TITLE: **Targeted Treatment of Obstructive Sleep Apnea to Reduce Cardiovascular Disparity**

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PROTOCOL NO. 1IK2CX001026-01

SPONSOR: VA Clinical Science Research and Development

DATE OF VERSION: **April 8, 2019**

VERSION: 1.8

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PI SIGNATURE SHEET

I have read the attached protocol and agree that it contains all the necessary details for performing the study.

I will provide copies of the protocol and of the preclinical and clinical information on the test article to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the test article and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB)/ethics committee, I will not modify this protocol without obtaining the prior approval of the Sponsor and of the IRB/ethics committee. I will submit the protocol modifications and/or any informed consent modifications to the Sponsor and the IRB/ethics committee, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to it, the principles of Good Clinical Practice [GCP; current International Conference of Harmonization (ICH) guidelines], and the Declaration of Helsinki (1964) including all amendments up to and including the Scotland revision (2000) and notes of clarification added in 2002 and 2004.

PI's Signature Date

LIST OF ABBREVIATIONS

AA	African Americans
AASM	American Academy of Sleep Medicine
ABP	ambulatory 24- hour blood pressure
AE	adverse event
AHI	apnea-hypopnea index
AIMs	Ancestry Informative Markers
ANOVA	analysis of variance
BSQ	Berlin Sleep Questionnaire
CABP	central aortic blood pressure
CCI	Charlson Comorbidity Index
CFR	Code of Federal Regulations
AUTOPAP	Autoadjusting positive airway pressure
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CVD	cardiovascular disease
DCF	data clarification form
DDD	Defined Daily Dose
EDS	excessive daytime sleepiness
ESS	Epworth Sleepiness Scale
EA	European Americans
FDA	Food and Drug Administration
FOSQ	Functional Outcomes of Sleep Questionnaire
LC/MS	Liquid chromatography/mass spectrometry
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
ICF	informed consent form
ICH	International Conference of Harmonization
IRB	Institutional Review Board
OSA	obstructive sleep apnea
PAP	positive airway pressure
PHI	Protected Health Information
PSG	polysomnography
PVT	Psychomotor Vigilance Test
SAE	serious adverse event
SNA	sympathetic nervous system activity
STE	speckle-tracking echocardiography

PROTOCOL SYNOPSIS

Sponsor:	VA CSR and D
Title of Study:	Targeted Treatment of Obstructive Sleep Apnea to Reduce Cardiovascular Disparity
Number of Study Centers:	1 JBVAMC
Objectives:	**Primary Objectives:** 1. To assess the efficacy of –autoadjusting positive airway pressure (AUTOPAP) treatment in reducing 24-hour ambulatory blood pressure (ABP) in African American (AA) vs. - other (race) veterans with newly-diagnosed obstructive sleep apnea (OSA); 2. To assess the efficacy of –autoadjusting positive airway pressure (AUTOPAP) treatment in reducing central aortic blood pressure (CABP) in AA vs. other (race) veterans with newly-diagnosed obstructive sleep apnea (OSA); 3. To assess the efficacy of –autoadjusting positive airway pressure (AUTOPAP) treatment in reducing urinary biomarkers implicated in the pathogenesis of hypertension in OSA: 1) sympathetic nervous system activity (SNA; 24-hour urinary catecholamines) and 2) oxidative stress (overnight urinary 8-isoprostane) in AA vs. other (race) veterans with newly-diagnosed obstructive sleep apnea (OSA). 4. To test daytime dysfunction (excessive daytime sleepiness; EDS) as a moderator of reduction in ABP and CABP in AA and other (race) veterans following 3 months of AUTOPAP treatment. EDS will be measured objectively with psychomotor vigilance task (PVT) and subjectively with Epworth sleepiness scale (ESS). **Secondary Objectives:** 5. To examine the association of reduction in ABP and CABP with genetic ancestry (using ancestry informative markers; AIMs in DNA), as genetic admixture is expected among self-identified ethnic groups (i.e., AA and other (race) veterans). 6. To analyze abnormalities in myocardial mechanics on transthoracic echocardiography (TTE) in obstructive sleep apnea and its reversibility with AUTOPAP treatment. **Ancillary Objective:** 7. Tissue banking (whole blood processed for DNA, RNA, serum, and plasma) for future genomic studies.
Study Design:	This is an interventional parallel group (AA and other (race) veterans) efficacy study of AUTOPAP (standard first- line) treatment in 220 veterans with OSA. Patients will be enrolled consecutively to reach target enrollment of 110 in each group. All outcomes assessment will be done 3 months after AUTOPAP treatment initiation. **Recruitment Site:** Sleep, Tele-sleep (for Adam Benjamin Jr CBOC), Pulmonary, and Primary Care Clinics at JBVAMC. In addition, local IRB approved study flyers will be posted in sleep and pulmonary clinics at Hines and North Chicago VA. **Initial Screening Visit #1:** At the first clinic visit, each patient will be provided with oral and written information (informed consent form) describing the study and will

Study Design (continued):	have any questions answered. Patients will be specifically consented for blood draw for tissue banking and for TTE and patients will be able to participate in the primary study without participating in the secondary and ancillary measures (blood draw for genetic testing, tissue banking, and TTE). Consenting patients will undergo preliminary eligibility assessments, including Berlin Sleep Questionnaire (BSQ), Epworth Sleepiness Score (ESS), Functional Outcomes of Sleep Questionnaire (FOSQ), medical history and physical examination, and CABP measurements. All eligible patients will perform a 10-minute psychomotor vigilance task (PVT). Specifically, consenting patients will have venous blood sampling (35 mL) for processing (DNA, RNA, serum, and plasma) and storage at UI Biorepository as well as a TTE scheduled at JBVAMC. A urine pregnancy test will be done for women of childbearing potential. All enrolled patients will initiate 7-day actigraphy with concurrent sleep logs (to estimate average nightly sleep duration), 24-hour urine collection, and 24-hour ABP recordings at completion of visit 1 and will be scheduled for an overnight portable monitoring (PM) test at Jesse Brown VAMC (Visit 2) *or* at home. If the PM test fails to record data due to technical or application errors, the patients may be asked to repeat this test once. **Visit #2:** For patients performing **home PM**, visit 2 will be in the morning and PM device, actigraphy, sleep log, urine sample, and ABP monitor will be collected and processed/downloaded. Patients performing PM test at JBVA will arrive for the overnight PM. Actigraphy, sleep log, urine sample, and ABP monitor will be collected and processed/downloaded in the morning. All patients with apnea hypopnea index (AHI) ≥ 15/hour on the PM test will be invited to continue. AUTOPAPAUTOPAPAUTOPAPAUTOPAPRATIONALE ** We have eliminated polysomnography (VISIT 3) at Hines for two reasons: 1. We noted refusals to participate in this project in the past year (45/190 Veterans approached) due to burden of traveling to Hines. To improve recruitment for this study and to meet target enrollment (n = 220) over next two years, we have therefore eliminated the requirement for polysomnography. The PM test will be used to diagnose patients with moderate to severe OSA; as is done in clinical practice and many research studies. 2. The primary goal of performing polysomnography was to obtain gold-standard diagnostic and electroencephalography data from polysomnography. However, we have noted suboptimal data quality from the 16 polysomnographies completed at Hines thus far. Substantial scientific advantage is not conferred to this project and its objectives with these polysomnographies. **Visit #3:** Patients will return to the clinic for AUTOPAP treatment set-up and education. AUTOPAP treatment adherence will be optimized with follow-up telephone contacts as specified in full protocol below. **Visit #4:** Patients will return to clinic for repeat PVT, FOSQ, ESS, CABP measurements and to initiate 24-hour ABP and urine collection. Patients will also be scheduled for repeat TTE and blood draw for tissue banking **Final Visit # 5:** Patients will return to clinic in the morning after initiating 24-hour ABP and urine collection, which will be collected and processed/downloaded. Patients

	will be given instructions regarding follow-up care for OSA with sleep medicine or primary care provider.
Duration of Patient Participation:	**Visits 1- 3** over 7-10 days **Visits 4-5** over 90-100 days
Study Duration:	**Total duration:** 100-110 days (4 months)
Number of Patients to initiate AUTOPAP Treatment:	220 Our sample calculations and current dropout rate 2/16 (12.5%, lower than the anticipated 20%) suggest n = 220 will provide 80% power to detect between group difference at two-sided alpha of 0.05.
Eligibility Criteria:	**Inclusion Criteria:** 1. Self-identified African American and Other Veterans 2. Age: 18-70 years 3. Hypertension 4. Apnea hypopnea index (AHI) ≥15/hour on portable monitoring (PM) RATIONALE ** We have modified inclusion criteria from self-identified African American and European American Veterans to African American and "Other" for the following reasons: • Our primary goal is to identify treatment response in AA compared to other race (Hypothesis: response of hypertension to AUTOPAP treatment will be higher in AA than other groups). • To improve recruitment for this study and meet target enrollment (n = 220) over next two years. **Exclusion criteria:** 1. Past/current treatment of OSA or other primary sleep disorders 2. Active uncontrolled medical or psychiatric conditions 3. Shift work in preceding 6 months 4. Current illegal drug or daily alcohol use 5. Pregnancy
Treatment Effectiveness Endpoints:	1. Change in 24-hour ABP measures from baseline to after 3 months of AUTOPAP treatment. 2. Change in CABP measures from baseline to after 3 months of AUTOPAP treatment. 3. Change in 24-hour urinary catecholamine's and 8-isoprostane measures from baseline to after 3 months of AUTOPAP treatment. 4. RATIONALE ** We have changed CPAP to AUTOPAP treatment as this treatment can be instituted after PM testing and eliminates the need for polysomnography guided CPAP titration. Moreover, AUTOPAP has been shown to equivalent in efficacy to CPAP in terms of symptom resolution and systemic blood pressure response.
Sleepiness as Moderators of Effectiveness:	5. Test association of baseline ESS and PVT with effectiveness endpoints 1 to 3. 6. Test association of change in ESS and PVT from baseline to visit 5 with effectiveness endpoints 1 to 3.

Exploratory Endpoints:	7. Test association of genetic ancestry with endpoints 1 to 3. 8. Test association of myocardial mechanical data from TTE with AHI (OSA severity). 9. Generate effect size estimates for change in myocardial mechanics with 3 months of AUTOPAP treatment.

1. INTRODUCTION

Obstructive sleep apnea (OSA) increases the risk of hypertension, cardiovascular disease (CVD), and premature death. African Americans (AA) are at higher risk of OSA compared to European Americans (EA) due to differences in craniofacial morphology and other genetic factors independent of obesity. This is particularly important as poorly controlled hypertension and CVD are more prevalent among AA than the general population. Continuous positive airway pressure (AUTOPAP) treatment of OSA reduces ambulatory 24-hour arterial blood pressure (ABP) and central aortic blood pressure (CABP; measured non-invasively by brachial artery/upper arm cuff). Despite this, the impact of AUTOPAP treatment on control of hypertension and biological factors that determine this response among AA remain understudied. This lack of knowledge poses a serious problem as it hinders the implementation of targeted treatment of OSA and attendant cardiovascular morbidity in this high-risk population.

Hypertension response to AUTOPAP treatment is moderated by age, level of obesity (body mass index; BMI), severity of hypertension (baseline ABP), severity of OSA (apnea hypopnea index; AHI), and objective adherence to AUTOPAP. Our retrospective study of Veterans with hypertension and OSA demonstrates that AUTOPAP treatment reduces blood pressure and this effect is larger in AA compared to EA, controlling for the known moderators of treatment-response. Our central hypothesis is that AA with OSA experience greater improvement in hypertension with AUTOPAP treatment compared to EA. The specific scientific objective is to examine key biological and clinical factors underlying hypertension response to AUTOPAP treatment in self-identified AA vs. Veterans of another race.

2. STUDY OBJECTIVES

2.1 Primary Objectives

The primary goal of this study is to quantify and compare change in blood pressure (ABP and CABP) following 3 months of AUTOPAP treatment in Veterans with hypertension and newly-diagnosed OSA. We will examine changes in ABP and CABP in the context of key biomarkers implicated in the pathogenesis of hypertension in OSA: 1) sympathetic nervous system activity (SNA; 24-hour urinary catecholamines) and 2) cumulative oxidative stress (overnight urinary 8-isoprostane). ABP, CABP, and the biomarkers will be measured pre-treatment and after 3 months of AUTOPAP treatment.

2.2 Secondary Objectives

Studies in EA suggest the presence of excessive daytime sleepiness (EDS; symptom of OSA) is associated with a more robust hypertension response to AUTOPAP treatment. Our preliminary data indicate a higher prevalence of objective EDS in AA with OSA compared to EA after adjusting for socio-demographic factors. Thus, we will examine EDS as a moderator of hypertension response to AUTOPAP treatment and identify EDS by the Psychomotor Vigilance Test; PVT (higher number of lapses per trial is objective EDS) and ESS questionnaire.

There is significant genetic admixture among self-identified ethnic groups which introduces biological heterogeneity and potential confounding. We will identify genetic ancestry by known and specific DNA sequence variation(s), called Ancestry Informative Markers (AIMs) and explore the role of genetic (i.e. biologic) ancestry as a moderator of ABP and CABP outcomes.

The effects of OSA on the heart muscle (myocardium) have not been well characterized. The repetitive apnea and/or hypopnea that occur during sleep in OSA can result in a mismatch of myocardial oxygen supply and demand. However, the left ventricular dysfunction seen in OSA is not typically reflected in the left ventricular ejection fraction which is usually normal. Subclinical left ventricular dysfunction has been observed in OSA manifesting as changes in both systolic and diastolic dysfunction in the presence of normal overall ejection fraction. Cardiac ventricular function is most commonly evaluated by echocardiography, but this is limited by its inability to easily quantify global and regional changes in myocardial tissue lengthening or shortening (i.e. contraction or relaxation; also known as myocardial deformation). More recently, an echocardiographic imaging technique named speckle-tracking echocardiography (STE) has been developed that allows for quantification of myocardial deformation in any plane, thus has the advantage of being 'angle independent'. STE can be performed on any standard two-dimensional echocardiographic images (does not require special equipment to obtain the images). Multiple studies in various cardiac disease states have demonstrated the ability of STE to quantify and detect subtle preclinical changes in myocardial deformation. The effects of OSA on myocardial deformation by STE have not been well-characterized and its relationship to the severity of OSA has been inconsistent. Additionally, limited data suggests that acute treatment with continuous positive airway pressure may improve myocardial mechanics. The current study will evaluate the effects of the severity and treatment of OSA on subclinical cardiac function by STE.

2.3 Ancillary Objective

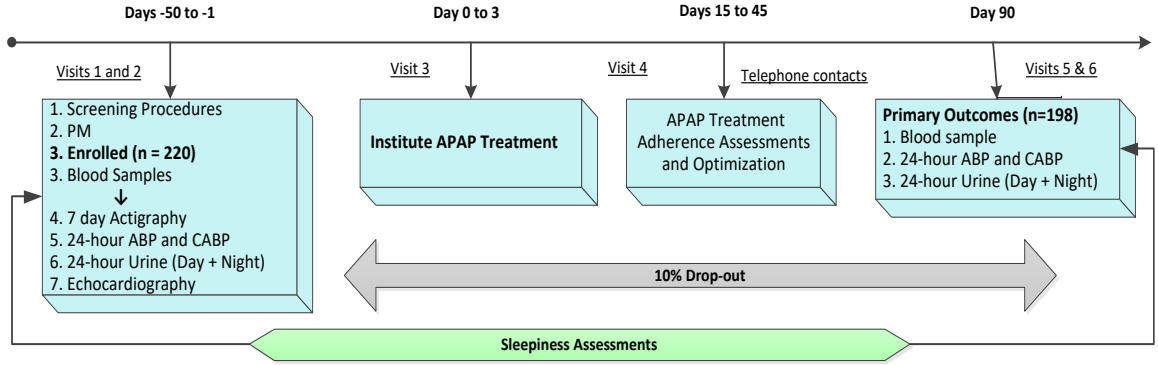
We will perform tissue banking of DNA, RNA, serum, and plasma samples derived from venous blood sampling at UI Biorepository for future genomic studies. As we are interested in examining AUTOPAP treatment related with genomic studies, we will repeat blood sample collection once at end of treatment period (visit5).

3. STUDY DESIGN

This is a parallel groups interventional study consisting of 4 main sections: 1. Screening for OSA and enrollment of moderate to severe OSA in AA and other Veterans-, 2. Pre-treatment measurement of outcomes (ABP, CABP, SNA, oxidative stress, TTE/STE) and postulated moderators (PVT lapses/ESS and genetic ancestry), 3. Institution of laboratory titrated AUTOPAP treatment with adherence optimization, and 4. Assessment of AUTOPAP treatment effects after 3 months on outcomes and EDS measures, namely PVT and ESS.

Figure. Research Protocol Timeline

Key: ABP = 24-hour ambulatory blood pressure, CABP = Central aortic blood pressure, APAP = Auto-adjusting positive airway pressure; STE = Speckle-tracking echocardiography; PM = portable monitoring



Potential study participants will be identified from sleep, tele-sleep, and pulmonary specialty clinics at JBVAMC (second floor Ogden Pavilion). Additionally, we will post study information flyers in Primary Care clinics (first floor Ogden Pavilion). All study related patient visits will be at JBVAMC. All study related patient visits during normal business/clinic hours will be in (rooms 2295, 2292, and 2291, Taylor Pavilion, JBVAMC). The overnight visits for testing (Visit 2) will be in Hospital, Damen Pavilion, 9 West (rooms Room 9508, 9511, 9514, 9515, and 9516). Visit 3 (polysomnography) will be at Hines VA hospital, Building 200, Room # 1421, 5000 S 5th Ave, Hines IL 60141.

Initial Screening Visit #1: All eligible patients identified from the clinics noted above will be provided with the study information sheet by the PI/Study coordinator. Each patient that verbally assents will be provided with oral and written information (informed consent form) describing the study and will have all questions answered. Patients will be specifically consented for blood draw for AIMs measurement, tissue banking, and echocardiography. Patients who want time to decide regarding participation in this study will contact the research coordinator by telephone. The potential participants will be screened with the use of a telephone screening script. *Patients will be able to participate in the primary study without participating in these measures (venous blood draw and echocardiography)*. Consenting patients will undergo preliminary eligibility assessments, including Berlin Sleep Questionnaire (BSQ), Epworth

Sleepiness Score (ESS) questionnaire, Functional Outcomes of Sleep Questionnaire (FOSQ), complete medical history and physical examination, and CABP measurements. Enrollment of patients who have severely elevated blood pressure (>200/120 mm Hg) will be deferred and they will be referred to their primary care provider/urgent care as clinically appropriate.

All antihypertensive medications will be recorded (number, dose, and refill information) for each patient at baseline and at the end of the study (final visit). This information will be confirmed with the JBVAMC/or outside pharmacy for accuracy. Patients will be encouraged to follow up with primary physician per usual schedule and to avoid taking over-the-counter medications that may affect blood pressure (e.g., some cold medications) for 24 hours before and during the ABP monitoring.

All eligible patients will perform a 10-minute psychomotor vigilance task (PVT). Specifically, consenting patients will have venous blood sampling (35 mL) for processing (DNA, RNA, serum, and plasma) and storage at UI Biorepository as well as an echocardiography scheduled at JBVAMC. A urine pregnancy test will be done for women of childbearing potential. All enrolled patients will initiate 7-day actigraphy with concurrent sleep logs (to estimate average nightly sleep duration), 24-hour urine collection, and 24-hour ABP recordings at completion of visit 1 and will be scheduled for an overnight portable monitoring (PM) test at Jesse Brown VAMC (Visit 2) or at home. If the PM test fails to record data due to technical or application errors, the patients may be asked to repeat this test once. We will schedule an echocardiography test at JBVAMC for consenting patients (to be completed before visit 4).

Each measure to be performed is briefly described below. All are validated and commonly used in clinical practice and research:

i. **PVT**: A 10-minute PVT192 test with a hand-held device for sustained attention will be conducted (Ambulatory Monitoring, Inc., Ardsley, NY), with a preceding 1-minute practice exercise. Participants will be using the hand held device and respond to the stimuli presented on the screen at random intervals by pressing a button. The PVT indicator of EDS will be frequency of lapses (instances where the patient fails to respond to the stimulus in a timely manner; Reaction Time, RT > 500 milliseconds).

ii. **ESS** is a self-administered questionnaire used to estimate sleepiness for eight different real-life situations. A score of ≥ 11 is indicative of EDS, while 0-10 is defined as normal.

iii. **FOSQ10**: is a 10-item self-administered questionnaire to assess disease specific quality of life.

iv. **Actigraphy** will measure and account for daily sleep duration objectively with 7-day recordings (Actiwatch 2Ò, MiniMitter, Philips Respironics, Andover, MA) in conjunction with a *sleep log*, a subjective daily log of sleep-wake schedule used for final scoring of actigraphy. This is important as sleep duration will affect daytime sleepiness assessments.

v. **CABP** will be assessed using a brachial (upper arm) cuff-based FDA approved system; SphygomoCor XCEL (Atcor medical, Australia). This will be measured in a seated position after 5 minutes of rest at visits 1 and 5.

vi. **ABP** will be recorded with the 90217 Ultralite ambulatory blood pressure monitor (Spacelabs, Issaquah, WA). Blood pressure measurements will be taken every 20 minutes in the day and every 30 minutes at night (9 PM to 6 AM, to minimize sleep disruption) for a 24-hour period. Recordings will be considered valid when at least 70% of expected measurements are available. Patients will be asked to repeat the ABP for failed recordings.

vii. **24-hour Urine collection:** Patients will provide urine samples collected over 24 hours as two samples (after first void in the morning to 10 PM and from 10 PM to first void the morning). The patients will be provided appropriate urine collection system and overnight samples will be collected on ice and stored at the JBVAMC room 7200, Taylor Pavillion for isoprostanes to be performed. The overnight urine samples to be stored at JBVAMC will be labeled with unique study ID number and sample type only. 24-hour urinary excretion of catecholamines (SNA) will be measured from the pooled sample and this will be performed outside JBVAMC laboratory per standard clinical procedure.

Tissue banking: blood processing for DNA, RNA, serum and plasma isolation and storage will be performed at UI Biorepository, under the supervision of Dr. Rich Minshall, Dr. Klara Valyi-nagy and Vaiva Liakaite (Clinical Science Building North, 820 S. Wood Street, W-17 Chicago, IL 60612). The blood samples to be carried outside JBVAMC will be labeled with unique study ID number only. Only the processing of blood will be done outside JBVAMC and the blood draw procedure will only be done at JBVAMC. The processing for JBVAMC is done at the Madison/Milwaukee VA lab. To avoid delays loss of sample or sample integrity, we want to hand-deliver the samples without individual identifiers to UIC for prompt processing.

viii. **Echocardiogram** data will include myocardial mechanical data obtained by clinical software that is used in the clinical workstation in the JBVAMC echocardiography laboratory. This software is called Velocity Vector Imaging (VVI; Siemens Medical Solutions USA, Inc., Mountain View, CA). Dr. Mayank Kansal, JBVAMC cardiologist and collaborator will provide interpretation of these data.

Visit #2: Patients performing home PM will be instructed to begin ABP monitoring and urine sample collection the morning prior to the scheduled visit 2. Visit 2 will be in the morning and PM device, actigraphy, sleep log, urine sample, and ABP monitor will be collected and processed/downloaded. Patients performing PM test at JBVA will be instructed to begin ABP monitoring and urine collection the morning of scheduled visit 2. Patients will arrive for the overnight PM at JBVAMC. Actigraphy, sleep log, urine sample, and ABP monitor will be collected and processed/downloaded in the morning after completion of the PM test. PM tests will be performed using ALICE PDX (Phillips Respironics) or WatchPAT200 (Itamar Medical). PI has experience with both these devices that are available to us, are validated, used routinely in

clinical practice, and provide similar information with respect to screening for OSA. All patients with apnea hypopnea index (AHI) ≥ 15/hour on the PM test will be invited to continue. This additional screening step with PM testing will reduce screen failure rates with PSG, which is more costly. We propose to allow both home PM and PM at JBVAMC in order to minimize scheduling conflicts between clinical and research tests scheduled.

Previously scheduled Visit 3 will be eliminated to reduce participant burden and improve recruitment to meet target sample of 220 over the next two years.

Visit #3: Patients will return to the clinic for AUTOPAP treatment set-up and education by trained study coordinator. Patients and their bed partner will receive a one-on-one standardized education session describing the method of applying the device and individualized mask fitting will be performed; (4) patients will be called twice weekly in the first two weeks and once weekly thereafter to troubleshoot barriers to AUTOPAP use and encourage daily treatment application including during daytime naps.

Visit #4: Patients will return to clinic for repeat PVT, FOSQ, ESS, CABP measurements and to initiate 24-hour ABP and urine collection. Patients will also be scheduled for repeat echocardiography test in the following 3 months, if they had a pre-treatment echocardiography (to assess AUTOPAP treatment effects). No caffeine will be permitted during visits 1 and 5 to minimize interference with EDS assessments. Patients will also do venous blood sampling (35 mL) for processing (DNA, RNA, serum, and plasma) and storage at UI Biorepository

Final Visit # 5: Patients will return to clinic in the morning after initiating 24-hour ABP and urine collection, which will be collected and processed/downloaded. AUTOPAP devices contain a software system that records the duration of AUTOPAP use each day. These data will be accessed and recorded as final measure of AUTOPAP treatment adherence.

Patients will be given instructions regarding follow-up care for OSA with sleep medicine or primary care provider.

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

A patient may be included in this study if he or she meets all the following criteria:

1. Self-identified African American and Other (race)Veterans;

2. Age: 18-70 years, < 18 and > 70 years of age patients will be excluded due to higher prevalence of circadian phase misalignment and aging-related changes in sleep architecture (respectively), which would influence the sleepiness assessments;

3. Hypertension: will be defined as pre-existing physician diagnosis of hypertension, current antihypertensive drug therapy, or average blood pressure of systolic ≥ 140 or diastolic ≥ 90 mm Hg on seated resting cuff blood pressure measurements x 3 (5 minutes apart). While we will not employ an upper limit of blood pressure as an exclusion criterion, we will defer screening and enrollment of patients with systolic > 120 or diastolic > 120 mm Hg and refer them to their primary care physicians or urgent care as clinically appropriate;

4. Apnea hypopnea index (AHI) ≥15/hour on portable monitoring (PM);

5. Able to understand and complete informed consent and all study assessments and forms.

4.2 Exclusion Criteria

A patient will be excluded from this study if he or she meets any of the following criteria:

1. Past/current treatment of OSA or other primary sleep disorders;

2. Active uncontrolled medical or psychiatric conditions (for e.g. heart failure, chronic lung disease, depression etc.);

3. Shift work in past 1 month;

4. Current Illegal drug use, daily alcohol use, or a positive urine drug screening test in the preceding 6 months;

5. Pregnancy (every woman of childbearing age group will have a urine pregnancy test on visit 1);

6. Use of dietary supplements which in the judgment of the PI may impact sleep or breathing behaviors;

7. Average daily caffeine consumption > 500 mg/day (~5 cups of coffee);

5. CONCOMITANT MEDICATIONS (ANTIHYPERTENSIVES)

Antihypertensive medications will be recorded (number, dose, and refill information) for each patient at baseline and at every visit thereafter. This information will be confirmed with the patient's pharmacy for accuracy at visit 1 and 6. Patients will be encouraged to follow up with primary physician per usual schedule and to avoid taking over-the-counter medications that may affect blood pressure (e.g., some cold medications) for 24 hours before and during the ABP

monitoring. Adherence to medications for hypertension will be defined by the Medication Possession Ratio (MPR; performed by examining pill vials at visits 1, 2, 6, and 8). MPR calculation results in values of 0-1.0 (1.0 = perfect adherence). An MPR will be calculated for each antihypertensive drug, and the mean MPR for all antihypertensive drugs will be used as a measure of adherence to medication treatment. To adjust for changes in antihypertensive regimen or dosage of drugs that may occur during the study, we will use the Defined Daily Dose (DDD). The World Health Organization; WHO DDD methodology (http://www.whocc.no/atcddd/) allows for comparison of BP medications across antihypertensive regimens using "the average maintenance dose per day for a drug." Because no class of antihypertensive drugs is known to be superior in OSA, this approach will allow examination of the potential blood pressure lowering effect of AUTOPAP across the inter-individual and intra-individual variations in antihypertensive medication regimens.

6. STUDY DISCONTINUATION

The PI may recommend discontinuation of participation of individual patients. Reasons for early patient discontinuation may include: inability to tolerate AUTOPAP, development of severe blood pressure elevation (> 200/120 mm hg) or other unstable medical conditions during study participation, request to discontinue the trial from the patient or a regulatory authority, or protocol violations.

7. ADVERSE EVENTS

7.1 Definition- An AE is any adverse change from the patient's baseline condition, including any clinically significant signs and symptoms that occurs during the course of the study after the patient is enrolled, whether the AE is considered expected/unexpected and related to the treatment or not.

7.2 Adverse Event Reporting

7.2.1 Routine **Reporting of Adverse Events**

All AEs encountered during the clinical study that are considered study related, will be recorded in the source documents. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after enrollment through the period of protocol-specified follow-up (visit 6) will be recorded.
The information to be recorded for each AE includes:

1. Medical diagnosis of the event in standard medical terminology (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);

2. Date of onset of any new AE or the date of worsening of a previously observed AE;

3. Date of resolution of the event;

4. Severity of the event;

5. Assessment of the attributability of the event to the study protocol;

6. Whether the event is serious;

7. Whether the event is expected;

8. Description of action taken in treating the event (include medications or therapies administered, whether hospitalization was required, diagnostic procedures performed, and whether the patient was discontinued from the study);

9. Description of outcome: resolved or complete return to baseline, resolving, unknown/lost to follow-up, AE persisting or resolved with sequelae.

All AEs will be followed until resolution or stabilization of the event. This may require additional clinical assessments. The follow-up results will be recorded in the patient's source documentation.

7.2.2 Expedited Reporting of Serious Adverse Drug Experiences

No serious adverse events (SAE) are expected as the intervention(s) are standard in clinical practice and non-invasive. Nevertheless, study coordinator/PI will be informed of a SAE via a telephone call and preliminary information will be obtained. If all information is not known at the time of initial reporting, an initial report will still be made. The PI is responsible for following up on completion of the SAE Form. The PI will submit substantiating data in hard copy form, such as diagnostic test reports and progress notes, as requested.

During the initial telephone call, the study coordinator /PI will require the following information about the patient and the reported SAE:

1. Patient identification including participant identification number, initials, and date of birth;

2. Date of onset of the event;

3. Date of resolution of the event (or confirmation ongoing);

4. Severity of the event;

5. Assessment of the attributability of the event to study participation;

6. Whether the event is expected;

7. Action taken in treating the event and (including concomitant medications or therapies administered, whether hospitalization or prolongation of hospitalization was required, diagnostic procedures performed, and whether the patient was discontinued from the study);

8. All concomitant medications (including doses, routes, regimens, and indications);

9. Pertinent laboratory data;

10. Medical history (including time on study prior to adverse experience).

The PI will review each SAE report and evaluate the relationship of the adverse experience to underlying disease. Based on the PI's assessment of the adverse experience, a decision will be made concerning the need for further action. The primary consideration governing further action will be whether findings affect the safety of patient(s) participating in the clinical trial.

Further action that may be required includes the following:

1. Alteration of existing research by modification of the protocol;

2. Discontinuation or suspension of the study;

3. Alteration of the informed consent process by modification of the existing consent form and informing current study participants of new findings;

2. Modification of previously identified expected adverse experiences to include adverse experiences newly identified as study-related.

PI will promptly report all SAE to the I.R.B.

7.3 Withdrawal from Study Treatment Due to Adverse Experience

Patients withdrawn from receiving additional study drug due to an AE will be followed by the PI until resolution or stabilization of the event. Additional reports will be provided to the regulatory authorities when requested. Every effort will be made to follow the patient for the full study period as per the schedule of study visits.

8. Final Outcomes Assessment:

Table. Outcomes and covariates
Outcomes
Specific Aim 1. Between Group Comparisons (African Americans vs. Other): **Measure *change* from pre-treatment to 3 months after AUTOPAP treatment:** (a) Primary- average 24-hour systolic and diastolic BP (b) Secondary- central aortic blood pressure ii. average of 24-hour mean BP = diastolic + 1/3x (systolic diastolic) iii. 24-hour urine norepinephrine & normetanephrine iv. Overnight urine 8-isoprostane levels
Specific Aim 2. (change from pre-treatment to 3 months after AUTOPAP treatment): (a) Primary (*between-group comparison*)- change in number of PVT lapses (b) Secondary (*within-group comparisons*)- i. Association of change in PVT lapses with primary outcome of specific aim 1. ii. Association of change in PVT lapses with secondary outcomes of specific aim 1. iii. Association of change in ESS scores with outcomes of specific aim 1.
Exploratory Aim 3. i. Within-group analysis of change in specific aim 1. primary and secondary outcomes (i. and ii.) with addition of Individual Ancestry as a covariate. ii. Velocity Vector Imaging (VVI) from echocardiography correlation with AHI. iii. Generate effect size estimates for AUTOPAP treatment on VVI.
Covariates: Specific Aims 1 and 2
Socio-demographic Characteristics • Age (continuous) • Daily sleep duration on actigraphy (continuous) • Gender (categorical, 2 levels) • Education (categorical, 2 levels; ≤ high school, > high school) • Income per annum (categorical, 5 levels; <30K, 30-50K, 51-75K, 76-100K, >100K) • Smoking status (pack-years; continuous) • Alcohol use (number of drinks/week; continuous)
OSA Disease Severity • AHI • Time below 90% oxygen saturation (T<90)
Co-morbid Medical Conditions • Obesity: Body Mass Index (BMI) • General Health Status: Charlson Comorbidity Index (CCI)
Treatment Related Measures • Antihypertensive drugs: Adherence and Regimen Medication Possession Ratio (MPR) and Defined Daily Dose (DDD) • Adherence to AUTOPAP: Objective device download data at 90 days

9. DATA ANAYLSIS

9.1 Case Report Forms

All data required by the study protocol will be entered onto CRFs. Only authorized research personnel will enter data on the CRFs. All entries must be legible and made using indelible

black ink. If an entry error is made, a single line will be placed through the incorrect entry. The correct entry will then be made, with the correction dated and initialed by the person making the entry. Any corrections to data entered into the CRF must be made in such a way that the original entry is not obscured.

CRFs must be completed for every patient who signs an informed consent and has screening procedures performed. The CRFs will be signed and dated by the study coordinator/PI. The original completed CRFs should be kept in a locked study file and handled in accordance with JBVAMC R&D policies.

9.2 Data Quality Assurance

CRFs will be checked for correctness against source document. Once the CRF page is complete, it will be delivered to data management personnel for entry into an electronic database. Prior to data entry, the completed CRF will again be reviewed for completeness, consistency and legibility. Any discrepancies will be noted on a data clarification form (DCF). The discrepancy will be clarified on the DCF and placed in patients research record.

9.3 Data Management

PI will check data for quality control, weekly for the first month, and then biweekly. Electronic data (Microsoft office Excel) will be stored on a password-protected computer in a locked office and hard copies of data will be stored in a locked file cabinet in a locked office (PI's office, JBVAMC, Damen pavilion, Room 8508). Identifying information (MASTER LIST) will be stored separately in a designated locked cabinet at aforementioned location. Data will be backed up regularly on VA network (vhachsprasab2). Only the study coordinator/PI will have access to the keys for this cabinet. For analysis, data will be stripped of all individual identifiers and exported from Excel to the Statistical Analysis System (SAS Institute, 2011, version 9.3 or later), where appropriate labels and formats will be applied. The de-identified SAS dataset will be provided to UIC Design and Analysis Core, Center for Clinical and Translational Science for statistical analysis on VA encrypted flash drive. All scripts for statistical analysis will be documented with file headers and embedded comments; all data and analysis files will be preserved securely per JBVAMC R&D regulations. Prior to beginning analysis, descriptive statistics will be prepared for all variables and collected in a codebook. Any "wild" data values discovered will be resolved. After data analysis is complete, the electronic data will be returned to PI on the VA encrypted flash drive and no copied data will be retained on non-VA computer. If key authorized personnel leave the study/VA employment their access to research data/locked file/computers will be terminated immediately. PI will report incidents, i.e. theft or loss of data or storage media, unauthorized access of sensitive data or storage devices or non-compliance with security controls to JBVAMC R&D office/other regulatory authorities in accordance with VA policy.

9.4 Statistical Analyses

9.4.1 Power and Sample Size Estimation: The primary goal of this initial study is to estimate differences in the effect size of AUTOPAP treatment on ABP and CABP in AA and EA's with moderate to severe OSA. Our preliminary data shows a reduction in office BP 3 months after AUTOPAP treatment and the effect size difference between-groups (by ethnicity; using differences in mean ± SD change of BP and t-tests in PASS program, http://www.ncss.com/pass.html) was 0.42 for systolic and 0.53 for diastolic blood pressure. We determined that 99 patients in each group (total n = 198) will provide power of 0.8 in two-tailed tests with an alpha of 0.05 to observe a difference in effect size between groups of 0.4. Based on our prior experience we anticipate an attrition rate of 10-20%. Therefore, a total of 220 (198/0.90) patients enrolled will achieve the target of 198 patients completing the study. Our preliminary study of PM in our clinical population (selected with a positive BSQ) shows approximately 95% sensitivity and specificity of PM determined AHI of ≥ 15/hour in terms of predicting PSG-determined AHI of ≥ 15/hour (inclusion criteria). Therefore, we expect to screen approximately 260 patients (220/0.95).

9.4.2 Data analysis: A p-value < 0.05 will be considered significant. All variables and analysis goals are listed in table above. **Specific Aim 1.** Within group changes in primary and secondary outcomes will be assessed with repeated measures ANOVA implemented with PROC MIXED in SAS. A two-sample t-test (PROC TTEST in SAS) will be used to examine between-group differences in the treatment-related changes in primary and secondary outcomes. **Specific Aim 2.** Primary outcome will be assessed with a two-sample t-test (PROC TTEST in SAS). Secondary outcomes will be assessed with partial correlations implemented with PROC CORR in SAS.

Exploratory Aims 3. Effect of Individual Ancestry on changes in primary and secondary outcomes of specific aim 1 within group will be assessed with ANCOVA implemented with PROC GLM in SAS. Correlations (of VVI with AHI and change in VVI with change in AHI) will assessed within groups.

Residuals and outliers: Linear models require residuals from model fits to follow a normal distribution and be free of outliers. We will examine residual distributions and apply transformations to outcomes and explanatory variables as warranted to achieve normality. Further model checks will employ partial regression plots; outliers will be dealt through case removal, influence down-weighting (robust M-estimation), or bootstrapping of estimators.

Missing data: We will examine patterns of missingness with respect to covariates and include any covariates differentially associated with missing data. Correctly specified linear mixed models yield unbiased estimated for data missing at random (MAR); multiple imputation can also be employed. It is possible that subjects' treatment experiences will affect missingness (dropout), in which case data is not missing at random (NMAR) or nonignorable, and requires additional modeling, such as pattern-mixture models.

Moderators: Whether a moderator variable Z affects the association of explanatory variable X and outcome Y will be examined by introducing Z and the Z*X interaction in the linear model. When Z is a (0,1)-indicator variable (e.g., gender), the coefficient for Z is the adjustment to the intercept and the coefficient for Z*X is the adjustment to the slope appropriate for the group coded 1; the latter coefficient tests the moderator effect.

Mediators: None hypothesized.

10. ETHICAL CONSIDERATIONS

10.1 Declaration of Helsinki

The study will be conducted in accordance with the Declaration of Helsinki (1964) including all amendments up to and including the Scotland revision (2000) and notes of clarification added in 2002 and 2004 as described in Appendix D.

10.2 Good Clinical Practice and Regulatory Compliance

This study will be conducted in accordance with the principles of GCP (per the current ICH guidelines) and the requirements per JBVAMC R&D policies regarding the conduct of clinical trials and the protection of human patients.

10.3 IRB/Ethics Committee Approval

The PI will submit the protocol, the ICF, HIPAA and any other material used to inform patients about the trial to the IRB for approval prior to enrolling any patient into the trial. Approval must be in the form of a letter signed by the Chairperson of the IRB or the Chairperson's designee, must be on IRB stationary and must include the protocol by name and/or designated number. The protocol and ICF must be approved by the IRB committee before patients are screened for enrollment.

The PI will not amend the protocol without approval from the JBVAMC R&D office and IRB. Changes to the protocol must be in the form of a written amendment. These changes must be submitted by the PI to the IRB and such amendments will only be implemented after written approval of the requisite IRB. All amendments will also be submitted to VA regulatory authorities as required by JBVAMC R&D office.

The PI will submit all periodic reports and updates that the IRB/ JBVAMC R&D office may require, including any final reports.

10.4 Informed Consent

No study related procedures will be performed until a patient has given written informed consent. The ICF will clearly describe the potential risks and benefits of the study, in a language that the patient understands, and each prospective participant must be given adequate time to discuss the study with the PI or study coordinator and to decide whether or not to participate. Each patient who agrees to participate in the trial and who signs the ICF will be given a copy of the signed and dated document. The original copy of the signed and dated ICF will be retained by the PI in the study files.

The PI will also obtain authorization from the patient to use and/or disclose PHI in compliance with the HIPAA. Written HIPAA authorization will be obtained separately from the ICF.

If a protocol amendment substantially alters the study design or potential risk to the patient, the ICF will be revised and submitted to the IRB for review and approval; the revised form will be used to obtain consent from patients currently enrolled in the study if they are affected by the amendment; and the new form must be used to obtain consent from new patients prior to enrollment.

10.5 Departure from Protocol

When an emergency occurs that requires a departure from the protocol for an individual, a departure will be only for that patient. The PI in such an emergency will, if circumstances and time permit, contact the IRB/JBVA R&D office immediately by telephone. Such contacts will be made as soon as possible to permit a decision as to whether or not the patient (for whom the

departure from protocol was affected) is to continue in the study. The CRF and source documents will completely describe the departure from the protocol and state the reasons for such departure.

11. ADMINISTRATIVE

11.1 Study Monitoring

The study monitoring will be through regularly scheduled Data Safety Monitoring Committee (PI and Study coordinator/other key personnel, TBD) meetings and as per the JBVAMC R&D office regulations. They may include review of various aspects of the trial including, but not limited to, screening logs, compliance with the protocol and with the principles of GCP, completion of CRFs, source data verification, study data security and storage, facilities and staff.

11.2 Source Documents

The PI will maintain records separate from the CRFs in the form of clinical charts, laboratory reports, etc. (i.e., source documents). The PI will document in the clinical chart the name and number of the trial and the date on which the patient signed the ICF prior to the patient's participation in the study. Source documents will reflect the nature and extent of the patient's medical care relevant to this research protocol and will be available for source document verification against entries in the CRFs.

11.3 Record Retention

The PI will be responsible for maintaining the study records and the location and security of the study record storage. All records will be retained by the PI indefinitely per VA regulations after the completion of the study and in accordance with VHA's Records Control Schedule (RCS 10-1). PI will consult JBVAMC R&D office and VACO CSR&D (funding agency) prior to destruction of any research records. If paper records are destroyed, these paper documents will be placed in the secured document destruction box has been placed in room 6204, JBVAMC R&D.
Tissue samples collected for this research will be stored indefinitely at UI Biorepository.

11.4 Confidentiality

Patient medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited.

Medical information resulting from a patient's participation in this study may be given to the patient's personal physician or to the appropriate medical personnel responsible for the patient's welfare.

Patient's medical information may be reviewed by government regulatory agency auditors, designated study auditor, or the IRB committee in the course of monitoring the conduct of the study. Every reasonable effort will be made to maintain such information as confidential.

The results of the study may be presented in reports, published in scientific journals or presented at medical meetings; however, patient names will never be used in any reports about the study.

12. REFERENCES

American Academy of Sleep Medicine Task Force. 1999. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep 22(5):667-89.

Prasad B, Carley DW, Krishnan JA, Weaver TE, and Weaver FM, Effects of positive airway pressure treatment on clinical measures of hypertension and type-2 diabetes.J Clin Sleep Med.,2012.8:481-7.

Abstract and Oral presentation: "Effect of Ethnicity on Excessive Daytime Sleepiness in Obstructive Sleep Apnea". Associated Professional Societies of Sleep Medicine SLEEP 2013

Patel SR, Goodloe R, De G, Kowgier M, Weng J, Buxbaum SG, Cade B, Fulop T, Gharib SA, Gottlieb DJ, Hillman D, Larkin EK, Lauderdale DS, Li L, Mukherjee S, Palmer L, Zee P, Zhu X, and Redline S,Association of genetic loci with sleep apnea in European Americans and African-Americans: the Candidate Gene Association Resource (CARe).PLoS One,2012.7:e48836.

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APPENDIX I: SCHEDULE OF EVENTS

Table. Patient Schedule						
Procedures	**Visits**					
	1	**2**	**3**	**4**	**5**	**6**
Week	**0-2**				**3-16**	
Informed consent	×					
History and physical, anthropometrics	×					
Home portable monitoring (PM) test	×					
Determine AHI at termination of PM (≥ 15/hour; enrolled)		×				
Central aortic blood pressure measurement (with brachial cuff), PVT x 2	×	×			×	
Venipuncture for blood sample (genotyping for AIMs)		×			x	
Start 7-day actigraphy recording with sleep log		×			×	
Initiate 24-hour urine collection		×			×	
Initiate 24-hour ABP		×			×	
Collect and process: 24-hour urine, 24-hour ABP			×			×
Download 7-day actigraphy recording			×			×
Initiation of AUTOPAP with subject education				×		
Telephone contacts for AUTOPAP adherence optimization	Twice weekly x 4, then once/week					
Schedule Echocardiography		×			×	
Final visit and AUTOPAP adherence data download						×

APPENDIX II: QUESTIONNAIRES and TEST BATTERY

1. Epworth Sleepiness Scale (ESS)

This is a well-validated patientive rating scale for sleepiness, comprising 8 items each rated from 0 (no chance of dozing) to 3 (high chance of dozing). A total score of >10 is generally taken to indicate significant sleepiness. Patients for this protocol will be required to have and ESS score of ≥12. The standard instruction asks a patient to refer to "your usual way of life in recent times", when answering. This instruction will be modified, asking patients to refer to "the past two weeks", which will be the interval between successive visits.

THE EPWORTH SLEEPINESS SCALE

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life during the past two weeks. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation:

0 = no chance of dozing
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

SITUATION	CHANCE OF DOZING
Sitting and reading	_____
Watching TV	_____
Sitting inactive in a public place (e.g. a theater or a meeting)	_____
As a passenger in a car for an hour without a break	_____
Lying down to rest in the afternoon when circumstances permit	_____
Sitting and talking to someone	_____
Sitting quietly after lunch without alcohol	_____
In a car, while stopped for a few minutes in traffic	_____
Total	_____

2. Functional Outcomes of Sleep – Short Form (FOSQ-10)

The FOSQ-10 is a 10-item questionnaire, derived from the original 30-item FOSQ instrument, that has been specifically validated in patients with OSAS (Chasens et al. 2009). The FOSQ-10 is a quality of life instrument with subscales addressing the impact of sleepiness on: general productivity, activity level, vigilance, social outcomes, and intimacy and sexual relationships. Each subscale has a possible range of 1 – 4 and the total score has a possible range of 5 – 20. Higher scores indicate less impairment.

FUNCTIONAL OUTCOMES OF SLEEP QUESTIONNAIRE (FOSQ)

Some people have difficulty performing everyday activities when they feel tired or sleepy. The purpose of this questionnaire is to find out if you generally have difficulty carrying out certain activities because you are too sleepy or tired. In this questionnaire, when the words "sleepy" or "tired" are used, it means the feeling that you can't keep your eyes open, your head is droopy, that you want to "nod off", or that you feel the urge to take a nap. These words do not refer to the tired or fatigued feeling you may have after you have exercised.

DIRECTIONS: Please put a (_) in the box for your answer to each question. Select only one answer for each question. Please try to be as accurate as possible. All information will be kept confidential.

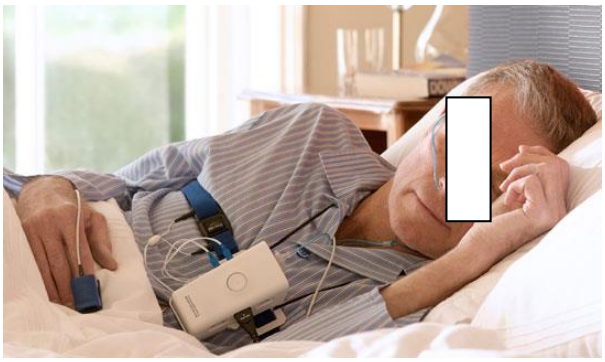
	(0) I don't do this activity for other reasons	(4) No difficulty	(3) Yes, a little difficulty	(2) Yes, moderate difficulty	(1) Yes, extreme difficulty
1. Do you have difficulty concentrating on the things you do because you are sleepy or tired?		❒	❒	❒	❒
2. Do you generally have difficulty remembering things, because you are sleepy or tired?		❒	❒	❒	❒
3. Do you have difficulty operating a motor vehicle for short distances (less than 100 miles) because you become sleepy or tired?	❒	❒	❒	❒	❒
4. Do you have difficulty operating a motor vehicle for long distances (greater than 100 miles) because you become sleepy or tired?	❒	❒	❒	❒	❒

Psychomotor Vigilance Task (PVT)

The PVT is a motor reaction time task and a test of sustained vigilance. The patient is given a hand-held device with a screen and is instructed to press the "space bar" every time a flash of numbers appears in the center of the screen. The intervals between these stimuli are randomized and when the space bar is pressed, the number displays the reaction time in milliseconds for a brief interval before the screen again goes blank. The task continues for 10 minutes.

Portable Monitoring (PM)

1. ALICE PDX



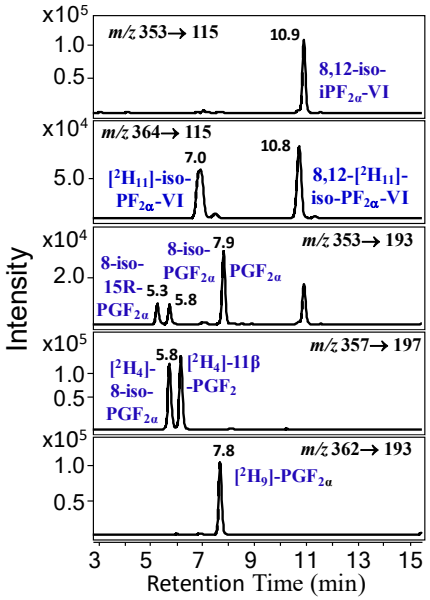
2. WP200

Shown above are pictures of the two validated and non-invasive portable monitors that will be used during this study. They will yield equivalent results that are comparable PSG. Two monitors are chosen for efficiency of screening as 1 of each is available to the PI. The PSG has to follow the PM test as it will serve as the confirmatory gold standard diagnostic test and will allow for titration of AUTOPAP treatment.

URINE SAMPLE

Urine Samples for Sympathetic Nervous System Activity: Patients will provide urine samples collected over 24 hours as two samples (after first void in the morning to 10 PM and from 10 PM to first void the morning). The overnight samples will be collected on ice 24-hour urinary excretion of catecholamines (SNA) will be measured from the pooled sample
The samples will be homogenized into aliquots of 50 ml. The catecholamine assay will be performed per the UIC Clinical Science Building.
The assay analysis of the urine will be done at the UIC Clinical Science Building because the turnaround time is quicker at the same cost. Samples will be analyzed for norepinephrine and 3-methylnorepinephrine (normetanephrine) using high performance liquid chromatography (HPLC) with electrochemical detection and expressed in µmol•mol−1 of creatinine83. HPLC offers the advantage that commonly used anti-hypertensive drugs do not interfere with the results.

Urine Samples for 8-isoprostane: (oxidative stress related to OSA) from the overnight samples.



Total (free + esterified) isoPs will be analyzed. Esterified isoP-containing lipids in urine (0.5 mL) will be hydrolyzed with β-glucuronidase from Helix pomatia (10 µL, 100 units/ µL) at 37 oC under argon for 20 h. Lipid hydrolysis will be conducted in the presence of [2H11]-iso-PF2a-VI, [2H11]-8,12-iso-PF2a-VI, [2H4]-8-iso-PGF2a, [2H9]-PGF2a, and [2H4]-11□-PGF2 as stable isotopically-labeled internal standards (10 ng of each), butylated hydroxytoluene (to prevent autoxidation) and deferoxamine (to prevent Fenton chemistry). The free isoprostanes that are released together with free isoPs that were present original in the urine (total isoPs) will be analyzed by negative ion LC-SRM/MS (Figure x). The assay will simultaneously analyze the four major isoprostanes found in serum lipids (iso-PF2a-VI, 8,12-iso-PF2a-VI, 8-iso-15(R)-

PGF2a, and 8-iso-PGF2a), as well as PGF2a, and 11□-PGF2. After hydrolysis, the urine will be diluted with 1 M phosphate buffer pH 6.8 (1 mL) and acidified to pH 5.5 with 1 M formic acid. Lipids will be extracted with methyl tertiary-butyl ether/hexane, the organic layer evaporated to dryness, the residue re-constituted in 30 % aqueous acetonitrile (50 µL). An aliquot (10 µL) will be analyzed by LC-SRM/MS under negative electrospray ionization conditions using the transitions shown in Figure above. Chromatography will be conducted on a Phenomenex KinetexXB C18 (2) column (150 x 2.1 mm internal diameter, 2.6 µm, 100 Å). A linear gradient will be used with a mobile phase of water with 2 mM ammonium hydroxide and acetonitrile and a flow of 0.2 mL/min.

APPENDIX III: BLOOD SAMPLING WITH DNA, RNA, SERUM, and PLASMA PROCESSING AND STORAGE

A single venous blood sample of approximately 35 ml or two tablespoons will be collected during visit 1and visit 5. Samples will be transported with labels that state study ID together with a sample requisition form (with title of study, name of PI and coordinator, date of collection, and number and types of test tubes delivered; see below). These samples will be processed as specified below and stored indefinitely at UI Biorepository. These samples will remain linked to clinical information gathered for research by study ID, which will be maintained indefinitely at the UI Biorepository.

REQUISITION FOR LABORATORY SERVICES

STUDYTITLE : **Targeted Treatment of Obstructive Sleep Apnea to Reduce Cardiovascular Disparity**

IRB Protocol #

Patient Study ID: _____

Performance Site (circle one): Jesse Brown VA Medical Center

Contact: Bharati Prasad 312-996-8039, bpradsad@uic.edu

Collection Date: _____Collection Time:_____

Approved Test: mark all appropriate

	Test Name	Tube
☑		↑
☐	Serum	1 Red Top, 6ml 3 Purple Top, 6ml
☐	DNA	1 Pax Gene
☐	PBMC	↓
☐	Plasma	
☐		

*Top 4 boxes should always be marked

**RRC Contact Info: 312-996-3807 / dnas.blood@gmail.com

Delivered to RRC (CSBN W-17)? Yes___Time of delivery _____No ___If no, why?

___

Urine REQUISITION FOR LABORATORY SERVICES

STUDY TITLE: **Targeted Treatment of Obstructive Sleep Apnea to Reduce Cardiovascular Disparity**

IRB Protocol #

Patient Study ID:

Performance Site (circle one): Jesse Brown VA Medical Center

Contact Bharati Prasad 312-996-8039,

Collection Date: **Collection**

Approved Test: mark all

	Test Name	Tube
☑		▲
☐	isoprostane	2 6 ml tubes on dried ice ▼
☐		
☐		
☐		
☐		

Delivered to JBVAMC (Room 7200), Taylor Pavillion

Yes___Time of delivery _____No ___If no, why? ___

24-hour urine REQUISITION FOR LABORATORY SERVICES

STUDYTITLE: **Targeted Treatment of Obstructive Sleep Apnea to Reduce Cardiovascular Disparity**

IRB Protocol #

Patient Study ID: _____

Performance Site (circle one): Jesse Brown VA Medical Center

Contact: Bharati Prasad 312-996-8039, bpradsad@uic.edu

Collection Date: _____Collection Time:_____

Approved Test: mark all appropriate

	Test Name	Tube
☑		
☐	Dopamine	2 6ml tubes on dried ice
☐	Norepinephrine	
☐	epinephrine,	
☐		
☐		

*Top 3 boxes should always be marked

Sally Opell contact info: 312-355-2248

Deliver to CSB (room 215) __ Yes___Time of delivery _____No ___If no, why? _
CSB Staff Initials_____

The processing and storage will be performed at UI biorepository per protocol specified below.

Five tubes of blood will be collected; these include two 8-ml BD Vacutainer® CPT Mononuclear Cell Preparation Tube for the isolation of lymphocytes and monocytes, two 6-ml purple top vacutainers (K_2EDTA additive) for plasma and DNA recovery, and one 6-ml red top vacutainer (no additives) for serum recovery. These samples will be collected and stored at room temperature until processing. Processing for all derivatives will be performed within four hours of the initial blood draw.

Whole blood collected in the CPT vacutainers will be centrifuged according to the manufacturer's instructions, and after the centrifugation, the overlying plasma will be decanted. The buffy coat remaining above the gel barrier will be removed from the two CPT vacutainers and transferred to a sterile 50-ml tube and washed with Hank's Buffered Salt Solution (HBSS). At this point, Peripheral blood mononuclear cell (PBMC) number will be measured using a Millipore Scepter Handheld Automated Cell Counter. The PBMCs will then be pelleted by centrifugation, and the HBSS removed. The PBMCs will then be lysed in 2 ml of TRIzol solution and stored at -80C in a sterile 2-ml 2D barcoded tube.

Blood from the two purple top vacutainers will be fractionated as following: Duplicate DNA extractions will be performed on 350 microliters of whole blood using the iPrep Purification Instrument with the iPrep™ PureLink™ genomic DNA (gDNA) Blood kit (Invitrogen). The gDNA will be eluted in 200 microliters of buffer, analyzed using a NanoDrop 1000 spectrophotometer and aliquoted. Six aliquots of 30 microliters will be produced for future distribution, and one aliquot of 200 microliters (likely 10-20 micrograms of DNA) will be retained for future whole genome sequencing, if requested. These will be stored in sterile, barcoded tubes of 0.5-ml volume. In addition, 750 microliter aliquots of whole blood will be stored in sterile, barcoded tubes of 1-ml volume. The remaining whole blood will be centrifuged at 4°C for 10 minutes at 1,500 RPM, and the overlying plasma will be recovered and aliquoted into six 1-ml barcoded tubes.

Blood from the single red top vacutainer will be stored at room temperature for 15 minutes before centrifugation at 4°C for 10 minutes at 1,500 RPM. Subsequently, the overlying serum will be recovered and aliquoted into four 1-ml barcoded tubes.

In total, 20 tubes of derivatives of whole blood will be generated: (1) 2-ml tube with PBMCs in TRIzol, (2) 0.75-ml whole blood in 1-ml tubes, (6) 0.03-ml gDNA in buffer in 0.5-ml tubes, (1) 0.2-ml gDNA in buffer in a 0.5-ml tube, (6) 0.50-ml plasma in 1.0-ml tubes and (4) 0.50-ml serum in 1.0-ml tubes.

Gene and microRNA Expression Profiling- This proposal will utilize the GeneChip® Human Gene 1.0 ST Array by Affymetrix (details at the website) got gene expression profiling, covering > 28,869 genes represented on the array by approximately 26 probes spread across the full length of an individual gene. The Human Gene 1.0 ST Array has greater than 99 percent coverage of

sequences present in the RefSeq database. To determine microRNA differences, this proposal plans to use the mercury LNA microRNA array offered by Exiqon. The array provides access for examination of over 1300 human microRNAs with as little as 30ng total RNA. RNA for both of these arrays will be isolated from the blood collected from these patients in this study as described earlier. Details of this technology are also provided at http://www.exiqon.com.

3) Serum specimens

Serum specimens are primarily collected for future proteomics analysis. At this stage of planning, specimens are prepared anticipating subsequent analysis by a modified form of Isotope Coded Affinity Tagging. As new techniques become available or new biomarkers are identified, these specimens may be analyzed by alternative methods.
One 8 ml red-top tube specimen will be centrifuged at room temperature at approximately 1000 x g for 5 minutes. The serum supernatant will be removed and stored at –80ºC in 125µl aliquots.

4) Plasma specimens

Plasma specimens are primarily collected for conventional analysis by immunosorbent or biochemical assay. As platelet contamination, proteolysis and other aspects of handling can contribute to variable assay results, specimens will be handled according to the following protocol. The technician will retrieve one 4 ml lavender-top tube and centrifuge for 15 minutes at 4ºC at 1000 x g within 30 minutes of collection. The plasma supernatant will be removed to a fresh tube and centrifuged an additional 10 minutes at 1000 x g before removal and storage by the same method for the serum above.

5) AIMs analysis in isolated DNA at Kittles laboratory

Autosomal SNP AIMs will be genotyped using the Sequenom MassARRAY™ platform and iPLEX™chemistry. Individual Ancestry will be estimated from the genotype data using the Bayesian Markov Chain-Monte Carlo (MCMC) method implemented in STRUCTURE version 2.1. STRUCTURE will be run under the admixture model using prior population information and independent allele frequencies. The MCMC method will be run using K=2 and K=3 parental populations and a burn-in length of 30,000 for 70,000 repetitions. The best model of K to use will be determined and principal components based on the total AIMs using EIGENSTRAT will be computed.

APPENDIX IV: HUMAN SUBJECTS

A. Risks to Subjects

A.1 Human Subjects Involvement and Characteristics

After completing the informed consent process, the proposed study will enroll a total of 220 self-identified African American and Other(race), Veterans with pre-existing hypertension and newly diagnosed (and treatment-naïve) moderate to severe obstructive sleep apnea. These adult Veterans will not have debilitating physical or mental health problems. We will not recruit pregnant women, children, prisoners, institutionalized individuals, or other vulnerable populations. During preliminary screening, subjects will be informed of the study purpose, the risks and benefits pertaining to their participation, the enrollment process, the proposed testing, and the necessary time commitment.

Inclusion criteria briefly noted above are summarized in section C.4 (Table 7) of the research plan for this proposal. *Exclusion criteria* include: 1. Past/current treatment of OSA or other primary sleep disorders; 2. Active uncontrolled medical conditions; 3. Shift work in past 6 months; 4. Current drug use, and pregnancy. At screening visit blood sample (15 ml, for genetic ancestry testing), urine pregnancy test (if applicable), and disease specific screening questionnaires, and a brief vigilance test with a hand-held device will be obtained. In addition, information regarding personal and family medical history, medication use, sleep-wake schedule and work schedule will be collected, and a physical examination will be performed. The physical examination will include central aortic blood pressure measurement (performed with a cuff on the upper arm) similar to the process utilized routinely in clinical practice. All subjects with baseline peripheral blood pressure values of > 180 mm Hg systolic or > 110 mm Hg diastolic will be referred for urgent medical care and their enrollment will be deferred until medically cleared by their primary care provider.

All eligible subjects will undergo standard clinical care and research related procedures: (1) polysomnography for diagnosis of obstructive sleep apnea and confirmation of disease severity, (2) 7-day actigraphy, 24-hour ambulatory blood pressure monitoring, and 24-hour urine sample collection, Epworth sleepiness questionnaire, and a psychomotor vigilance test will be performed at baseline (pre-treatment) and three months after institution of treatment for obstructive sleep apnea, (3) standard treatment of obstructive sleep apnea i.e., polysomnography guided continuous positive airway pressure device treatment will be provided to all subjects. <u>All research related subject visits will take place at the Jesse Brown VAMC</u>.

A.2 Potential Risks

General: We see no psychological, social or legal risk associated with this research, beyond those of participation in health-related research in general.

Blood sampling: There is a small risk of local hematoma (1 in 10 cases), and an extremely small risk (1 in 1000 cases) of infection associated with insertion of needles for blood withdrawal. An experienced technician, using aseptic techniques, will minimize these risks.

Urine collection: No risks are identified except loss of confidentiality (addressed below).

Polysomnography: Is a routine laboratory-based procedure attended by a trained technologist. Minor skin irritation may occur from the electrode's application, in addition to fragmentation of sleep. These complications are rarely observed, and every effort will be made to minimize the associated discomfort.

Actigraphy: Is a wrist-watch and maybe associated with minor skin irritation and wrist discomfort.
24-ambulatory blood pressure monitoring with an inflatable cuff on the non-dominant upper extremity: The monitor itself is light and fits inside a shirt pocket. The cuff inflations can cause local discomfort. Therefore, we will limit our sampling rate to every 20 minutes during the day and every half-hour at night.
Central aortic blood pressure measurement will be performed non-invasively with a cuff on the subject's upper arm that is inflated briefly. This cuff is attached to a special monitor (Sphygomocor). The procedure in terms of subject risk is the same as a regular blood pressure check in their doctor's office.
Continuous Positive Airway Pressure (AUTOPAP) device treatment: Is the first-line treatment for obstructive sleep apnea and its safety and efficacy are well established. Complications skin irritation of the face at the site of mask application, nasal congestion/oral dryness, and claustrophobia. We will use standard measures used to address these issues promptly, such as individualized education, mask-fitting, and use of heated humidity.
Confidentiality: It is possible that subject confidentiality could be breached. However, all data will be coded and direct linkages between the subject and the data from that subject will be broken once the necessary clinical information has been obtained. No publications lists or stored data will directly link a study subject with his or her data.

A.3 Sources of Material
The research data for Aim 1, 2, and 3 include questionnaire data, medical histories, physical examination and measurements, blood and urine tests, polysomnography, actigraphy, and 24-hour ambulatory blood pressure monitoring. A portion of the urine sample will be collected on ice and carried by study coordinator to JBVAMC room 7200, Taylor Pavillion to be stored in -80 degrees Celsius freezer for later systemic oxidative stress biomarker measurement. The single blood draw of 35 ml will be carried by study coordinator to UI Biorepository, Clinical Science Building North 820 S. Wood Street, W-17 Chicago, IL 60612 for DNA, RNA, serum, and plasma processing and storage. The DNA data generated will be hand carried by PI/study coordinator to UIC Institute of Human Genetics at 3302 MBRB, MC 767, 900 S Ashland Ave, Chicago IL 60612 for genetic ancestry analysis. All data collected will be securely stored, de-identified, and used for research purposes only.

A.4 Therapeutic Risk
The treatment intervention (AUTOPAP) is the standard treatment for sleep apnea of all severity. Therefore, the risks incurred are no more than in routine clinical care and include complications skin irritation of the face at the site of mask application, nasal congestion/oral dryness, and claustrophobia.
Polysomnography will be performed once (for diagnosis confirmation and AUTOPAP titration) as is done in routine clinical practice. Minor skin irritation may occur from the electrode's application, in addition to fragmentation of sleep.

A.5 Research Risk
24-ambulatory blood pressure monitoring with an inflatable cuff on the non-dominant upper extremity will be performed twice by the subjects for research purposes only. Cuff inflations can cause local discomfort and we will limit our sampling rate to 20 minutes during the day and

every 30 minutes at night. Ambulatory blood pressure is a more sensitive measure of blood pressure control and cannot be replaced with single measurements.

B. Adequacy of Protection Against Risks

B.1 Recruitment and Informed Consent

The proposed study will obtain approval from the Jesse Brown VAMC I.R.B governing clinical research activity both at Jesse Brown VAMC and ABJ CBOC, prior to initiation of the project. All participating PIs will be required to submit documentation of completing human subject research training. Consent will be obtained with an I.R.B. approved consent form.

The subjects in this study will be recruited through direct contact during regular Jesse Brown VA Medical and Sleep Clinic visits. The principal PI or research assistant will explain the goals and procedures of the study. Interested subjects will be invited to participate.

Informed Consent: All subjects will undergo informed consent in accordance with the requirements of the I.R.B governing clinical research activity both at Jesse Brown VAMC and ABJ CBOC. This will involve a complete discussion of the study procedures, risks, exclusion criteria. A discussion of possible risks will include discomfort associated with study procedures including venipuncture, polysomnography, actigraphy, 24-hour ambulatory blood pressure monitoring, and AUTOPAP treatment. In addition to medical evaluation at screening visit subjects will be asked to disclose any information that may preclude safe participation. The process will be documented with an approved consent form signed by the study subject and the principal PI/research assistant. Subjects will be informed that all medical information obtained will only be used for study purposes.

B.2 Protection Against Risks

Jesse Brown VAMC has established procedures to ensure the privacy of study subjects and the maintenance of strict confidentiality and this study will follow these same procedures. Maintenance of confidentiality is accomplished in several ways. First, all research materials (data and biological specimens) are maintained in a manner inaccessible to anyone other than the study PIs who have been trained in the protection of human subjects. All source documents and specimens are identified by unique study code number, the keys to which are kept in locked file cabinet and on password protected electronic media. Second, the transmission of data to and from the Sleep Laboratory occurs via secure web-based connections or using encrypted electronic media. No results will be reported in a personally identifiable manner.

General: To protect against or minimize potential risk, subjects will be carefully evaluated and closely supervised during all study visits. The subjects will be screened to exclude those with medical problems that might increase the risks of developing complications, such as uncontrolled high blood pressure. Before being considered for participation in the study, the subjects will undergo a physical examination and be medically cleared to participate by the principal PI. Subjects will be excluded if they are found to have any medical problems or other lifestyle behaviors described above in section A.1.

Blood Sampling: An experienced technician using aseptic techniques will minimize the associated risks.

Polysomnography, actigraphy, central aortic blood pressure measurement, and 24-hour ambulatory blood pressure monitoring: The potential for a serious adverse event to occur during these procedures will be minimized by prior medical screening and by monitoring of all study related visits and scheduled phone calls between study visits.

Confidentiality: To maintain confidentiality, all of the data collected on the subjects will be coded with letters and numbers. Access to the data file that matches an individual with an identifying code will be password protected and restricted to the PIs. Subject records will be kept in locked file cabinets within the respective laboratories in which they are collected. There are no alternatives to these procedures that would permit the acquisition of the information required for these research studies. The alternative is not to participate, and that option is always available to study volunteers.

C. Potential Benefits of the Proposed Research to the Subjects and Others

The proposed research might not be of direct benefit to the subjects but is of potential benefit to society by increasing our understanding of the effectiveness of AUTOPAP treatment in reducing adverse cardiovascular outcomes of obstructive sleep apnea. Potential benefits to the subjects in this preliminary study include favorable effects on nightly sleep, excessive daytime sleepiness, blood pressure control, and cardiovascular risk. Moreover, if data obtained from the subjects in this preliminary study shows improvement of biomarkers implicated in progression of blood pressure and ambulatory blood pressure levels, this will lead to the design and implementation of larger studies to evaluate the effect of this AUTOPAP intervention on cardiovascular disease progression. Overall, we feel that the benefits of this research greatly outweigh the risks to the subjects.

D. Importance of the Knowledge to be Gained

The overall objective of our proposal is to examine continuous positive airway pressure treatment-related effects on adverse cardiovascular effects (including 24-hour blood pressure profile) of obstructive sleep apnea in African Americans, a high-risk population. The ability to identify and effectively intervene for poorly-controlled hypertension in this population is of great relevance as it could help reduce the progression of organ injury.

E. Data and Safety Monitoring Plan

Safety Monitoring: Data from the study will be monitored on a continuous basis by the Data Safety Monitoring Committee [Dr. Bharati Prasad, principal PI (PI), Drs. David Carley, and Frances Weaver (mentors), study coordinator Tomas Mercado]. All serious adverse events (SAEs), adverse events (AEs), and laboratory values will be reviewed by the PI on an ongoing basis. The PI will be responsible for annual reports to the I.R.B and for reporting any adverse events (AEs) to the IRB. The IRB will be notified within 24 hours of any SAE occurring. In addition, all laboratory values outside of normal range will be discussed with the study subject, and appropriate arrangements will be made for treatment, if necessary. The data monitoring and safety committee composed of Drs. Carley, Weaver, and Prasad will meet on a monthly basis to discuss all safety issues. All key personnel involved in the conduct of the research proposed will receive the required education of the protection of human research subjects prior to starting work on this project.

Monitoring of Relevant Literature: The principal PI and mentors have expertise in the area of obstructive sleep apnea, cardiovascular disease and vascular function and continually monitor the scientific literature, serve on VA and NIH funded multicenter DSMB’s and the editorial boards of several high impact peer reviewed journals, and regularly attend scientific meetings in the field. Any new information that might affect subject safety or the risk-benefit ratio will be reported to the IRB and used to amend or discontinue study procedures. New information will

be immediately available and confirmed in the continuing progress reporting procedure to the IRB.

F. Inclusion of Women and Minorities

F.1 Inclusion of Women

Based on the demographics of Jesse Brown VAMC and ABJ CBOC (recruitment sites) we do not expect to enroll many women in this study. However, we will include consenting women Veterans based on our specified inclusion/exclusion criteria.

F.2 Inclusion of Minorities

Consistent with the Aims of this study will include self-identified African Americans and Caucasian Veterans. For future studies, all ethnic groups will be eligible to participate.

F.3 Targeted/Planned Enrollment

240 study subjects will be recruited from the Jesse Brown VA and ABJ CBOC sleep clinics, with a recruitment pool of more than 1400 potential subjects. Therefore, the planned recruitment of approximately 6 subjects per month over the anticipated enrollment period of 42 months is feasible and realistic. We will include consenting adult African American and Caucasian Veterans for this study.

F.4 Inclusion of Children

The proposed study is a study of health outcomes in adults, hence the topic is not relevant to children and none are included.