Metabolic Impact of Intermittent CPAP

Consent Form

Johns Hopkins IRB # NA_00086830

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Johns Hopkins Medicine - eForm A

- **Use the section headings to write the eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
- **When submitting eForm A (new or revised), enter the date submitted to the field at the top of eForm A.**

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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) causes repetitive airway closure during sleep, and is associated with metabolic and cardiovascular dysfunction. The manner in which OSA affects metabolism during sleep is not well studied, but could reveal mechanisms by which OSA promotes cardiovascular diseases. We have previously shown that OSA increases free fatty acids (FFA) in subjects with congestive heart failure (CHF)[1]. Excessive plasma FFA, even when transiently elevated, can induce metabolic and vascular dysfunction[2]. Hence, nocturnal FFA elevations could be responsible for the cardio-metabolic consequences of OSA.

In this study, we will examine if OSA increases FFA in healthy (non-CHF) subjects, and the effects of treating OSA with continuous positive airway pressure (CPAP). We will also determine whether changes in nocturnal FFA caused by OSA are associated with adverse vascular endothelial function and altered glucose metabolism. This pilot study is needed before determining whether FFA levels in OSA constitute a biomarker of cardio-metabolic risk.

2. Objectives (include all primary and secondary objectives)

Specific Aim 1: To examine whether OSA, elicited by CPAP withdrawal, causes lipolysis and cardio-metabolic dysfunction.
We hypothesize that OSA activates lipolysis during sleep, resulting in high levels of plasma glycerol and FFA, which are lowered by CPAP. We also hypothesize that CPAP withdrawal will impair glucose tolerance and endothelial function, and increase glucose production during sleep.

Specific Aim 2: To examine factors associated with lipolysis during sleep.
We hypothesize that patient specific and sleep-specific factors influence nocturnal lipolysis. Patient factors may include age, sex, or body mass index (BMI). Sleep-specific factors may include OSA severity and/or OSA-induced sympathetic activation, elapsed time, or sleep stage. Additional outcomes of interest are effects of OSA, CPAP on circulating genetic/epigenetic changes.

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)

OSA induces repetitive upper airway closure during sleep, and affects 24% of middle aged men and 9% of women in the United States[3]. Several studies suggest that OSA may induce cardio-metabolic complications such as insulin resistance, endothelial dysfunction, and dyslipidemia[4] culminating in cardiovascular disease and death[5]. Unfortunately, it remains unclear what mediates detrimental outcomes in OSA. We have shown that OSA causes rapid FFA elevations during sleep in heart failure subjects, which can be prevented by supplemental oxygen [1]. We speculate that stimulation of the sympathetic nervous system by OSA and intermittent hypoxia cause lipolysis, leading to excessive FFA mobilization from adipose tissue.

It is not known whether OSA causes surges of FFA in non-CHF patients, and whether CPAP can abolish the increase. It is also unclear which patient-related or sleep apnea features predict nocturnal FFA elevations, and

whether FFA elevations from OSA are associated acutely with detrimental physiologic changes. In subjects with a history of OSA, we will examine nocturnal FFA in the presence or absence of therapeutic CPAP. We hypothesize that CPAP withdrawal leads to increases in nocturnal FFA. We will also determine whether morning endothelial function and glucose tolerance correlate with nocturnal FFA changes.

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.

Experience with Procedures:
Members of the study team are experienced with sleep research and metabolism. This study is similar in design to a previous study (Western IRB #1040564) utilizing frequent blood sampling during sleep in patients with sleep apnea.

4. Study Procedures

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

Sequence and Timing of visits: Each subject will undergo up 2 visits to the lab. One sleep study will be conducted while wearing CPAP for the entire night. The second sleep study will be conducted using nasal dilator strips (NDS) as a placebo, instead of wearing CPAP, the entire night. NDS have been used in other studies as a CPAP placebo and have no impact on OSA severity [6]. The studies will be performed in random order, occurring >1 week apart and no longer than 3 months apart. To maximize chances of eliciting OSA during the non-CPAP study night, patients will be asked to refrain from CPAP use, for two nights preceding their non-CPAP study night. They will be provided with ClearPassage® NDS to use at home during CPAP withdrawal nights. This will ensure CPAP "washout" since therapeutic carryover effects of CPAP have been observed in some studies[7]. Patients who were previously enrolled in the single-night study (within-night CPAP withdrawal) will be invited to return for these additional sleep studies.

Study eligibility: To be enrolled in the study, participants will need to undergo a screening questionnaire and meet inclusion/exclusion criteria. They will need to provide written consent for participation. To avoid issues of CPAP intolerance, participants must also be accustomed to using CPAP, and report that they are capable of sleeping with CPAP on.

As of March 16, 2020 (or approval of this eFormA update), and in order to mitigate risks related to COVID-19 exposure to participants and study team, phone screening procedures will be completed from the study team members' home using a connecting service, such as Google Voice or Skype Voice-to-IP to protect study team members' personal contact information while allowing them to continue job functions from a safe location. Participants will be notified of the change in procedure.

Consent: In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants' risks. 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.

Covid-19 screening: Participants will be asked screening questions on the phone for Covid-19 symptoms and risk exposure at the time they are given an appointment and 24-48 hours prior to each study visit. If they screen positive, they will not be eligible to participate until they are deemed safe to return to work by CDC criteria.

Covid-19 testing: Prior to each sleep study at the CRU, Covid-19 testing will be performed within 72 hours of the admission. A negative result will be required for the participation. If the participants claim to have been tested, they will be asked to provide a written proof of such test with a negative result within 72 hours of the admission.

Standard Precautions to be followed by subjects and staff to minimize the risk of coronavirus transmission:
Subjects will be instructed to follow the visitor policy during each study visit. They will be given an Information Sheet about COVID-19 Related Risks through email prior to the visit. They will be instructed to come alone to the CRU, wear a face mask upon entry to the building, practice social distancing and hand hygiene. Study team will minimize face-to-face contact with the participants using all remote means possible during study visits. They will adhere to 6-foot distance between participants and staff or between staff and other staff whenever feasible. Staff will be trained to use a surgical or procedure mask with a face shield PPE at a minimum for all participant interaction. During a CPAP sleep study, Airborne and Contact Precautions with Eye Protection PPE will be used (isolation gown, double exam gloves, N95/Drager respirator with a face shield). The door of the patient room will be kept closed whenever possible during a CPAP sleep study.

Pre-sleep procedures. Subjects will undergo a brief history and physical examination. They will report to the clinical research unit and undergo anthropometric assessment including weight, height, neck, and waist circumference. 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. At approximately 17:30, 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 IV's will be placed for blood sampling during sleep, with one as a backup in case of failure of one IV. IV tubing will be extended to an adjacent control room so that blood samples can be obtained without disturbing the subject.
PSG setup. On each study night from 22:40 until 06:40, 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 CO2 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.

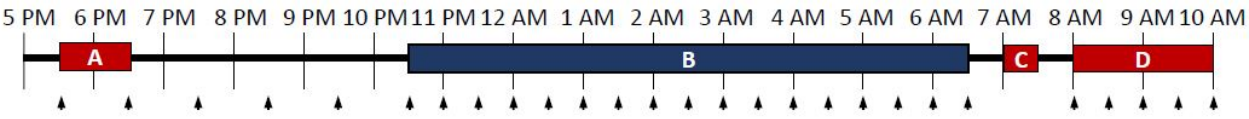
During the CPAP night, patients will sleep using CPAP set to their prescribed home CPAP settings. Airflow will be recorded with a pneumotachograph in the CPAP circuit. CPAP pressure will be titrated if necessary to prevent

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.

Blood work and urine collection
All samples will be obtained via the indwelling IV, without awakening the subject. Blood samples will be drawn at 30 to 60 minute intervals beginning at 5:30 PM and continuing until the next morning at 6:30 AM as shown in the figure below. At 10:30 PM, when the sleep study starts, blood is sampled at 30 minute intervals in order to capture dynamic changes in FFA metabolism during sleep. 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. If necessary, based on nursing schedules, the timing of sampling and sleep procedures can be shifted up to 1 hour later or earlier.

Following centrifugation, plasma will be transferred to cryovials for storage. The final morning sample at time of awakening (6:30 AM) will be 12 cc (3 tubes) since we plan additional assays with this sample (lipid panel, inflammatory markers) and 4 cc will be retained as whole blood for future potential analysis. 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 since this volume is required for isolation of peripheral mononuclear cells. In total, the blood collected will be approximately 174 cc. For patients that consent to isolation of genetic material, the total will be 190 cc. This quantity is much less than during a blood donation. Samples will be frozen at -80°C for research pertaining to metabolic effects of OSA, with the consent of participants. A morning urine sample will be collected for measurement of catecholamines.

Morning procedures:
The PSG montage will be disconnected. One IV is removed, leaving the other IV in place for use during later glucose tolerance test. Then peripheral artery tonometry, DEXA scanning, and OGTT will be performed as follows:



Each study night, participants will undergo the following procedures:
A. Research diet and IV access
B. Sleep study, (CPAP, OSA or Supplemental oxygen)
C. EndoPAT
D. OGTT (all visits) and DEXA (1 visit)
Blood is sampled from the IV at time points indicated by the arrowheads.

Peripheral arterial tonometry (EndoPAT). The subject will be 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 reactive hyperemia index (RHI) and the augmentation index (AI) will be derived by software and used as metrics of endothelial function and arterial stiffness respectively.

Oral glucose tolerance test (OGTT). From the indwelling antecubital IV, baseline insulin (gold top tube, 4 cc) and glucose (grey top tube, 4 cc) 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 at 30, 60, 90, and 120 minutes after drinking the glucose solution. They will be asked, if possible, to remain awake during the OGTT.

Dual-energy X-ray absorptiometry (DEXA). After the baseline glucose level for the OGTT has been drawn and the patient has consumed the glucose solution, they will undergo a DEXA scan. The DEXA scan will be used to estimate fat mass, which will be one of the variables analyzed as a predictor of FFA elevation.

Subjects may eat breakfast after the OGTT testing is completed

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 testing:
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

If the initial sleep study meets 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 initial study.

Scoring. Sleep will be scored according to Rechschaffen and Kales criteria. 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.

Data Analysis and Anticipated Outcomes:

The primary outcomes is nocturnal plasma FFA levels. The main independent variable is OSA versus CPAP exposure during sleep. Since 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. 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. As a secondary analysis, we will also examine whether there are patient-specific ("static") determinants of FFA level during sleep such as age, sex, or body mass index and whether these variables interact with sleep ("dynamic") variables such as OSA severity. We hypothesize that OSA exposure increases FFA, compared to CPAP. We will then examine whether sleep physiology (e.g. AHI, arousals, heart rate) is dynamically associated with FFA, and whether urine catecholamine levels are associated with increases in FFA. For variables measured once (RHI, morning lipid profile) or averaged (e.g. nocturnal FFA 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. Data analysis will be performed with a biostatistician and described under the "Study Statistics" section.

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

Each study subject will make two overnight visits to the sleep lab. Subjects will be screened with an initial telephone interview. During this screening, only information necessary to assess eligibility for the study will be collected, or information voluntarily provided by the patient to initiate or maintain correspondence. Subjects without any of the self-reported exclusionary criteria will be scheduled for a face-to-face visit for further screening. At this visit, eligibility for

participation in the study protocols will be confirmed. Urine screening for pregnancy in women of child-bearing age will also be performed.

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

To prevent CPAP "carry-over" effects, preceding the non-CPAP night, participants will be requested to abstain from CPAP use for 2 nights, if possible. If they have historically been unable to discontinue CPAP for this length of time, they may continue CPAP until the in-lab study. For CPAP withdrawal nights, NDS will be used as a placebo. This will control for unintended effects of stopping CPAP such as anxiety before sleep. Lab assays will be performed by a technician blinded to the CPAP status of participants.

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

This study is being conducted to examine the effects of OSA and its treatment on lipid metabolism. The rapid kinetics of FFA turnover requires careful attention to dynamic changes in plasma FFA during sleep with or without CPAP. Studying patients with known OSA, adherent to CPAP therapy, has several advantages. First, CPAP-naïve patients may not tolerate CPAP, and experience paradoxically increased sleep loss, stress, and sympathetic stimulation of lipolysis. Second, we will greatly reduce time and resource costs needed to identify undiagnosed subjects with OSA. Third, CPAP withdrawal has been utilized in other studies, yielding highly reproducible physiological effects of OSA[8]. Finally, intermittent/inconsistent CPAP use is common in clinical practice, and the metabolic consequences are not known. After CPAP withdrawal (total of 3 nights), patients may resume their routine CPAP use.

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.

f. Definition of treatment failure or participant removal criteria.

If participants cannot tolerate CPAP off, they will be removed from the study.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.
Following the non-CPAP night, subjects may resume routine CPAP use.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:
We will include adults of all races ≥ 20 and ≤ 75 years old, with a history of mild to severe OSA (AHI or RDI> 10). They must also own a CPAP machine and report the ability to tolerate sleeping with or without CPAP during the night.

Exclusion Criteria:

1. Uncontrolled hypertension with systolic blood pressure >170 or diastolic blood pressure > 110
2. Congestive heart failure
3. Use of, clonidine, or nicotinic acid medication
4. Diabetes requiring the use of insulin
5. Pregnancy, by urine testing in women of child-bearing age

6. Patients with a prior history of falling asleep while driving, involved in a near-miss or motor vehicle accident, or and those in high-risk occupations such as commercial drivers or pilots.
7. Hemoglobin < 10 g/dL on point of care screening.

6. Drugs/ Substances/ Devices

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

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[6]. By including NDS as a placebo, we will avoid potential confounding such as anxiety caused by stopping CPAP.

b. Justification and safety information if non-FDA approved drugs without an IND will be administered. N/A

Study Statistics

The overall analysis plan has been presented in section 4, "Data Analysis and Anticipated Outcomes". In this section we briefly address the statistical methods to be used for analysis separated by specific aim:

Specific Aim 1: To examine whether OSA, elicited by CPAP withdrawal, causes lipolysis and cardio-metabolic dysfunction.

Primary outcome: FFA level, comparing between CPAP and OSA nights, analyzed using linear mixed effect models. Independent variable: CPAP/OSA exposure.

Specific Aim 2: To examine factors associated with lipolysis during sleep.

Primary outcome: FFA level

Independent variables: OSA severity (AHI, arousal index, heart rate, urine catecholamines), Patient factors (age, sex, body mass index/fat distribution).

These analyses are primarily designed to understand the heterogeneity of lipolysis during CPAP withdrawal.

- *Sex-specific effects*: This will be assessed by (1) comparing mean change in FFA level during CPAP withdrawal between men and women (unpaired T-test); (2) modeling mean FFA level as a function of sex (as well as sex x BMI interactions) in multivariable regression. We hypothesize that men will exhibit greater OSA-induced FFA elevation than women, with or without adjustment for OSA severity and fat distribution.
- *Effects of OSA severity or sympathetic activation:* We will apply linear mixed models with FFA as the outcome, OSA severity or SNS variables (e.g. AHI, ODI, arousal index, HR, urine catecholamines) as fixed factors and subject ID as the random effects variable. Iterative time lags will be used to account for potential delayed effects of dynamic variables. We hypothesize that plasma FFA level will be independently associated with AHI, arousals from sleep, HR elevation, or urine catecholamine level.
- *Identification of robust "responders":* We will examine FFA elevation as a dichotomous outcome, defining "FFA responders" as those who exhibit ΔFFA$\geqslant$10% during CPAP withdrawal (subject to change depending on the distribution of responses; the 10% threshold is based on a median increase in nocturnal FFA of ~8.9% in our preliminary data). OSA severity, sleep architecture, sex, age, and fat mass will be compared between responders and non-responders.
- *Identification of potential "downstream" effects of FFA elevation.* We will test the association between nocturnal FFA profile and other cardiometabolic outcomes (e.g. morning OGTT, endothelial function). Multivariable regression will be used to adjust for potential confounders such as age, sex, and BMI.
- *Identification of potential circulating genetic/epigenetic changes.* 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: One of the stated goals of this study is to examine sex-specific lipolytic responses to CPAP withdrawal. We base our sample size on the ability to detect a difference in FFA elevation between men and women. In the 20 non-diabetics of our preliminary data, the average ΔFFA during CPAP withdrawal was 0.047 ± 0.04 mmol/l in men (n=13), and 0.021 ± 0.04 mmol/l in women (n=7). This yields a medium effect size (Cohen's d = 0.65). To detect a sex difference at 80% power at a significance level of 0.05, requires a sample size of 78 (39 men and 39 women). We anticipate potential technical problems (e.g. IV failures, hemolysis, and poor sleep efficiency) leading to loss of 15% data. Therefore, our target enrollment will be 92 subjects.

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

7. Risks

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

Venipuncture: May be associated with discomfort and bruising. Infection or irritation (phlebitis) can occur at the IV site.

Phlebotomy: In total, the blood collected will be approximately 174 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.

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, heart rate, and triglycerides[7]. Risks of stopping CPAP for 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.

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 inferior to CPAP for treating OSA.

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

Oral glucose tolerance test: There are no significant risks to this standard clinical test other than the associated phlebotomy.

EndoPAT: There are no significant risks to this test other than arm discomfort during the procedure.

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.

Veni-Puncture: 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 appropriate.

Withdrawal of CPAP. 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. 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.

DEXA scan: DEXA uses a beam of very low radiation, delivering <10% radiation as during a clinical chest X-ray.
NDS: This is only being used for a few nights as a CPAP placebo.

Oral glucose tolerance test: The phlebotomy associated with this procedure will be performed by experienced personnel.

EndoPAT: This procedure will be performed only by trained study team members or Core physiology lab members. Any complications will be evaluated by a physician. In the case of an adverse event, the subjects will be referred to the appropriate clinical specialist, either outpatient or inpatient for care. Adverse events related to the study procedures will be reported to the IRB.

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.

8. 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 OSA causes nocturnal FFA elevations and whether CPAP is effective in preventing the elevations.

9. 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 $600 for completing all parts of this study, based on the following procedures:

- $200 for each sleep study (2 visits)
- $25 for each oral glucose tolerance test and EndoPAT test (done after each of the 2 visits)
- $50 for a DEXA scan (only done once)
- $100 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.

10. 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

Reference List

1. Jun, J.C., et al., *Effects of sleep apnea on nocturnal free fatty acids in subjects with heart failure.* Sleep, 2011. **34**(9): p. 1207-1213.
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3. Young, T., et al., *The occurrence of sleep-disordered breathing among middle-aged adults.* N.Engl.J.Med., 1993. **328**(17): p. 1230-1235.
4. Jun, J. and V.Y. Polotsky, *Metabolic consequences of sleep-disordered breathing.* ILAR.J., 2009. **50**(3): p. 289-306.
5. Punjabi, N.M., et al., *Sleep-disordered breathing and mortality: a prospective cohort study.* PLoS.Med., 2009. **6**(8): p. e1000132.
6. Amaro, A.C., et al., *The use of nasal dilator strips as a placebo for trials evaluating continuous positive airway pressure.* Clinics (Sao Paulo), 2012. **67**(5): p. 469-74.
7. Rossi, V.A., et al., *Is continuous positive airway pressure necessarily an everyday therapy in patients with obstructive sleep apnoea?* Eur Respir J, 2014. **43**(5): p. 1387-93.
8. Kohler, M., et al., *Effects of continuous positive airway pressure therapy withdrawal in patients with obstructive sleep apnea: a randomized controlled trial.* Am J Respir Crit Care Med, 2011. **184**(10): p. 1192-9.