRB. We will use the following definitions and grading scales for monitoring purposes:

Definition of adverse event (AE): any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure

Definition of serious adverse event (SAE): any event that is fatal or life-threatening, that is permanently disabling, requires or extends hospitalization of the subject, represents a significant overdose or breach of protocol, suggests that a drug, device, or procedure used in a research protocol has produced a congenital anomaly or cancer, or in the opinion of the investigator, represents other significant hazards or potentially serious harm to the research subject or others

Adverse events will be graded as (a) mild (adverse event of little clinical significance), (b) moderate (adverse event between mild and severe – causing some limitation of usual activities), or (c) severe (an event that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, represents a significant overdose or breach of protocol, results in congenital anomalies/birth defects or produces cancer, or in the opinion of the investigator, represents other significant hazards or potentially serious harm to the research subject or others), and their attribution will be classified as (a) not related (clearly not related), (b) possible (may be related), (c) probable (likely related), (d) definite (clearly related) or (e) unable to assess.

Study participants will be monitored throughout the study for adverse events, both anticipated and unexpected. The Principal Investigator will serve as the monitor. Adverse events will be reviewed by the Principal Investigator, managed according to standard clinical practice, and classified for severity and

attribution. Serious adverse events or unexpected adverse events will be recorded on the appropriate IRB form and reported to the IRB and RSA as required. All adverse events will be tracked to resolution.

The principal investigator will provide an interim report of all adverse events to the IRB at the time of continuing review. In addition, the Principal Investigator will provide the RSA a tabulation of the number of subjects enrolled, number of specific adverse events and a summary of the study at regular intervals. The RSA in collaboration will monitor the frequency of adverse events and serious adverse events

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Breach of confidentiality would result in unauthorized individuals having access to information about the participant's medical history. To prevent unauthorized access, (1) all files related to study subjects will be locked in file cabinets; (2) digital files will be restricted to study investigators and associated staff, on a need-to-know basis. (3) Subject data will be stored with unique identifiers and will be password protected. Encryption algorithms that can only be reversed with password access will be implemented. All computers will require log on passwords.

e. Financial risks to the participants.

Study-related assessments will not be charged to the subject. Parking fees will be paid. Subjects will be responsible for any treatment of conditions that are uncovered as part of the study evaluations or if they are injured as a result of being in the study.

9. Benefits

a. Description of the probable benefits for the participant and for society.

There are no direct benefits to participants for being in this study. This study will help researchers learn whether propranolol attenuates glucose or FFA elevations caused by OSA, which will demonstrate the potential mechanism by which OSA may foster diabetes and cardiovascular disease.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will be paid up to $1000 for completing all parts of this study, based on the following procedures:

- $250 for each sleep study (3 visits) = $750
- $25 for each EndoPAT test (done after each of the 3 visits) = $75
- $50 for a DEXA scan (only done once)
- $125 study completion bonus for participants who are able to keep their appointments as originally scheduled. They will forfeit this bonus if they must reschedule their visit(s).
- They will also receive parking reimbursement so there is no fee to come to our laboratory
- NOTE: If a participant has completed parts of the study before this change in research is approved, we will still offer them the increased payment system above.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

Participants will not be billed for study procedures. Polysomnograms and blood tests will be billed to the study at standard CRU rates.

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