Pharmacologic Interventions for Cardiovascular Disease in Obstructive Sleep Apnea

A Phase II, randomized, double-blind, placebo-controlled, multi-center study of the effects of losartan and allopurinol on chemoreflex responses, sympathetic output, vascular function, severity of sleep apnea and blood pressure in hypertensive patients with obstructive sleep apnea.

John M. Dopp, Pharm.D.
Barbara J. Morgan, Ph.D., PT
James Stein, M.D.
Jerome Dempsey, Ph.D.
Ailiang Xie, M.D., Ph.D.
David DeMets, Ph.D.
Christine Sorkness, Pharm.D.
Mihaela Teodorescu, M.D.
Scott Hetzel, M.S.
Paul Smith, M.D.
Debra Kruser, RN, BSN, MS
MJ Washburn RRT, CCRC

University of Wisconsin Schools of Pharmacy and Medicine and Public Health
Institute for Clinical and Translational Research
University of Wisconsin-Madison
Madison, WI

Jaime Boero, M.D., Ph.D.
Marshfield Clinic Research Foundation
Marshfield Clinic
Marshfield, WI

Daren Tobert, M.D.
Gundersen Medical Foundation
Gundersen Health System
LaCrosse, WI

James Gapinski, M.D.
Aurora Bay Care Sleep Center
Green Bay, WI

Scott Sands, PhD
Brigham and Women's Hospital
Boston, MA

Version: 23, 7-25-17
Initial version: [01-26-10]

Amended: [06-17-10]
Amended: [09-14-10]
Amended: [03-05-11]
Amended: [07-20-11]
Amended: [08-03-11]
Amended: [08-19-11]
Amended: [10-22-11]
Amended: [11-29-11]
Amended: [01-09-12]
Amended: [03-18-12]
Amended: [04-20-12]
Amended: [07-13-12]
Amended: [08-22-12]
Amended: [04-26-13]
Amended: [07-15-13]
Amended: [08-16-13]
Amended: [11-13-13]
Amended: [06-25-14]
Amended: [09-18-14]
Amended: [12-22-14]
Amended: [03-03-15]
Amended: [12-05-16]

Table of Contents

List of Abbreviations

A-II = angiotensin II
AE = adverse event
AT1R = angiotensin type 1 receptor
ATP = adenosine triphosphate
CCR = carotid chemoreceptor
CIH = chronic intermittent hypoxia
CO2 = carbon dioxide
CPAP = continuous positive airway pressure
CRU = clinical research unit (at UW-Madison)
CV = cardiovascular
DMC = data monitoring committee (at UW-Madison)
ICTR = institute for clinical and translational research (at UW-Madison)
MSNA = muscle sympathetic nerve activity
NO = nitric oxide
O2 = oxygen
OSA = obstructive sleep apnea
PI = principal investigator(s)
PRC = Pharmaceutical Research Center (at UW-Madison)
ROS = reactive oxygen species
SAE = serious adverse event
SaO2 = oxygen saturation
UIP = University of Iowa Pharmaceuticals
WiNHR = Wisconsin Network for Health Research
XO = xanthine oxidase

Study Summary

Title	*Pharmacologic Interventions for Cardiovascular Disease in Obstructive Sleep Apnea*
Protocol Number	
Phase	*Phase II*
Methodology	*Randomized, double-blind, placebo-controlled, parallel group study*
Study Duration	*4 years*
Study Site(s)	*Multi-Center* *-University of Wisconsin, Madison, WI* *-Marshfield Clinic, Marshfield, WI* *-Gundersen Health System La Crosse, WI* *-Aurora Bay Care, Green Bay, WI* *-Brigham and Women's Hospital, Boston, MA (Data analysis only)*
Objectives	*The objective of this application is to investigate the potential utility of angiotensin receptor blockade and xanthine oxidase inhibition in targeting and reversing putative mechanisms of hypertension in patients with OSA.*
Number of Subjects	*N=100*
Diagnosis and Main Inclusion Criteria	*Hypertensive adult patients with obstructive sleep apnea using continuous positive airway pressure*
Study Product, Dose, Route, Regimen	*Losartan (Cozaar) 50-100 mg orally daily, Allopurinol 300 mg orally daily*
Duration of administration	*6-weeks*
Reference therapy	*Placebo*
Statistical Methodology	*ANOVA and chi-square tests where appropriate*

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Obstructive sleep apnea (OSA) is a condition that is increasingly associated with adverse effects on cardiovascular (CV) health. It is estimated that up to 18 million Americans have OSA, many of whom remain undiagnosed (22). The U.S. population is becoming increasingly overweight and is aging, both of which are alarming trends because obesity and aging are two of the strongest risk factors for the development of OSA (23-25). As more people develop OSA, the prevalence of CV disease is expected to increase. Untreated OSA is associated with the development of hypertension and other CV diseases (26-30). Discovery of treatable causes of CV disease in OSA may lead to reductions in morbidity and mortality. Increased sympathetic traffic and endothelial dysfunction are well-documented in OSA and are believed to play a significant role in the development of hypertension. Continuous positive airway pressure (CPAP), the therapy of choice for OSA, has beneficial effects on sympathetic activity, endothelial function, and blood pressure (31-33); however, up to 30 percent of patients do not adhere to CPAP therapy (17). Therefore, development of other treatments that specifically target mechanisms of CV disease and hypertension is warranted.

Sympathetic Nervous System in OSA: Evidence from animal models suggests that carotidchemoreceptors (CCR)s (via intact sympathetic nerves) mediate changes in sympathetic output that result in hypertension (34-36). Patients with OSA have elevated sympathetic nerve activity while exposed to intermittent hypoxia during sleep and also during daytime waking hours when blood oxygen levels are normal (37). Plasma and urinary norepinephrine concentrations are also elevated in patients with OSA (38). Hypoxia, rather than hypercapnia, is primarily responsible for increases in sympathetic nerve activity (39) that occur during apneas in patients with OSA. Narkiewicz et al demonstrated that inhibiting the CCR with hyperoxia reduced mSNA in patients with OSA, suggesting that tonic activation of CCR afferents contributes to elevations in daytime mSNA (2). Furthermore, OSA patients have CCR hypersensitivity to subsequent hypoxia exposure (3). Treatment of OSA with CPAP or oxygen normalizes sympathetic activity, blood pressure, and CCR hypersensitivity (3,31,32). These data suggest that intermittent episodes of hypoxia mediate increased sympathetic traffic in patients with OSA via sensitization of the carotid chemoreflex and that this alteration is reversible.

Role of A-II and XO in Carotid Body Function: Heightened sensitivity of carotid chemoreflex regulation of sympathetic discharge is one putative mechanism of sympathetic overactivity in patients with OSA. Changes in CCR sensitivity appear to be driven by exposure to intermittent hypoxia, rather than hypercapnia or arousal from sleep (40). Experimental animal studies have demonstrated that chronic intermittent hypoxia (CIH) increases CCR sensitivity to subsequent hypoxic episodes (41-43). The signal for increased CCR sensitivity during hypoxia appears to involve endothelin-1 and angiotensin II (A-II) via superoxide and other reactive oxygen species (ROS)(44). The importance of Angiotensin II's (A-II) role in enhancing chemoreceptor sensitivity to hypoxia is becoming increasingly recognized. Injection of A-II directly into isolated carotid bodies elevates nerve activity in the carotid sinus nerve (44). Additionally, carotid afferent nerve activity is increased in ex vivo studies by application of physiologic concentrations of A-II (45). Application of an A-II antagonist, losartan reverses the sympathoexcitation of A-II, indicating involvement of the A-II type 1 receptor (45). Hypoxia increases expression of carotid body A-II

type 1 receptors (44) and A-II receptor mRNA (46). Most importantly, our recent laboratory findings demonstrated that CIH-induced chemoreflex hypersensitivity was prevented by administration of losartan before and during CIH exposure (See Preliminary Studies Section).

Previous studies have shown that CIH increases generation of ROS in the carotid body (40). Furthermore, administration of superoxide scavengers prevents CIH-induced CCR sensitization (40), indicating this anion plays a pivotal role in sensitization of the carotid chemoreflex. Likely sources of superoxide in the carotid body include NADPH-oxidase and XO, which can be upregulated by hypoxia (47-49). Preliminary data from our laboratory illustrates that XO and A-II inhibition prevent CIH-induced increases in carotid body superoxide production (see Preliminary Studies). Taken together, these data suggest that superoxide is a key player in the signaling pathway that mediates enhanced CCR sensitivity caused by CIH and that A-II and XO increase carotid superoxide production under these conditions. Thus, targeting A-II with an ARB and XO with allopurinol would appear to be a logical strategy to reduce the chemosensitizing effects of CIH in the carotid body. Evaluation of XO and A-II inhibition in human subjects with OSA is warranted to determine if these preliminary findings have clinical relevance. If XO and A-II inhibition can reduce CCR sensitivity, they may also reduce sympathetic overactivity and the risk of developing hypertension.

Role of Nitric Oxide (NO) in Carotid Body and Vascular Endothelium: NO decreases CCR sensitivity and appears to work in a counter-regulatory fashion to A-II (50). Two isoforms of nitric oxide synthase (eNOS and nNOS) are present in the carotid body (51). In our preliminary studies, exposure to CIH downregulated carotid body nNOS, potentially removing an important effect of NO in maintaining normal CCR sensitivity (52). Our preliminary data demonstrate that inhibition of XO and A-II partially prevents CIH-induced reductions in nNOS expression (see Preliminary Studies section). These findings are in accordance with previous studies demonstrating that inhibition of XO and A-II increases NO availability in patients with OSA (53). Thus, XO and A-II inhibition may boost NO availability in the carotid body and reduce pathological CCR hypersensitivity produced in response to OSA.

Reduced availability of NO is thought to play a role in the development of vascular abnormalities in patients with OSA (54). These individuals demonstrate impairments in endothelium-dependent vasodilation in numerous vascular beds (55-57), and treatment with CPAP improves these impairments (32). Animal data demonstrate that CIH reduces acetylcholine-induced endothelium-dependent vasodilation in gracilis arteries and middle cerebral arteries (58). These impairments likely represent reductions in NO availability since endothelium-independent vasodilation to exogenous nitrates is not impaired in subjects with OSA or animals exposed to CIH (55,58). ROS are a likely trigger for OSA-induced endothelial dysfunction. ROS, *e.g.* superoxide anion, produced in the vessel wall can decrease the availability of NO via scavenging and by combination with NO to form peroxynitrite which leads to NOS uncoupling (59). Markers of oxidative stress, including neutrophil superoxide production and lipid peroxidation, are elevated in patients with OSA (60,61). Angiotensin II and XO are involved in the production of ROS in the vascular endothelium (12,62). Plasma concentrations of A-II are elevated in OSA and the activity of XO is increased by hypoxia (48,49,13). In patients with OSA, XO inhibition improves NO-mediated vasodilation. We have shown that XO inhibition and A-II inhibition both can prevent the CIH induced endothelial impairments and improve NO-mediated vasodilation in ex vivo animal studies (See Figure 5, Preliminary Studies Section).

Remodeling as a cause of impaired vasodilation: Increased carotid intima-media thickness (63,64) and increased arterial stiffness (64-69) are observed in individuals with OSA. Blood levels of NO and ET-1, endothelium-derived regulators of vascular stiffness with opposing actions (70,71), are decreased and increased, respectively (54,72). In rat gracilis arteries, we have shown

that CIH exposure causes a leftward shift in the stress-strain curve, consistent with an increase in arterial stiffness (73).

Mitogenic factors (*e.g.*, vascular endothelial growth factor (VEGF), basic fibroblast growth factor, platelet-derived growth factor) are up-regulated by hypoxia (74,75) and during inflammation (76). In patients with OSA, elevated plasma Ang II levels (13,64) have been implicated in increased VEGF expression (77).

Chronic sympathetic activation causes vascular remodeling via release of catecholamines that induce vascular wall growth (78). In pulmonary arteries, ATP released from adrenergic nerves during hypoxia-induced sympathetic stimulation causes proliferation and migration of adventitial fibroblasts into the intima and media (79). This mechanism is a putative cause of remodeling in the systemic circulation (80). In addition, surges in sympathetic outflow during episodes of apnea produce cyclical increases in arterial pressure and blood flow that may trigger adaptations in endothelial cells, vascular smooth muscle, and extracellular matrix aimed at normalizing wall stress (81,82).

Heightened Chemoreflex Sensitivity and Diminished Cerebrovascular Reactivity Destabilize Breathing during Sleep: Unstable ventilatory control during sleep in individuals with upper airways that are anatomically susceptible to collapse may precipitate OSA (7,8,83,84). Further, limited evidence points to a significant prevalence of unstable ventilatory control (*i.e.* high control system "loop gain") in patients with severe OSA, secondary in part to increases in CO_2 sensitivity (increased controller gain) (8,85). Augmented carotid chemoreflex sensitivity secondary to intermittent hypoxia (86) might also contribute to increased ventilatory instability and, in turn, to cyclical obstructions in OSA patients. Accordingly, reducing this important component of increased controller gain by preventing intermittent hypoxia-induced increases in carotid body sensitivity may also reduce the severity of sleep disordered breathing concomitantly with a reduction in sympathetic nervous system activity. We emphasize that prevention of ventilatory instability, *per se*, is most likely to reduce cyclical apneas in patients with mild to moderate levels of airway collapsibility, and less likely in those with severely compromised airway anatomy (7,8).

We have demonstrated that OSA impairs hypercapnic vasodilation in the cerebral circulation (87). In health, cerebral resistance arteries are exquisitely sensitive to changes in $PaCO_2$ (88). This important characteristic of the cerebral circulation minimizes the change in brain PCO_2 during fluctuations in arterial PCO_2. Reductions in cerebrovascular CO_2 responsiveness could exacerbate breathing instability during sleep by exaggerating the accumulation and also the washout of CO_2 from central chemoreceptors during fluctuations in ventilation and arterial PCO_2. We have shown, in healthy humans during wakefulness, that cerebral CO_2 responsiveness is an important determinant of eupneic ventilation and also the ventilatory response to hypercapnia (89). Furthermore, we have shown during sleep in healthy subjects, that reducing the cerebral vascular response to CO_2 with indomethacin decreases the CO_2 reserve below eupnea ($\Delta PaCO_2$, eupnea-apnea) and increases the susceptibility to apnea (90).

In patients with OSA, attenuated cerebrovascular CO_2 reactivity and exaggerated carotid chemoreflex sensitivity are both reversible when sleep disordered breathing is eliminated with CPAP (3,87,91). We hypothesize that pharmacological measures targeting the mechanisms involved also may reverse these impairments and lead to a reduction in the severity of sleep disordered breathing in selected patients.

1.2 Preclinical Data

N/A

1.3 Dose Rationale and Risk/Benefits

Drug selection and dose rationale: In our rat studies, an allopurinol dose of 65 mg/kg/day (~300mg/day in humans) (134) effectively reduced AT1R and superoxide concentrations in the

rat CCR (see Preliminary Studies). Subjects in our study will take allopurinol 300 mg daily for six weeks (dose will remain unchanged during four-week period). Allopurinol is quickly converted in vivo to its active metabolite, oxypurinol. Oxypurinol is responsible for the majority of the effects of allopurinol and has a half-life of 17-21 hours (135). Steady state blood concentrations of oxypurinol occur in approximately 105 hours (4.4 days). A study demonstrating vascular benefits of XO inhibition in patients with OSA was two weeks in duration, and our rat study used two weeks of CIH. We propose to have subjects take six weeks of study medication to increase the likelihood of observing significant sympathetic, vascular and blood pressure changes and to determine if preliminary changes seen after two weeks are sustained. A 300 mg daily dose will sufficiently inhibit XO because the IC_{90} of oxypurinol for XO is approximately 5 µg/mL, and a single 300 mg dose of allopurinol produces peak levels of 5-7 µg/mL (136-138). At steady state, peak drug concentrations are higher than peak concentrations from single doses, and > 90% of XO will be inhibited. Losartan will be used in the doses that are clinically used to treat hypertension (50-100 mg daily by mouth). E-3174 is the active metabolite responsible for the clinical effects of losartan and E-3174 has a half-life of 4-9 hours (139). Thus, steady-state concentrations of E-3174 are quickly achieved after approximately 20-45 hours (139). Use of this dose is based on data demonstrating that 100 mg doses produce plasma concentrations > 1000 ng/mL of E-3174 (140). The ability of E-3174 to block the pressor effects of an intravenous angiotensin II challenge plateaus between 500-750 ng/mL (141). Thus, we feel our proposed doses will effectively block the actions of A-II.
We have chosen a six week treatment period because in our previous study, vascular endothelial function was improved after 6 weeks of treatment and did not further improve after 12 weeks of treatment. Furthermore, three weeks of losartan therapy successfully prevented chemoreflex sensitization in adult male, Sprague-Dawley rats.

Risks to subjects are reasonable in relation to the anticipated benefits because the two medications are FDA approved with significant clinical history to base safety upon. If the study drugs are proven to be effective for the primary endpoint, they may undergo further trials as potential interventions for prevention of hypertension and cardiovascular disease in patients with OSA.

2 Study Objectives

1. Determine if treatment with losartan, an angiotensin type I receptor (AT_1R) antagonist, or allopurinol, a xanthine oxidase (XO) inhibitor, normalize chemoreflex control of sympathetic outflow and ventilation and improves local vascular regulation and stiffness.
2. Determine if these interventions reduce the severity of sleep disordered breathing and lower diurnal blood pressure.

3 Study Design

3.1 General Design

The proposed project is a randomized, double-blind, placebo-controlled, parallel three-treatment arm phase II clinical evaluation of allopurinol, losartan, and placebo in hypertensive patients with severe OSA. The proposed trial is based on preliminary data suggesting that both of these available medications may reduce chemoreflex sensitivity and endothelial function in patients with OSA. Subject enrollment will be conducted on a rolling basis during the three year study period and subjects will be randomized (stratified by baseline CPAP adherence) to one of the three treatments. Randomization will be stratified based on three categories of baseline CPAP

adherence: < 1 hr, 1 – 4 hrs, and > 4hrs of CPAP use on average per night. This will ensure balanced CPAP use between treatment arms. Allopurinol will be initiated at 300 mg daily and the dose will remain constant for the six-week study session. Losartan will be initiated at 50 mg daily and the dose will be increased to 100 mg after two weeks if systolic blood pressure is above 120 mm Hg and as long as serum creatinine and potassium do not significantly increase. Subjects with SBP between 120-100 mm Hg may increase to 100 mg if approved by physician. If the subject's serum creatinine (> 0.5 mg/dL) or serum potassium (above 5.0 mEq/L) significantly increase, or if the subject experiences a significant side effect from the medication (e.g. maculopapular rash) the subject will be discontinued from the study. If the subject does not tolerate the losartan dose increase to 100 mg, his/her dose will be reduced back to 50 mg by study staff at request of subject. If the subject tolerates the dose increase, the losartan dose will then remain unchanged from the second to sixth week of the study. Patients in the placebo group will receive placebo capsules daily for six weeks that are identical in appearance to the overencapsulated losartan and allopurinol.

Hypertensive patients who have severe sleep apnea (apnea hypopnea index or respiratory disturbance index > 25 events/hour) will be studied to allow the greatest likelihood of detecting significant differences in outcome variables. We will take patients who are and who are not using CPAP and will stratify their randomization based on baseline CPAP use. This will be done to simulate real-world treatments as best as possible and determine if our therapies' effectiveness is modified by CPAP use. All subjects will undergo a screening visit (Visit 1) at their local site to determine eligibility and collect pertinent data. Subsequently, subjects will be randomized to one of the three treatment groups (allopurinol, losartan, or placebo) and will have a physiology and vascular baseline study completed (Visit 3).

Subjects will have 24-hour ambulatory blood pressure and 24-hour Holter/Heart rate variability measurements taken* (Visit 2) on a day/night prior to Visit 3. Prior to starting study drug therapy, subjects will come to the Clinical Research Unit (CRU) at the University of Wisconsin for physiology/vascular study (Visit 3). Subjects recruited from the other recruitment sites will come to Madison for Visit 3. Subjects will arrive on day one of their study, and will have physiologic assessments taken before full overnight polysomnography and overnight Holter/Heart rate variability* is completed during night one (to determine severity of OSA). Prior to polysomnography, subjects will have muscle sympathetic nerve activity, forearm blood flow, cerebral blood flow velocity, plasma catecholamines, ventilation, blood pressure, and heart rate measured at rest under normoxic (room air) conditions and during graded levels of hypoxia (90%, 85% and 80% SaO2). Following this, subjects will be allowed to return to normoxia (room air levels of oxygen ~ 21% oxygen) and after dinner will be hooked up for polysomnography and Holter/Heart rate variability*. The morning after polysomnography, subjects will have assessment of brachial artery flow-mediated vasodilation and nitroglycerin induced vasodilation measured by ultrasound. Then subjects will have aortic pulse wave velocity and augmentation index measured by arterial tonometry. The 2nd night subjects will stay overnight again in the VA Research Unit and have control of breathing studies completed (Pcrit and apnea threshold). Subjects will initiate study drug treatment and will return two weeks later for a blood pressure and heart rate (office blood pressure) measurement, CPAP assessment, and safety assessment to measure serum creatinine and serum potassium concentrations. This is standard safety monitoring in patients taking ARBs, and to maintain blinding, all subjects will have this monitoring completed. Subjects who do have clinically significant increases in serum creatinine (an increase > 0.5 mg/dL) or whose serum potassium increases to a concentration > 5.0 mEq/L will have their participation in the study terminated. Subjects continuing in the study will then complete four additional weeks of drug therapy. At the end of six weeks of study medication, subjects will have 24-hour ambulatory blood pressure and 24-hour Holter/Heart rate variability assessed one day and night.

On a following night, subjects will return to Madison for physiology studies, overnight polysomnography and Holter/heart rate variability measurements*, vascular function measures and control of breathing studies the 2nd night to assess the study drug effects on variables measured at baseline. Subjects will be instructed not to consume caffeine (both solid and liquid forms) after midnight the night before they are admitted to the CRU and after 8pm on night 1. Subjects may consume caffeine after the physiology study on Day 1 and before 8pm on Day 1. Subjects will also be instructed not to smoke after 10 pm once they are admitted to the CRU until discharged. Subjects will not be allowed to participate in the study if they are unable to refrain from smoking after 10 pm for up to 24 to 48 hours once they are admitted to the CRU until discharged.

*Holter and Heart rate variability measurements are optional and will only be offered to UW subjects. They can choose to opt-in or opt-out of those procedures. Subjects who are non-CPAP users will have Holter monitoring done at Visits 3 and 6, but will not have Holter monitoring performed at Visits 2 and 5. The Holter monitors will be connected by UW Sleep Laboratory Technicians or study team personnel. Subjects may disconnect the Holter monitor on their own, or they may come back to UW-Madison to have the Holter monitor removed. A postage-paid envelope will be provided to subjects to return to Holter monitor by mail.

3.2 Primary Study Endpoints

The primary outcome variable is the difference in the individual slope of the MSNA – SaO_2 response curve at 6 weeks and baseline between treatment groups. Basal normoxic MSNA and MSNA responses during hypoxia will be compared between the three treatment groups. For purposes of quantification, MSNA is expressed as burst frequency (bursts/min), burst amplitude (arbitrary units) and total minute activity (burst frequency x mean burst amplitude). Total minute activity of mSNA is an effective way to capture the change in both burst frequency and burst amplitude. Both amplitude and burst frequency are influenced by disease states such as OSA and by environmental conditions such as hypoxia. Postganglionic mSNA in the right peroneal nerve will be recorded directly using the microneurography technique (95). The neural signals are passed to a differential preamplifier, an amplifier, a band-pass filter, and an integrator (time constant=100 ms). Placement of the recording electrode within a muscle nerve fascicle is confirmed by the presence of muscle twitches in response to electrical stimulation, the appearance of afferent activity in response to tapping or stretching of muscle, but not gentle stroking of skin, in the appropriate receptive fields, and other criteria. Once an acceptable neural recording (signal-to-noise ratio >3:1) is obtained, the subject is instructed to maintain the leg in a relaxed position for the duration of the study. Sympathetic bursts will be identified by computer-assisted inspection of the mean voltage neurogram.

3.3 Secondary Study Endpoints

Secondary outcome variables will include assessment measures of cerebral and forearm blood flow during basal conditions and during graded hypoxia before and after study drug treatment. Plasma catecholamine concentrations before and after study drug and change in plasma catecholamines during hypoxia will be assessed as a secondary indicator of sympathetic activation. Other secondary outcome variables will be assessed before and after study drug treatment, including: ventilation, aortic pulse wave velocity (a measurement of vascular stiffness), aortic augmentation index, % vasodilation during flow-mediated vasodilation (measurement of vascular endothelial function), apnea threshold, apnea-hypopnea index, % time spent below 90% oxygen saturation, heart rate variability, holter electrocardiogram measurements, and mean 24-

hour blood pressure, mean nighttime blood pressure, mean daytime blood pressure, blood pressure load, and night/day blood pressure ratio.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Inclusion Criteria
- Males and females between ages of 21 and 65 years inclusive
- Apnea hypopnea index or respiratory disturbance index (RDI) greater than or equal to 25 events/hour (diagnosed by Polysomnography or home study)
- Subjects eligible for CPAP or BiPAP therapy. If subject is on CPAP or BiPAP, he/she must be on treatment for a minimum of 3 months prior to Visit 2.
- Hypertension by clinical history of high blood pressure readings or diagnosis of hypertension (may be controlled with non-exclusionary medications) or average blood pressure ≥ 140/90 mm Hg (only require systolic OR diastolic to be above threshold, not both for inclusion). Blood pressures used for inclusion based on average blood pressure ≥ 140/90 mm Hg will be (using two measurements in prior 12 months – in PHI or other records OR 1 prior elevated blood pressure and 1 blood pressure at screening)

4.2 Exclusion Criteria

Exclusion Criteria
- If subject not using CPAP, having AHI > 60 events/hour or oxygen saturation ≤ 65% during sleep
- Presence of clinical CV disease (coronary artery disease, angina, arrhythmias (subjects with sinus arrhythmias will be reviewed by PI for enrollment), stroke, TIA, cor pulmonale, etc.), heart failure, bruits, or diabetes mellitus by clinical diagnosis/history
- Presence of pulmonary disease that results in significant hypoxemia (resting SaO_2 < 88%)
- Hypertriglyceridemia (triglycerides >300 mg/dL).
- Diabetes (fasting plasma glucose > 125 mg/dL)
- Subjects taking angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, renin inhibitors, potassium-sparing diuretics (without accompanying loop/thiazide diuretic), allopurinol, oxypurinol, febuxostat, amoxicillin, ampicillin, azathioprine or mercaptopurine. *(see below for examples)
- Subjects with chronic kidney disease (Serum creatinine >1.5 mg/dL) or history of significant hyperkalemia (Serum potassium > 5.2 mEq/L) with ARB therapy
- Subjects with history of angioedema
- Subjects with bilateral, modified radical or radical mastectomies
- Subjects who have a Serum potassium > 5.0 mEq/L at the screening visit
- Female subjects who are pregnant (determined by urine pregnancy test) or breastfeeding
- Subjects with active MRSA or VRE (vancomycin resistant enterococcus) infection
- History of adverse reaction to allopurinol,losartan, or zolpidem**
- Subjects who are currently receiving cancer treatment or have received cancer treatment in the past 1 year.
- Subjects who cannot swallow oral capsules
- Subjects who are hospitalized or who have been recently hospitalized (last 2 weeks)

- Inability to comply with or complete the protocol or other reasons at the discretion of the investigators

*angiotensin converting enzyme inhibitors (lisinopril, enalapril, etc.)
Angiotensin receptor antagonists (losartan, valsartan, etc.)
Renin inhibitors (aliskiren)
Potassium sparing diuretics (spironolactone, eplerenone, triamterene without hydrochlorothiazide, etc.)

**If adverse reaction to zolpidem, may opt out of control of breathing study

4.3 Subject Recruitment and Screening

Screening Visit (Visit 1): Subjects will come to the CRU or their local recruitment site for a brief screening visit. **Subjects should be instructed to be fasting for at least 12 hours prior to their visit and patients should hold any blood pressure medications on the morning of VISIT 1 (they may take at end of VISIT or after).** Investigators will explain the study, answer questions, and complete the informed consent and HIPAA process. Potential subjects identified through sleep databases and registries will be contacted via telephone to obtain verbal consent or declination for participation in this study. A waiver of written documentation of informed consent has been approved by the IRB to obtain consent via telephone. At the in person screening visit, the subject will then sign a consent form witnessed by the study coordinator or investigator. The subject will then receive a signed copy of this consent form. All copies of the consent forms will be maintained in the subject's binder. The subject will then undergo:

- a history and physical with the CRU nurse practitioner
- undergo a 12-lead electrocardiogram (if the electrocardiogram has abnormalities of clear clinical significance, then a copy of the EKG with a letter explaining those abnormalities will be sent to the subject with instructions to share with his/her physician).
- take a urine pregnancy test (for females of child-bearing age),
- have a fasting blood sample taken to measure serum cholesterol, potassium, creatinine, uric acid, and glucose concentrations

If the subject declines participating in the study, any personal and health related information will not be retained and will be destroyed upon completion of the telephone call.

Subjects will not receive study medications until the end of Visit 3 and will be instructed to bring their study medication bottles with them to Visit 4 (two weeks after starting medication) and Visit 6 (6 weeks after starting medication). Study drug diary evaluation will be used to evaluate study drug adherence. Subjects will receive an auto-CPAP machine or CPAP compliance card to use during the study. Subjects will not receive payment for Visit 6 if they do not return the CPAP machined or CPAP compliance card. Subjects will be instructed to maintain the baseline CPAP use throughout the study. Subjects will complete baseline 24-hour ambulatory blood pressure monitoring (Visit 2) prior to receiving either study drug. This will take place prior to their overnight visit to Madison (Visit 3).

Study subjects will be recruited from the Wisconsin Sleep Center in Madison, Marshfield Clinic in the greater Marshfield, WI area, Aurora Bay Care in Green Bay, and Gundersen Health System in La Crosse, WI. Wisconsin Sleep initiated a research subject registry and database in August, 2008, and the subject registry invites all patients referred to clinic or laboratory to consider being contacted about research projects. Data that is maintained in the subject database includes patient name, date of birth, gender, contact information, sleep study data, and diagnoses. Drs. Dopp and Teodorescu will also recruit subjects at Wisconsin Sleep in their own clinics. Subjects will be recruited in clinics at Wisconsin Sleep and after their sleep study at Wisconsin Sleep or

after a home study done by Wisconsin Sleep. Additional advertisements will be made available to subjects at other Sleep laboratories and clinics in the area, as well as at other clinics and medical centers in the greater Madison area (including, but not limited to: Baraboo sleep lab/clinic, Sauk prairie hospital sleep lab/clinic, St. Mary's sleep lab/clinic, Portage sleep lab/clinic, Reedsburg sleep lab/clinic, etc. Permission will be obtained prior to posting flyers or advertisements. An opt-out letter will be sent to qualifying subjects and the subjects will have 7 days to opt out before we will contact them to provide further information about the study and inquire as to their interest. Additional subjects who have participated in previous studies by investigators or other research groups who have expressed interest in future sleep apnea studies will be contacted about this study. Recruitment will also be completed with the use of social media and the internet. We will advertise on the UW Job Board, Craigslist, and Wisconsin Sleep's website. We will implement an email blast to the UW Madison students, faculty and staff as well as use campus and local newspapers and other media.

Recruitment at other sites:

We will also recruit from the sleep laboratories and sleep clinics at Marshfield Clinic, Marshfield, WI, Gundersen Health System, La Crosse, WI, and Aurora Bay Care, Green Bay, WI. Subjects will be recruited by the responsible investigators at the individual sites with the help of study coordinators. Subjects will be identified following sleep studies in the sleep laboratories and during visits to clinic. Subjects will be contacted and approached about study participation by individuals involved in his/her clinical care. Subjects may also be identified using advertisements in the community and at the local medical centers if necessary.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Allopurinol
If a subject experiences an adverse effect that is thought to be significant or serious (e.g. renal impairment, maculopapular rash, etc.) subjects' participation in the study will be ended.

Losartan
If a subject experiences clinically significant/serious adverse effects (angioedema, myalgias, acute renal failure), their participation will be ended. If the subject experiences increases in serum creatinine (> 0.5 mg/dL) or serum potassium (above 5.0 mEq/L) the subject's participation in the study will be ended. Blood pressure will also be measured at the two week monitoring visit (Visit 4). The dose of losartan will increase to 100mg daily if systolic blood pressure is > 120 mm Hg. For a systolic blood pressure between 100-120 mm Hg the subject may have dose increased if approved by study physician. If the subject's blood pressure is < 100/60 mm Hg and if the patient is dizzy and symptomatic during the study, the subject will discontinue study medication and go off study. This can be done via a phone encounter or via an additional (unexpected) visit. It is possible the subject's participation will be terminated if the study physicians feel that is appropriate.

Subjects may also be withdrawn from the study if they are diagnosed with a new and significant medical problem (e.g. they are hospitalized for an acute illness or major medical condition). Subjects may also be withdrawn from the study if they consistently fail to adhere to the protocol requirements. Study physicians will communicate with subjects' regular physician about care plans upon their withdrawal from the study.

Additional (Unexpected) Visit:
If a subject experiences an adverse event that requires them to be seen by study staff, they should be scheduled for a visit to their local center as soon as is feasible. The PIs should be notified immediately and the physician at the local site should see the subject during the visit. Depending on the complaint, the AE should be evaluated with appropriate objective measures (blood tests, blood pressure/heart rate measurements, etc.) and the case report forms for "Additional Visit" should be filled out (in subject binder). These forms include:

AE form
Vitals form
Additional visit form
AE log

The study physician will determine if the subject should be discharged from the study (if subject is discharged, then complete the "subject off study" form). If the subject is discharged from the study, then he/she should have the 30-day post study evaluation performed 30 days after their study discharge (see below).

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

We do not expect mortality or significant morbidity from the study. However, all subjects will be instructed to contact investigators if any problems arise after study participation is discontinued. Investigators will contact subjects who are withdrawn from the study 30 days after study discontinuation and periodically thereafter per study physician discretion if necessary. Data will be obtained and kept from any follow-up communications.

5 Study Drug

5.1 Description

Allopurinol and losartan are both FDA approved medications. Losartan is FDA approved for the treatment of hypertension. Allopurinol is approved for the treatment of gout. Study medications will be over encapsulated with lactose and all subjects will receive placebo with their active study medication in a double dummy design. Zolpidem will also be given to subjects (upon discharge from the CRU at Visits 3 and 6) to help them sleep during the control of breathing studies.

5.2 Treatment Regimen

Losartan 50 mg daily for two weeks, then increased to 100mg daily for 4 weeks

OR

Allopurinol 300 mg daily for 6 weeks

OR

Placebo capsule daily for 6 weeks

All three medications will be taken orally (in capsules) by subjects. The treatment regimen is summarized in Table 1.

Table 1. Treatment Regimen

Arm	Treatment	Administered as:			
PRODUCT TYPE		Active Losartan 50mg Capsule Bottle A	Active Losartan 100mg Capsule Bottle B	Placebo	Allopurinol 300mg Capsule
Group I	Losartan	1 capsule daily on Days 1-14	1 Capsule daily on Days 15-42	None	None
Group II	Allopurinol	None	None	None	1 Capsule Daily on Days 1-42
Group III	Placebo	None	None	1 Capsule Daily on Days 1-42	None

Losartan subjects will take capsules from Bottle A for days 1-14, and capsules from Bottle B for days 15-42. If the subject's systolic blood pressure is > 120 mm Hg, the subject will continue to take capsules daily from Bottle A (days 15-42). If SBP is between 100-120 mm Hg, the subject may switch to bottle B with physician approval.

Allopurinol and Placebo will also have Bottle A and B for each treatment and will contain identical drug in Bottle A and Bottle B. Subjects will transition from Bottle A to Bottle B after day 14 in the allopurinol and placebo group. This is done to maintain study blinding.

5.3 Method for Assigning Subjects to Treatment Groups

Randomization will be performed by the Pharmaceutical Research Center (PRC) using a scheme designed by the PRC and ICTR statisticians. Subjects will be randomized and stratified based on their baseline use of continuous positive airway pressure (permuted block randomization). Subjects will be classified into the following three categories of baseline CPAP use: 1) < 1 hour per night; 2) between 1 and 4 hours per night; and 3) > 4 hours per night. Subjects not using CPAP will be classified in the < 1 hour per night group. Subjects will be instructed to continue the same CPAP use during the study as they were using at study entry.

5.4 Preparation and Administration of Study Drug

Study drug will be over encapsulated by the University of Iowa Pharmaceuticals (UIP) and stored and distributed by the University of Wisconsin Pharmaceutical Research Center and blinded according to a pre-designed scheme. Subjects will be randomized at the end of visit 1 (screening visit) and will receive study drug at their baseline physiology study visit (visit 3). UIP is an FDA-registered facility compliant with 21 CFR parts 11, 210, and 211, licensed by the DEA to handle controlled substances (Schedules I-V), and capable of handling potent/cytotoxic materials. UIP has been a cGMP facility for over 30 years.

5.5 Subject Adherence Monitoring

All subjects will receive instructions on how to take and record medication adherence in a diary. Subjects will not receive study medications until the end of the Physiology Visit in Madison (Visit 3) and will be instructed to bring their study medications with them to Visit 4 (two weeks after starting medication at local study site) and Visit 6 (Six weeks after starting study medication – in Madison). Serum uric acid concentrations and plasma renin activity will be measured at baseline and after 6 weeks of study medication and will provide an indirect measure of drug adherence in subjects receiving allopurinol and losartan. Adherence to CPAP therapy will be monitored at Visit 4 (two weeks after starting medication) and Visit 6 (Six weeks after starting study medication). CPAP adherence can be determined from the electronic data card in the CPAP machine and downloaded into a printable report. Each site will have a card reader and software for

downloading the information from subjects' CPAP machines at Visit 4. Subjects on BiPAP will use their machines instead of ours, and we will attempt to get their adherence data from them.

5.5.1 CPAP Before and During Study

Subjects who are using CPAP or BiPAP prior to the study will be asked to provide adherence data for the time period prior to them entering the study. Subjects who require CPAP or BiPAP must be on treatment for a minimum of 3 months prior to Visit 2. This information will be obtained from their CPAP/BiPAP machine or from their home health provider that services their CPAP/BiPAP machine. We will also ask for their permission to contact their sleep physician to obtain a copy of their sleep study report. This will help us to determine how severe their sleep apnea is. Consent to obtain this information from home health providers and sleep physician will be obtained in the informed consent document. Subjects who qualify for the study will be instructed to continue to use CPAP/BiPAP the same amount during the study as they were using prior to the study. If subjects are not using CPAP/BiPAP prior to study entry, they will be instructed to continue not using CPAP/BiPAP during the study.

At Visit 3, subjects will be given a CPAP machine or CPAP compliance card to use during the study. Subjects using BiPAP will continue to use their own BiPAP machine. We will set the pressure on this machine to the same pressure the subject was using on their machine at home. Subjects will bring their CPAP/BiPAP machine or compliance card with them to Visit 4 (at local site) and Visit 6 (in Madison) to have the adherence data checked. Investigators should remove the compliance card from the CPAP machine and download the data at Visits 4 and 6. After the download, the cards should be returned to the subjects' CPAP machines. Subjects will use the mask they had previously been using with the CPAP/BiPAP machine issued to them. The Respironics CPAP machines given to subjects during the study will be capable of measuring residual breathing events to determine how effectively the machine is treating a subject's sleep apnea.

5.6 Prior and Concomitant Therapy

Information about all medications (prescription, OTC and supplements) being taken will be collected at Study visits 1, 1A, 2, (if more than 90 days after Visit 1), 3, 4, 5, 6, and 7. Subjects are instructed to let the study coordinator know if they start any new medication while they are enrolled in the trial. Other antihypertensive agents (diuretics, calcium channel blockers – see Appendix 3 for allowable blood pressure medications) will be allowed but doses should remain constant during the study period. Subjects taking non-hypnotic benzodiazepines, narcotic analgesics, quetiapine, or barbituates, on a daily basis or have taken them within 5 days (when using them p.r.n. (pro re nata or as needed) before the control of breathing study will not be allowed to participate in the control of breathing study (night 2, visits 3 and 6). However, if a subject is taking a medication for sleep (e.g. hypnotic benzodiazepines, zaleplon, eszopiclone, etc.), they may participate in the control of breathing study if zolpidem may be taken instead of their usual sedative hypnotic for the control of breathing study. Subjects should not take PDE-5 inhibitors (ie. sildenafil (Viagra), vardenafil (Levitra), or tadalafil (Cialis) within 72 hours (3 days) before or after taking nitroglycerin. If a subject has taken any of these medications within that timeframe he/she will not be able to participate in the Stein lab portion of Visit 3.

The following medications are not allowed to be taken:
Angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, renin-inhibitors, potassium-sparing diuretics, allopurinol, oxypurinol, febuxostat, amoxicillin, ampicillin, azathioprine or mercaptopurine.

5.6.1 Pregnancy Testing and Birth Control

Women of child-bearing age are eligible subjects for this study. However, since study drugs are pregnancy rate "D" (losartan) and "C" allopurinol, female subjects will take a urine pregnancy test at Visit 1 to ensure they are not pregnant. A urine pregnancy test will be repeated at Visit 1A if the study visit occurs more than 90 days after Visit 1. Furthermore, a urine pregnancy test will also be given at Visit 3 prior to study drug dispensing. The Pregnancy and birth control case report form should be completed at Visit 1, Visit 1A (if applicable) and Visit 3. On this case report form the method of birth control used should be documented and subjects are instructed to keep their birth control consistent throughout the study. Methods of birth control that are acceptable while participating in this study include oral contraceptives, hormonal contraceptives, hormonal implants, contraceptive patches, NuvaRing, intrauterine device hormonal, intrauterine device non-hormonal, barrier method, spermicide, post-menopausal for greater than or equal to two (2) years, tubal ligation, bilateral oophorectomy, or hysterectomy, vasectomy, or abstinence. If a female is practicing abstinence, she should confirm this, including duration, and initial and date the case report form.

5.7 Blinding of Study Drug

Allopurinol and Losartan tablets will be over encapsulated and dispensed to subjects with a placebo using a double-dummy design. Both subjects and investigators will be blinded to study drug assignment. The PRC will have unblinding information should the need arise to unblind a subject's study drug assignment. To obtain unblinding information, please call the University of Wisconsin Pharmaceutical Research Center at (608) 263-8863.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Drug Supplies

The PRC will order, receive and store all study medications for the trial. The PRC complies with all Federal and State regulations and current good manufacturing practices.

5.8.2 Dispensing of Study Drug

The PRC performs all required study drug reconciliation and inventory procedures on a regular basis. PRC drug record logs record the movement of study drugs out of PRC storage and all medications are checked by a PRC pharmacist prior to dispensing to the subject and study staff. Amount of study drug on hand and dispensed is constantly tracked in PRC records. All study drugs will be dispensed by the PRC to subjects during Visit 3 in Madison. Subjects will receive all study drugs for the 6-week treatment period at Visit 3 and will receive instructions from investigators prior to starting therapy. Zolpidem (supplied by the PRC) will be given to subjects upon discharge from the CRU at VISITS 3 and 6 to be taken immediately before (9-10pm) the control of breathing study on night two.

6 Study Procedures

See Appendix 2

6.1 Study Personnel

Study subjects will be recruited from the Wisconsin Sleep Center in Madison (Dr. John Dopp, Dr. Barbara Morgan, and the study coordinator(s)); Marshfield Clinic in the greater Marshfield, WI area (Dr. Boero and study coordinator(s); Aurora Bay Care in Green Bay (Dr. Gapinski and study coordinator(s)); and Gundersen Health System in La Crosse, WI (Dr. Tobert and study coordinator(s)). Dr. Dopp and Dr. Morgan will lead the overall project as coinvestigators at the University of WIsconsin-Madison. WiNHR Central Administration Staff (Deb Kruser, Director, MJ Washburn, Regional Research Networks Coordinator, and Kelly Miller, Senior Research Coordinator) in collaboration with the UW Site Study Coordinator will also assist with data management, site communication and oversight of study conduct. As described in section 8.7, CRIS Study Monitoring Personnel will perform monitoring throughout the project.

6.2 Summary of Visits

Subjects may complete some, but not all procedures without a protocol deviation occurring or affecting their study eligibility. For logistical or other reasons, subjects may complete some of the procedures (but not all procedures) (i.e. in cases of non-exclusionary subject disabilities that preclude them from having certain procedures done or body habitus that does not allow completion of certain procedures.). Reimbursement for participation will be pro-rated for Visits 3 and 6 as in protocol section 11.2.

Visit 1: Screen Visit (will be performed at each respective recruitment site)

- informed consent, height and weight, heart rate, blood pressure, temperature, and electrocardiogram recorded, blood samples, urine pregnancy test, brief history (performed by study coordinator and local PI)
- physical examination (local PI and UWHC medical staff (i.e., physician, physician's assistant, or nurse practitioner) for Madison subjects)

Visit 1A: If visit 2 will occur more than 90 days after visit 1, subjects will undergo repeat screening procedures in a separate visit to the local study site (Visit 1A) including:

Medical history, medication history, birth control assessment, blood samples, urine pregnancy test, AE assessment, electrocardiogram recorded (performed by the local PI, study coordinator, or medical staff (i.e., physician, physician's assistant, or nurse practitioner)

Visits 2 and 5: Home Blood Pressure Monitoring (will be performed at each local site where subjects were originally enrolled)

- Receive blood pressure monitor and Holter/heart rate variability monitor* to wear for 24 hours along with the diary recording of activity each time they engage in activity and each time the blood pressure cuff inflates; subject returns to the study site the following day (all activities performed by coordinator--local PI consulted if necessary). Subjects at UW-Madison will come to the School of Pharmacy Clinical research space of the Office of Clinical Trials Examination Space (H6/136 CSC) to be set up with their 24-hour blood pressure cuff and Holter/heart rate variability monitor. Adverse events will be assessed at visits 2 and 5.

*Holter and Heart rate variability measurements are optional and will only be offered to UW subjects. They can choose to opt-in or opt-out of those procedures. Subjects who are non-CPAP users will have Holter monitoring done at Visits 3 and 6, but will not have Holter monitoring performed at Visits 2 and 5." The Holter monitors will be connected by UW Sleep Laboratory Technicians or study team personnel. Subjects may disconnect the Holter monitor on their own, or they may come back to UW-Madison to have the Holter monitor removed. A postage-paid envelope will be provided to subjects to return to Holter monitor by mail.

Visits 3 and 6: (each visit requiring a one or two night stay in Madison at the CRU at UW and the VA Hospital for all subjects enrolled in the study)

- Day 1: Vital signs, Heart Rate; Forearm Blood Flow; Blood Samples; Cerebral blood flow; Nerve activity; Reduced oxygen exposure and increased oxygen breathing; overnight sleep study and heart rhythm measurements at the CRU to measure brain waves, respiration, blood oxygen level, snoring, leg movements, heart rhythm, and heart rate variability* (performed by PIs and sleep technician). A physical exam will also be obtained. If bruit are noted on physical exam the subject will no longer be able to participate in the study. The presence of a bruit would potentially indicate previously unrecognized cardiovascular disease.
- Day two: Ultrasound of blood vessels and heart; non-invasive tonometry; assessment of CPAP use; assessment of daytime sleepiness by completing the Epworth sleepiness scale; Study Medication dispensation (V3) and collection (V6); Control of Breathing Experiments during sleep stay at VA hospital. Subjects will receive zolpidem (sleeping medication) upon discharge from the CRU to be taken around 10pm at the start of the control of breathing study. (Ultrasounds performed in Dr. Stein's laboratory; control of breathing studies performed by sleep technician). Subjects who do not have someone to drive them home, do not want to take a complimentary cab home or do not want to go back to the CRU to sleep and have breakfast prior to having a driver/cab take them home from Visits 3 and 6 may opt out of the control of breathing study on the second night. When a subject arrives for Visits 3 and 6, he/she will be asked if they have a driver to take them home, need a cab ride home or if they agree to go back to the CRU to sleep and have breakfast prior to having a driver/cab take them home after the control of breathing study.
- Visit 6 will also include AE Assessment and diary review in addition to the procedures mentioned above.

*Holter and Heart rate variability measurements are optional and will only be offered to UW subjects. They can choose to opt-in or opt-out of those procedures. Subjects who are non-CPAP users will have Holter monitoring done at Visits 3 and 6, but will not have Holter monitoring performed at Visits 2 and 5." The Holter monitors will be connected by UW Sleep Laboratory Technicians or study team personnel. Subjects may disconnect the Holter monitor on their own, or they may come back to UW-Madison to have the Holter monitor removed. A postage-paid envelope will be provided to subjects to return to Holter monitor by mail.

Visit 4: Monitoring visit (performed at each local site where subject was enrolled)

- measurement of blood pressure, heart rate, temperature, and height and weight, blood sample collection, Study drug compliance and diary review, AE assessment (all performed by local site coordinator or CRU nurses for Madison subjects)

Visit 7: One month Follow-up (performed at each local site where subject was enrolled)

- Subjects will be contacted by phone for completion of a final AE assessment (performed by local site coordinator)

6.3 Sample collection

Blood samples collected during Visits 1 and 4 will be processed for the specific laboratory parameter at the local site and assayed in the clinical labs (or couriered from LaCrosse to Marshfield to be assayed). Samples will be collected and sent to the clinical laboratory for measurement of serum creatinine, serum potassium, serum glucose, lipid panels, serum uric acid, etc. At Visits 3 and 6 at UW-Madison, blood samples will be drawn at rest while the subject is breathing room air. Uric acid will be collected and assayed in the UW Hospital and Clinics Clinical Laboratory and other samples will be collected for research use. These samples collected for research use will be centrifuged, aliquoted, and stored in the locked freezer space at the University of Wisconsin School of Pharmacy for future assay. Stored blood samples will be stripped of identifiers and only labeled with study subject number and Visit number. Plasma will be sent to the TTR Analytical Laboratory Core at the University of Iowa College of Pharmacy for plasma catecholamine measurement. Other plasma will be assayed at the UW School of Pharmacy for oxidative stress markers.

6.4 Study communication and oversight

At the start of the trial, study binders will be set up for each study site with the current protocol version (will include protocol number and date). At any time during the study when the protocol changes, a new protocol will be sent to each site to be included in the study binder and to be kept in a relevant study folder electronically with the study coordinator. The new protocol will be sent along with a summary of protocol changes denoted separately to the study coordinator, the IRB and co-Investigators. Discussion of protocol changes will also be an item of discussion at the bi-weekly WiNHR coordinator conference calls.

When the UW HS IRB approves amendments to the protocol, copy of the approval will accompany the summary of changes approved and the new protocol version sent to the study sites. Electronic copies of the approval documents will be forwarded to each site with the other electronic documents and stored electronically and in paper form in an IRB folder at each study site. At each biweekly WiNHR coordinator conference call, reconciliation of all current approvals will be performed by all the investigators and their study binders and electronic files.

Reporting of adverse events and unanticipated problems will be performed at all centers through the Principal Investigators to comply with the UW HS IRB requirements. Investigators at all study sites will be instructed to notify the Principal Investigators of all adverse events immediately upon awareness of their occurrence. The Principal investigators and the study coordinator in Madison will provide the information to the DMC and the IRB in the appropriate

time frame. Unanticipated problems will be discussed at each WiNHR biweekly coordinator conference call and at quarterly investigator meetings.

For events that are unexpected, immediately life threatening or severely debilitating to other current subjects, and caused by or probably related to the study drug, the DMC and IRB will be notified immediately.

Any deviations from the protocol at any of the research sites will be discouraged and if occur, will be reported to the UW HS IRB if the deviation or exception presents a significant safety risk to the subject or potential subject. As per Good Clinical Practices, protocol deviations will be documented and reviewed at the quarterly investigator meetings. If changes are necessary for the project, protocol modifications will be submitted to the HS IRB to change the protocol to fit the required changes.

6.5 Unexpected Findings

Whenever tests like the electrocardiogram (Visit 1), cardiac output (Visits 3 and 6), or serum laboratory tests are done, there is the chance of an unexpected finding. Subjects will be informed of all findings of clear clinical significance which may be revealed during the electrocardiogram, cardiac output procedure (limited echocardiography), and serum laboratory tests. Findings of clear clinical significance are those for which we generally know the risks of non-treatment and for which treatment may be available. If unexpected findings for the electrocardiogram, cardiac output, or serum laboratory tests are discovered, a letter describing the findings will be sent to the subject and to his/her physician with permission from the subject.

7 Statistical Plan

7.1 Sample Size Determination

The primary outcome will be the change in the slope response of MSNA during progressive hypoxia from baseline to 6 weeks. From previous data from our lab we have seen an approximate standard deviation for slope in OSA patients under similar progressive hypoxia of 8-10.

Based on interim data, a power calculation for the differences and standard deviations in primary outcome variable estimated that we would have 90% power (this is increased power from our original sample size calculation that used 80%) to determine a difference of 3.07 (1.13 vs -1.94) at a significance level of 0.017 (Bonferroni adjusted alpha for 3 tests) if we had complete MSNA slope response data from 20 subjects per group.

We had originally proposed to enroll 50 subjects per group. Our interim analysis determined that if we obtain complete data in 20 subjects per group, we are likely to find a significant difference. We will enroll as many subjects as we can in the remaining time frame to give us the best possible chance at finding a difference. Consistent with DMC recommendations, we will reduce our sample size to 100 subjects. To account for drop-outs, we will ask to enroll 120 subjects to get 100 subjects who proceed to randomization.

7.2 Statistical Methods

Data will be analyzed by a statistician blinded to drug assignment. Demographic comparisons to assess effectiveness of randomization will be examined between treatment arms by ANOVA and chi-square tests where appropriate. Differences in baseline values for all variables between treatment arms will be assessed using ANOVA. The paired differences in these same variables will be compared after the 6-week study period. These values include all measured physiologic variables, such as resting heart rate, blood pressure, plasma catecholamine concentrations, MSNA, cerebral blood flow, forearm blood flow, flow-mediated vasodilation, pulse wave velocity, augmentation index, polysomnographic measures, Pcrit, apnea threshold, and others.

The primary outcome variable is the difference in the individual slope of the MSNA – SaO_2 response curve at 6 weeks and baseline between treatment groups. The MSNA – SaO_2 response curve will be graphed with MSNA as the dependent variable and SaO_2 as the independent variable during progressive hypoxia. Slope response curves will also be calculated for heart rate, ventilation, forearm blood flow, cerebral blood flow, and blood pressure during hypoxia, hyperoxia and controlled breathing periods. MSNA will be expressed as burst frequency (bursts/min), burst amplitude (arbitrary units) and total minute activity (burst frequency x mean burst amplitude). MSNA will be averaged over the 5-min measurement periods at baseline, 90%, 85%, 80% hypoxia, and during controlled breathing. During hyperoxic trials, 15-sec averages of MSNA will be obtained, and an average of the 4 trials will be computed. The slope response of MSNA during progressive hypoxia will be calculated and will be analyzed using ANOVA. Changes in polysomnographic measures from baseline to the end of the 6-week treatment period between the treatment groups will be compared using ANOVA. If significant ANOVA p-values are found, then post-hoc multiple comparison Tukey HSD tests will examine differences between each arms. Multiple regression will be used to determine the influence of subject characteristics and hours of CPAP use on the primary outcome, and on the effect of treatment on the primary outcome.

7.3 Subject Population(s) for Analysis

This study will follow a Intent to Treat (ITT) philosophy. Loss to follow-up is a possibility and can cause issues with final analysis because the “missingness” of the data may not be random. We will try to determine if there is any cause for drop out and summarize the reasons accordingly. We have accounted for drop outs in our projected sample sizes to assure sufficient power to remain in our tests. We will test the sensitivity of the analysis results by comparing the analysis between dropping those with missing data and utilizing last observation carried forward. In regard to the measurements taken at each time period, if a subject is unwilling or unable to complete the full progressive hypoxia we will calculate the slope for that individual based on the data we were able to attain and use that in the final analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)

- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that fulfills at least one of the following criteria:

- is fatal
- is life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
Adverse events must be reported once the subject undergoes any study procedures and adverse events must be reported during the entire active study period (6 weeks) and for 30 days following the last administration of study treatment.

General Physical Examination Findings
At screening, any clinically significant abnormality shall be recorded as a preexisting condition. Throughout the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject,

or the subject's personal physician, believes might be related to participation in this study. The investigator should notify the study sponsor and IRB of any serious adverse event or death occurring up to 30 days after the subject has discontinued or terminated study participation that may be related to this study. Study staff will contact the subject 30 days after study participation has ended to ask about possible adverse effects after study drug discontinuation.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity (e.g. serum creatinine increases more than 0.4 mg/dL)
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstance:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document (AE Assessment form), and also in the appropriate ongoing subject AE log. All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs up to 30 days after Visit 6 and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

All serious adverse events (AE) will be reported to the appropriate authorities, including the UW IRB and DMC in the required timeframe. Adverse events and SAEs will be reported to the DMC and study sponsor (NHLBI) within the same time frame as required for the IRB (see 8.3.1 and 8.3.2 below).

Investigators must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
- Multiple occurrences of an AE that, based on an aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects.
- An AE that is described or addressed in the investigator's brochure, protocol, or informed consent documents that occurs at a specificity or severity that is inconsistent with prior observations OR for which the rate of occurrence in the study represents a clinically significant increase in the expected rate of occurrence.
- Any AE or safety finding (e.g., based on animal or epidemiologic data) that would cause the sponsor to modify the investigator's brochure, study protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects.

(see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Study AE Assessment form
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the IRB

All adverse events and serious adverse events will be reported to the IRB at the appropriate and required time intervals. This will include acutely and yearly at the annual protocol renewal.

Will be reported immediately:

- Any serious adverse event that occurs any time during or up to 30 days after the research study, which in the opinion of the principal investigator meets all 3 of these criteria:
  1. Is Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)
  2. Is Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)
  3. Is Immediately life-threatening or severely debilitating to other current subjects

Will be reported within 14 days:

- Any other adverse events listed below will be reported within 14 days

1. A single occurrence of a serious, unexpected event that is uncommon and strongly associated with drug exposure (such as angioedema);
2. A single occurrence, or more often a small number of occurrences, of a serious, unexpected event not commonly associated with drug exposure, but uncommon in the study population.
3. Multiple occurrences of an AE that, based on an aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects (e.g. a comparison of rates across treatment groups reveals higher rate in the drug treatment arm versus a control).
4. An AE that is described or addressed in the protocol or informed consent documents that occurs at a specificity or severity that is inconsistent with prior observations OR for which the rate of occurrence in the study represents a clinically significant increase in the expected rate of occurrence.
5. Any AE or safety finding (e.g., based on animal or epidemiologic data) that would cause the sponsor to modify the investigator's brochure, study protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects.

Will be reported annually at continuing progress review:

- Occurred locally (or at any WiNHR study sites);
- Are related to the research study but unexpected;
- Were not assessed as placing subjects or others at increased risks (including physical, psychological, economic, or social harm) than was previously known or recognized;
- Were not assessed as resulting in new information that needed to be disseminated to participants; and

8.3.2 Sponsor reporting:

Notifying NHLBI

The investigators will appropriately notify NHLBI using expedited reporting to the NHLBI Program Officer for serious adverse events and unexpected problems. The reporting of adverse events and SAEs will be reported to NHLBI within the same timeframe as required by the IRB (see 8.3.1 above). Expedited safety reports will be sent concurrently to the DMC and NHLBI and the DMC response will be forwarded to NHLBI within 14 days.

8.3.3 Sponsor reporting: Notifying the FDA

The investigators will appropriately notify the FDA in safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening, and
- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,

- unexpected, and
- serious, but not fatal or life-threatening

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

8.4 Unblinding Procedures

Unblinding of the study medication will be done in emergent circumstances where the identity of the study medication needs to be known. All efforts will be made to maintain blinding except in the case of urgent medical necessity. If a subject needs to be unblinded, the study staff should contact the PIs and the PRC and the DMC for unblinding (using contact information below). The PI will work with the PRC to unblind the situation without compromising the blinding of remaining subjects. The investigator will follow all NIH and institutional rules for the timely and appropriate notification of the governing IRB. In most cases, the unblinding will be part of managing an SAE, and will be reported with the SAE, however, in cases where unblinding was not associated with an SAE, such actions should be reported in a timely manner.

Principal Investigators:
John Dopp: (608) 265-9352 (office phone); (608) 628-5756 (mobile)
Barbara Morgan: (608) 265-2087 (office phone)

Pharmaceutical Research Center (PRC): (608) 263-8863

Data Monitoring Committee (DMC): DMC contact Amy Siedschlag (608) 263-9508

8.5 Stopping Rules

In case there is a significant beneficial or harmful effect due to one or both of the treatment arms, which would require early termination of the study, we plan on having three interim data analyses. Interim analysis will be performed after approximately 33%, 66%, and 100% of the subjects complete the 6-week study period. Stopping criteria for benefit/harm will be based on the primary outcome variable analysis with O'Brien-Fleming alpha spending thresholds for three interim analyses. These thresholds are: p=0.0005 at look 1, p=0.01413 at look 2, and p=0.04507 at final analysis. The DMC, concurrent with and additional to the interim analyses, will meet on a quarterly basis to evaluate the protocol, subject accrual rates, subject retention, adverse events, and other aspects of the study. In those meetings which correspond to the times determined for interim analyses the DMC will also evaluate the benefit or harm of the treatments.

8.6 Independent Data and Safety Monitoring Board

The Data and Safety Monitoring Committee used for this trial will be the University of Wisconsin Institute for Clinical and Translational Research DMC. The project's statistician, Dr. DeMets sits on the committee along with other ICTR personnel to oversee the project. Mr. Hetzel (our study statistician) will make the data presentations to the ICTR DMC. Dr. DeMets will recuse himself from discussion of this project during DMC meetings to avoid conflict of interest for discussion of this protocol. In case there is a significant beneficial or harmful effect due to one or both of the treatment arms, which would require early termination of the study, we plan on having three interim data analyses. Interim analysis will be performed after approximately 33%, 66%, and 100% of the subjects complete the 6-week study period. Stopping criteria for benefit/harm will be

based on the primary outcome variable analysis with O'Brien-Fleming alpha spending thresholds for three interim analyses. These thresholds are: p=0.0005 at look 1, p=0.01413 at look 2, and p=0.04507 at final analysis. The DMC, concurrent with and additional to the interim analyses, will meet on a quarterly basis to evaluate the protocol, subject accrual rates, subject retention, adverse events, and other aspects of the study. In those meetings which correspond to the times determined for interim analyses the DMC will also evaluate the benefit or harm of the treatments.

Data will be summarized and provided directly to the DMC through the OnCore CRM clinical trial software used for data collection during the study. The DMC will keep minutes of its meetings and the PIs and all study staff will receive verbal and written summaries of their reviews and recommendations.

8.7 Study Coordination and Data Monitoring

The WiNHR Central Administrative Office personnel based at the University of Wisconsin, along with UW ICTR Clinical Research Infrastructure System (CRIS) Study Monitoring Service personnel, will complete Site Initiation Visit procedures at each performance site after IRB approval is confirmed and before enrollment of any subjects into the study. The Site Initiation Visit will include a summary of all monitoring activities and expectations during the study. The WiNHR Central Administrative Office personnel will monitor study activities at each site through biweekly study personnel conference calls and through weekly assessment of OnCore study data. In addition, the study will be formally monitored using the the (CRIS) Study Monitoring Service. Beyond the Site Initiation Visit activity described above, CRIS Monitoring personnel will perform study monitoring at each performance site following enrollment of their first subject, ongoing interim study monitoring every 3-6 months throughout the study, along with a closeout study monitoring visit upon completion of the study. Finally, this study will utilize the UW ICTR Data and Safety Monitoring Committee. The project's statisticians, Dr. DeMets and Dr. Hetzel will sit on the committee along with other ICTR personnel to oversee the project. Data will be summarized and provided directly to the DMC through the OnCore CRM clinical trial software used for data collection during the study. The DMC will keep minutes of its meetings and the PIs and all study staff will receive verbal and written summaries of their reviews and recommendations.

The University of Wisconsin will be the lead site of the study. Specific responsibilities for coordination inherent to being a lead site are as follows: 1) WiNHR will facilitate biweekly study coordinator conference calls for discussion of current accrual and study-specific questions; 2) WiNHR Central Administration personnel (Deb Kruser, WiNHR Director, MJ Washburn, WiNHR Regional Research Networks Coordinator, Kelly Miller, WiNHR Senior Research Coordinator and Greg Guilfoil, WiNHR Senior Research Coordinator.) will serve as first-line communication conduit for questions from outlying sites and will refer questions, as necessary, to the UW-Madison Investigators; 3) WiNHR Central Administration Personnel will provide regular updates to the WiNHR Advisory Committee regarding study progress and pertinent updates; 4) UW-Madison and other site Investigators will participate on WiNHR biweekly study coordinator calls, as needed; 5) UW-Madison Investigators, working with WiNHR Central Administration, will facilitate ongoing site Investigator teleconferences and/or live meetings for discussion of study progress; 6) UW Madison Investigators will participate with WiNHR Central Administration personnel in Site Initiation Visits which will be completed prior to the enrollment of subjects at any performance sites; 7) WiNHR Central Administration personnel will work with ICTR Study Monitoring Service personnel to coordinate their site visits; 8) the UW-Madison study coordinator and sleep tech will

perform study-specific activities with enrolled subjects and will communicate regularly with WiNHR Central Administration personnel and the Study Investigators.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). All subjects will sign an informed consent document and HIPAA authorization that names specific privacy and confidentiality rights. Study data will be maintained per Federal and State data policies. Subject data that is entered and stored in OnCore CRM database is coded with a study number generated by the OnCore system. The ICTR data management team will work with investigators to collect only relevant, applicable study data, which will be entered in a secure web-based system with restricted access.

OnCore is an electronic data management system that will be used to capture, edit, manage, and export study data for analysis.

The research team will manage study data through OnCore. Access to the OnCore system is restricted to those that have been granted access by the software administrators. User access requires supervisor approval, completion of HIPAA and Human Subjects Protection training, and completion of role-based training in the OnCore system. In addition, users' access is limited to protocols for which they have some responsibility of protocol, subject, or data management. Within those protocols, the ability to view and modify data is restricted based on their role in the conduct of the research project (e.g. regulatory staff do not have the privilege to view subject identifiable information).

In addition, the technical components of this software are managed by the UW-Madison's Bioinformatics Computing Group (for server maintenance, software upgrades, etc.), and security and software support is provided by ICTR administrative staff.

All communication between the clients and the OnCore application takes place via Hypertext Transfer Protocol over Secure Socket Layer or HTTPS. HTTPS provide the ability for normal web based communication over an encrypted Secure Socket Layer (SSL) connection. This ensures that data passing between the client and OnCore is protected from unauthorized attempts to access the data.

The OnCore system is web-based software, with data stored on secure servers. Data exported from the OnCore System, is exported with indirect identifiers (i.e. with study ID number per subject) for statistical and data monitoring purposes in an MS Excel or SAS format. Upon exportation, the clinical research management system has no control over how it is manipulated or managed.

9.2 Source Documents

All source data will be kept and merged and/or entered into the electronic clinical trial software (OnCore CRM). Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda,

subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a section of the CRF is left blank because the procedure was not done or the question was not asked, please document reason on the source document. If the item is not applicable to the individual case, document "N/A".

9.4 Data Collection at Sites

Data will be collected at Marshfield Clinic, Gundersen Health System, and Aurora Bay Care at the screening visit (Visit 1) and subject monitoring visit (Visit 4). In addition, 24-ambulatory blood pressure monitoring will be performed at each site (Visits 2 and 5). Data from these visits including subject demographic information, vital signs, laboratory test values, and other subject information will be uploaded into OnCore CRM clinical trial software. As a result all investigators will be able to access subject data from the screening visit and monitoring visit. It is vital that all investigators can access data from the monitoring visit for safety reasons and monitoring of the study medications. Data for the primary outcome variable will be uploaded into OnCore from Visit 3 and Visit 6 and will be made available for the data and safety monitoring committee when they meet quarterly and for interim/final analyses after 33%, 66%, and 100% of subjects have been enrolled.

9.5 Data analysis at Brigham and Women's Hospital

Dr. Scott Sands, at Brigham and Women's Hospital will analyze some study data by isolating individual waveforms obtained from sleep studies. Data will be transmitted to Dr. Sands by using a restricted Box folder within the password protected UW Box program. The data that will be shared with Dr. Sands consists of individual waveforms from sleep studies. The waveform files will be labeled with the subject's unique study ID number and will not contain any other identifiers. Coded sleep study files will be uploaded for Dr. Sands to access. He will extract data from individual waveforms, and then summarize and score the data, and make the scored files available on Box. Dr. Sands will not have access to the key that links the code to identifiable data. Brigham and Women's Hospital (Partners IRB) is deferring IRB oversight to the University of Wisconsin-Madison. UW-Madison IRB will serve as the IRB of record for this study.

9.6 Data sharing with 2016-0988 study (PI: Claudia Korcarz)

Study data collected in this study will be shared with the 2016-0988 study (PI: Claudia Korcarz). The rationale for using this data in the 2016-0988 study is that this data contains information about PAP compliance, risk factors, demographics, etc. IRB approval has already been obtained for the use of this data in the 2016-0988 protocol.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

The trial has been funded by the NIH – National Heart Lung and Blood Institute

11.2 Subject Stipends or Payments

Subjects will be reimbursed $50 for completing the screening visit (Visit 1), $25 for subjects undergoing re-screening procedures, if necessary (Visit 1A), and $50 for the 2-week monitoring visit (Visit 4). Subjects who choose to wear the Holter/Heart rate variability equipment will also be paid $25 for each night the equipment is worn at home. Subjects will receive up to $400 for each study visit they complete in Madison (2 night visit) (Visits 3 and 6). This amount may be prorated if the subject is only able to complete certain portions of the procedures. The prorated amounts are $100 for completion of the Physiologic Studies for day 1 of Visit 3 and 6, $100 for the completion of the sleep studies for night 1 of Visit 3 and Visit 6, $100 for the completion of the Vascular Studies in Dr. Stein's Lab for Visit 3 and Visit 6, and $100 for the completion of the Control of Breathing Studies on night 2 for Visit 3 and Visit 6. If the subject completes all procedures, they will receive $400 for each Visit 3 and Visit 6. In addition, subjects will receive $100 for completing the study and the post-study follow-up questions with study staff (Visit 7). Subjects would receive $1075 if they complete the entire study, including re-screening Visit 1A and the optional Holter procedures.

If subjects do not return the loaned CPAP machine or CPAP compliance card at Visit 6, they will not receive the payment for Visit 6 or the 30-day follow-up phone call until they return the loaned CPAP machine or compliance card.

Subjects who travel more than 40 miles one way to Madison for Visits 3 and 6 will be reimbursed for their travel. Subjects will be reimbursed $150 for traveling to visit 3 and visit 6 ($150 for each visit, $300 total). In addition, hotel accommodations at the Best Western Inn Towner (two blocks from UW Hospital) are available for a spouse/significant other and or family of subjects enrolled

at Aurora Bay Care, Gundersen Health System, and Marshfield Clinic for the 2- night study visit in Madison. This is provided at no cost to the subject.

12 Publication Plan

Study data and results will be published and shared as mandated by NIH and Federal data sharing regulations.

13 References

1. Das AM and Khayat R. Hypertension in obstructive sleep apnea: risk and therapy. *Expert Rev Cardiovasc Ther* 2009;7:619-626.
2. Narkiewicz K, van de Borne PJ, Pesek CA, Dyken ME, Montano N and Somers VK. Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation* 99: 1183-1189, 1999.
3. Imadojemu VA, Mawji Z, Kunselman A, Gray KS, Hogeman CS and Leuenberger UA. Sympathetic chemoreflex responses in obstructive sleep apnea and effects of continuous positive airway pressure therapy. *Chest* 2007;131:1406-1413.
4. Marcus N, Li Y, Bird C, Olson E, Smith S, Sorenson K, Schultz H and Morgan B. Chronic intermittent hypoxia alters chemoreflex control of lumbar sympathetic nerve activity and carotid body protein expression. *FASEB J* 2009;23: 1008.1.
5. Peng YJ, Overholt JL, Kline D, Kumar GK and Prabhakar NR. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: implications for recurrent apneas. *Proc Natl Acad Sci U S A* 2003;100:10073-10078.
6. Li Y-L, Gao L, Zucker IH, Schultz HD. NADPH oxidase-derived superoxide anion mediates angiotensin II-enhanced carotid body chemoreceptor sensitivity in heart failure rabbits. *Cardiovascular Res* 2007;75:546-554.
7. Warner G, Skatrud JB and Dempsey JA. Effect of hypoxia-induced periodic breathing on upper airway obstruction during sleep. *J Appl Physiol* 62: 2201-2211, 1987.
8. Wellman A, Jordan AS, Malhotra A, Fogel RB, Katz ES, Schory K, Edwards JK and White DP. Ventilatory control and airway anatomy in obstructive sleep apnea. *Am J Respir Crit Care Med* 170: 1225-1232, 2004.
9. Kelley EE, Hock T, Khoo NK, Richardson GR, Johnson KK, Powell PC, Giles GI, Agarwal A, Lancaster JR Jr, Tarpey MM. Moderate hypoxia induces xanthine oxidoreductase activity in arterial endothelial cells. *Free Rad Biol Med* 2006;40:952-959.
10. McCord JM. Oxygen-derived free radicals in post-ischaemic tissue injury. *N Engl J Med* 1985;312:159-163.
11. Landmesser U, Spiekermann S, Preuss C, Sorrentino S, Fischer D, Manes C, Mueller M and Drexler H. Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. *Arterioscler Thromb Vasc Biol* 2007;27:943-948.
12. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA and Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 1997;95:588-593.
13. Moller DS, Lind P, Strunge B and Pedersen EB. Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. *Am J Hypertens* 2003;16:274-280.
14. Hassoun PM, Feng-Sheng Y, Cote CG et al. Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1 and hypoxia. *Am J Respir Crit Care Med*. 158:299-305.

15. Kayyali US, Donaldson C, Huang H et al. Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. *J Biol Chem*. 2001; 276:14359-14365.
16. El Solh AA, Saliba R, Bosinski T et al. Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. *Eur Respir J*. 2006;27:997-1002.
17. Engleman HM, Wild MR. Improving CPAP use by patients with the sleep apnea/hypopnea syndrome. *Sleep Med Rev*. 2003;7:81-99.
18. Barone-Kribbs N, Pack A, Kline L et al. Effects of one night without CPAP treatment on sleep and sleepiness in patients with obstructive sleep apnea. *Am Rev Respir Dis.* 1993;47:1162-1168.
19. Weaver T, Kribbs N, Pack A, et al. Night-to-night variability in CPAP use over the first three months of treatment. *Sleep.* 1997;20:278-283.
20. Fitzpatrick MF, Alloway CE, Wakeford TM, MacLean AW, Munt PW, Day AG. Can patients with obstructive sleep apnea titrate their own continuous positive airway pressure? *Am J Respir Crit Care Med* 2003;167:716-722.
21. Grote L, Hedner J, Grunstein R, Kraiczi H. Therapy with CPAP: incomplete elimination of sleep related breathing disorder. *Eur Respir J*. 2000;16:921-927.
22. National Commission on Sleep Disorders Research. Wake up America: a national sleep alert. Washington, DC: Government Printing Office; 1993.
23. Strohl KP, Redline S. Recognition of obstructive sleep apnea. *Am J Respir Crit Care Med*. 1996;154:279-289.
24. Levinson PD, McGarvey ST, Carlisle CC et al. Adiposity and cardiovascular risk factors in men with obstructive sleep apnea. *Chest*. 1993;103:1336-1342.
25. Phillips B, Cook Y, Schmitt F et al. Sleep apnea: prevalence of risk factors in the general population. *South Med J*. 1989;82:1090-1092.
26. Peppard PE, Young, T, Palta, M et al. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med*. 2000; 342:1378-1384.
27. Dyken ME, Somers VK, Yamada T et al. Investigating the relationship between stroke and obstructive sleep apnea. *Stroke*. 1996; 27:401-407.
28. Partinen M. Guilleminault C. Daytime sleepiness and vascular morbidity at seven-year follow-up in obstructive sleep apnea patients. *Chest*. 1990; 97:27-32.
29. He J, Kryger MH, Zorick FJ et al. Mortality and apnea index in obstructive sleep apnea: experience in 385 male patients. *Chest*. 1988; 94:373-376.
30. Marin JM, Carrizo SJ, Vicente E et al. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet*. 2005;365:1046-1053.
31. Narkiewicz K, Kato M, Phillips BG et al. Nocturnal continuous positive airway pressure decreases daytime sympathetic traffic in obstructive sleep apnea. *Circulation*. 1999;100:2332-2335.
32. Ip MS, Tse HF, Lam B, Tsang KW, Lam WK. Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med*. 2004;169:348-353.
33. Becker HF, Jerrentrup A, Ploch T, Grote L, Penzel T, Sullivan CE, Peter JH. Effect of nasal continuous positive airway pressure treatment on blood pressure in patients with obstructive sleep apnea. *Circulation*. 2003;107:68-73.
34. Fletcher EC, Lesske J, Behm R, Miller CC 3rd, Stauss H, Unger T. Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. J Appl Physiol. 1992;72:1978-1784.
35. Fletcher EC, Lesske J, Culman J, Miller CC and Unger T. Sympathetic denervation blocks blood pressure elevation in episodic hypoxia. *Hypertension* 1992;20:612-619.
36. Fletcher EC, Lesske J, Qian W, Miller CC, III and Unger T. Repetitive, episodic hypoxia causes diurnal elevation of blood pressure in rats. *Hypertension* 1992;19:555-561.

37. Carlson JT, Hedner J, Elam M et al. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest*. 1993;103:1763-1768.
38. Dimsdale JE, Coy T, Ziegler MG, Ancoli-Israel S, Clausen J. The effect of sleep apnea on plasma and urinary catecholamines. *Sleep.* 1995;18:377–381.
39. Xie A, Skatrud JB, Puleo DS, Morgan BJ. Exposure to hypoxia produces long-lasting sympathetic activation in humans. *J Appl Physiol*. 2001;91:1555-1562.
40. Peng YJ, Overholt JL, Kline D et al. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: implications for recurrent apneas. *Proc Natl Acad Sci U S A*. 2003;100:10073-10078.
41. Greenberg HE, Sica A, Batson D et al. Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. *J Appl Physiol*. 1999;86:298-305.
42. Sica AL, Greenberg HE, Ruggiero DA et al. Chronic-intermittent hypoxia: a model of sympathetic activation in the rat. *Respir Physiol*. 2000;121:173-184.
43. Prabhakar NR, Peng YJ, Jacono FJ et al. Cardiovascular alterations by chronic intermittent hypoxia: importance of carotid body chemoreflexes. *Clin Exp Pharmacol Physiol*. 2005;32:447-449.
44. Lam SY, Fung ML, Leung PS. Regulation of the angiotensin-converting enzyme activity by a time-course hypoxia in the carotid body. *J Appl Physiol*. 2004;96:809-813.
45. Allen AM. Angiotensin AT1 receptor-mediated excitation of rat carotid body chemoreceptor afferent activity. J Physiol. 1998;510:773-781.
46. Leung PS, Lam SY, Fung ML. Chronic hypoxia upregulates the expression and function of AT(1) receptor in rat carotid body. J Endocrinol 2000;167:517-524.
47. Li Y-L, Gao L, Zucker IH, Schultz HD. NADPH oxidase-derived superoxide anion mediates angiotensin II-enhanced carotid body chemoreceptor sensitivity in heart failure rabbits. *Cardiovascular Res.* 2007;75:546-554.
48. Hassoun PM, Feng-Sheng Y, Cote CG et al. Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1 and hypoxia. *Am J Respir Crit Care Med*. 1998;158:299-305.
49. Kayyali US, Donaldson C, Huang H et al. Phosphorylation of xanthine dehydrogenase/oxidase in hypoxia. *J Biol Chem*. 2001; 276:14359-14365.
50. Valdes V, Mosqueira M, Rey S, Del Rio R, Iturriaga R. Inhibitory effects of NO on carotid body: contribution of neural and endothelial nitric oxide synthase isoforms. *Am J Physiol.* 2003;284:L57-L68.
51. Prabhakar NR. Neurotransmitters in the carotid body. *Adv Exp Med Biol.* 1994;360:57-69.
52. Weiss JW, Liu MD, Huang J. Sleep Apnoea & Hypertension: Physiological basis for a causal relationship of obstructive sleep apnoea to hypertension. *Exp Physiol*. 2007;92:21-26.
53. El Solh AA, Saliba R, Bosinski T et al. Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. *Eur Respir J*. 2006;27:997-1002.
54. Ip MS, Lam B, Chan LY, Zheng L, Tsang KW, Fung PC, Lam WK. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med*. 2000 Dec;162(6):2166-71.
55. Kato M, Roberts-Thomson P, Phillips BG et al. Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation*. 2000;102:2607-2610.
56. Kraiczi H, Caidahl K, Samuelsson A et al. Impairment of vascular endothelial function and left ventricular filling : association with the severity of apnea-induced hypoxemia during sleep. *Chest*. 2001;119:1085-1091.

57. Nieto FJ, Herrington DM, Redline S et al. Sleep apnea and markers of vascular endothelial function in a large community sample of older adults. *Am J Respir Crit Care Med*. 2004;169:354-360.
58. Phillips SA, Olson EB, Morgan BJ et al. Chronic intermittent hypoxia impairs endothelium-dependent dilation in rat cerebral and skeletal muscle resistance arteries. *Am J Physiol Heart Circ Physiol*. 2004;286:H388-H393.
59. Milstein S and Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Comm*. 1999;263:681-684.
60. Schulz R, Mahmoudi S, Hattar K et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. *Am J Respir Crit Care Med*. 2000;162:566-570.
61. Barcelo A, Miralles C, Barbe F et al. Abnormal lipid peroxidation in patients with sleep apnoea. *Eur Respir J*. 2000;16:644-647.
62. McNally JS, Davis ME, Giddens DP, et al. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol*. 2003;285:H2290-H2297.
63. Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Tanaka A, Oda N, Okada S, Ohta S, Naito H and Adachi M. Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005;172:625-630.
64. Tanriverdi H, Evrengul H, Kara CO, Kuru O, Tanriverdi S, Ozkurt S, Kaftan A and Kilic M. Aortic stiffness, flow-mediated dilatation and carotid intima-media thickness in obstructive sleep apnea: non-invasive indicators of atherosclerosis. *Respiration* 2006;73:741-750.
65. Baguet JP, Hammer L, Levy P, Pierre H, Launois S, Mallion JM and Pepin JL. The severity of oxygen desaturation is predictive of carotid wall thickening and plaque occurrence. *Chest* 2005;128:3407-3412.
66. Drager LF, Bortolotto LA, Figueiredo AC, Silva BC, Krieger EM and Lorenzi-Filho G. Obstructive sleep apnea, hypertension, and their interaction on arterial stiffness and heart remodeling. *Chest* 2007;131:1379-1386.
67. Kohler M, Craig S, Nicoll D, Leeson P, Davies RJ and Stradling JR. Endothelial function and arterial stiffness in minimally symptomatic obstructive sleep apnea. *Am J Respir Crit Care Med* 2008;178:984-988.
68. Phillips C, Hedner J, Berend N and Grunstein R. Diurnal and obstructive sleep apnea influences on arterial stiffness and central blood pressure in men. *Sleep* 2005;28:604-609.
69. Tsioufis C, Thomopoulos K, Dimitriadis K, Amfilochiou A, Tousoulis D, Alchanatis M, Stefanadis C and Kallikazaros I. The incremental effect of obstructive sleep apnoea syndrome on arterial stiffness in newly diagnosed essential hypertensive subjects. *J Hypertens* 2007;25:141-146.
70. McEniery CM, Qasem A, Schmitt M, Avolio AP, Cockcroft JR and Wilkinson IB. Endothelin-1 regulates arterial pulse wave velocity in vivo. *J Am Coll Cardiol* 2003;42:1975-1981.
71. Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP and Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. *Circulation* 2002;105:213-217.
72. Phillips BG, Narkiewicz K, Pesek CA, Haynes WG, Dyken ME and Somers VK. Effects of obstructive sleep apnea on endothelin-1 and blood pressure. *J Hypertens* 1999;17:61-66.
73. Phillips SA, Olson EB, Lombard JH and Morgan BJ. Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries. *J Appl Physiol* 2006;100:1117-1123.

74. Carmeliet P and Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249-257.
75. Duyndam MC, Hulscher TM, Fontijn D, Pinedo HM and Boven E. Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1alpha protein by the oxidative stressor arsenite. *J Biol Chem* 2001;276:48066-48076.
76. Brown NJ. Aldosterone and vascular inflammation. *Hypertension* 2008;51:161-167.
77. Takahashi S, Nakamura Y, Nishijima T, Sakurai S and Inoue H. Essential roles of angiotensin II in vascular endothelial growth factor expression in sleep apnea syndrome. *Respir Med* 2005;99:1125-1131.
78. Bleeke T, Zhang H, Madamanchi N, Patterson C and Faber JE. Catecholamine-induced vascular wall growth is dependent on generation of reactive oxygen species. *Circ Res* 2004;94:37-45.
79. Gerasimovskaya EV, Ahmad S, White CW, Jones PL, Carpenter TC and Stenmark KR. Extracellular ATP is an autocrine/paracrine regulator of hypoxia-induced adventitial fibroblast growth. Signaling through extracellular signal-regulated kinase-1/2 and the Egr-1 transcription factor. *J Biol Chem* 2002;277:44638-44650.
80. Stenmark KR, Gerasimovskaya E, Nemenoff RA and Das M. Hypoxic activation of adventitial fibroblasts: role in vascular remodeling. *Chest* 2002;122:326S-334S.
81. Tulis DA and Prewitt RL. Medial and endothelial platelet-derived growth factor A chain expression is regulated by in vivo exposure to elevated flow. *J Vasc Res* 1998;35:413-420.
82. Wilson E, Mai Q, Sudhir K, Weiss RH and Ives HE. Mechanical strain induces growth of vascular smooth muscle cells via autocrine action of PDGF. *J Cell Biol* 1993;123:741-747.
83. Hudgel DW, Gordon EA, Thanakitcharu S and Bruce EN. Instability of ventilatory control in patients with obstructive sleep apnea. *Am J Respir Crit Care Med* 1998;158:1142-1149.
84. Onal E, Burrows DL, Hart RH and Lopata M. Induction of periodic breathing during sleep causes upper airway obstruction in humans. *J Appl Physiol* 1986;61:1438-1443.
85. Younes M, Ostrowski M, Atkar R, Laprairie J, Siemens A and Hanly P. Mechanisms of breathing instability in patients with obstructive sleep apnea. *J Appl Physiol* 2007;103:1929-1941.
86. Prabhakar NR, Peng YJ, Jacono FJ, Kumar GK and Dick TE. Cardiovascular alterations by chronic intermittent hypoxia: importance of carotid body chemoreflexes. *Clin Exp Pharmacol Physiol* 2005;32:447-449.
87. Reichmuth KJ, Dopp JM, Barczi SR, Skatrud JB, Wojdyla P, Hayes JD and Morgan BJ. Impaired Vascular Regulation in Patients with Obstructive Sleep Apnea: Effects of CPAP Treatment. *Am J Respir Crit Care Med* 2009;180:1143-1150.
88. Brian JE, Jr. Carbon dioxide and the cerebral circulation. *Anesthesiology* 1998;88:1365-1386.
89. Xie A, Skatrud JB, Morgan B, Chenuel B, Khayat R, Reichmuth K, Lin J and Dempsey JA. Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *J Physiol* 2006;577:319-329.
90. Xie A, Skatrud JB, Barczi SR, Reichmuth K, Morgan BJ, Mont S and Dempsey JA. Influence of cerebral blood flow on breathing stability. *J Appl Physiol* 2009;106:850-856.
91. Foster GE, Hanly PJ, Ostrowski M and Poulin MJ. Ventilatory and cerebrovascular responses to hypercapnia in patients with obstructive sleep apnoea: Effect of CPAP therapy. *Respir Physiol Neurobiol* 165: 73-81, 2009.
92. Marcus N, Li Y, Bird C, Olson E, Smith S, Sorenson K, Schultz H and Morgan B. Chronic intermittent hypoxia alters chemoreflex control of lumbar sympathetic nerve activity and carotid body protein expression. *FASEB J* 2009;23:1008.1.

93. Spicuzza L, Bernardi L, Balsamo R, Ciancio N, Polosa R and Di Maria G. Effect of treatment with nasal continuous positive airway pressure on ventilatory response to hypoxia and hypercapnia in patients with sleep apnea syndrome. *Chest* 2006;130:774-779.
94. Wang D, Grunstein RR and Teichtahl H. Association between ventilatory response to hypercapnia and obstructive sleep apnea-hypopnea index in asymptomatic subjects. *Sleep Breath* 2007;11:103-108.
95. Vallbo AB, Hagbarth KE, Torebjork HE & Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 1979;59:919-957.
96. Dopp JM, Wiegert NA, Moran JJ, Muller D, Weber SM, Hayney MS. Humoral immune responses to influenza vaccinationin patients with obstructive sleep apnea. *Pharmacotherapy* 2007;27:1483-1489.
97. Hoffman RP, Sinkey CA, Dopp JM, Phillips BG. Systemic and local adrenergic regulation of muscle glucose utilization during hypoglycemia in healthy subjects. *Diabetes* 2002; 51:734-42.
98. Agapitov A, Correia ML, Sinkey CA, Dopp JM, Haynes WG. Impaired skeletal muscle and skin microcirculatory function in human obesity. *J Hypertens* 2002;20:1401-1405.
99. Hoffman RP, Sinkey CA, Dopp JM, Phillips BG. Lack of effect of alpha- and beta-adrenergic inhibition on forearm glucose uptake despite differences in forearm blood flow in healthy humans. *Metabolism* 2002; 51:1506-1513.
100. Ainslie PN, Ashmead JC, Ide K, Morgan BJ, Poulin MJ. Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol.* 2005;566:613-24.
101. Khayat RN, Przybylowski T, Meyer KC, Skatrud JB, Morgan BJ. Role of sensory input from the lungs in control of muscle sympathetic nerve activity during and after apnea in humans. *J Appl Physiol*. 2004;97:635-40.
102. Halliwill JR, Morgan BJ, Charkoudian N. Peripheral chemoreflex and baroreflex interactions in cardiovascular regulation in humans. *J Physiol*. 2003;552:295-302.
103. Przybyłowski T, Bangash MF, Reichmuth K, Morgan BJ, Skatrud JB, Dempsey JA. Mechanisms of the cerebrovascular response to apnoea in humans. *J Physiol*. 2003;548:323-32.
104. Derchak PA, Sheel AW, Morgan BJ, Dempsey JA. Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol*. 2002;92:1539-1552.
105. Katragadda S, Xie A, Puleo D, Skatrud JB, Morgan BJ. Neural mechanism of the pressor response to obstructive and nonobstructive apnea. *J Appl Physiol*. 1997;83:2048-2054.
106. Morgan BJ, Denahan T, Ebert TJ. Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea. *J Appl Physiol*. 1993;74:2969-2975.
107. Morgan BJ, Crabtree DC, Palta M, Skatrud JB. Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol*. 1995;79:205-213.
108. St Croix CM, Morgan BJ, Wetter TJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *J Physiol*. 2000;529:493-504.
109. Aaslid R, Markwalder T, Nornes H. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg.* 1982;57:769-774.
110. Otis SM, Ringelstein EB. The transcranial Doppler examination: principles and applications of transcranial Doppler sonography. In: Tegeler CH, Babikian VL, Gomez CR, *Neurosonology*, Mosby, St. Louis, MO, 1996, pp. 113-128.

111. Giller CA, Bowman G, Dyer H, Mootz L, Krippner W. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery*. 1993;32:737-742.
112. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke*. 2000;31:1672-1678.
113. Bishop CCR, Powell S, Rutt D, Browse NL. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke*. 1986;17:913-915.
114. Kirkham FJ, Padayachee TS, Parsons S, Seargeant LS, House FR, Gosling RG. Transcranial measurement of blood velocities in basal cerebral arteries using pulsed Doppler ultrasound: velocity as an index of flow. *Ultrasound Med Biol*. 1986;12:15-21.
115. Pyke KE, Hartnett JA and Tschakovsky ME. Are the dynamic response characteristics of brachial artery flow-mediated dilation sensitive to the magnitude of increase in shear stimulus? *J Appl Physiol* 2008;105:282-292.
116. Aeschlimann SE, Mitchell CK and Korcarz CE. Ultrasound brachial artery reactivity testing: technical considerations. *J Am Soc Echocardiogr* 2004;17:697-699.
117. Laurent S, Cockcroft J, Van BL, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I and Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;27:2588-2605.
118. Karamanoglu M, O'Rourke MF, Avolio AP and Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J* 1993;14:160-167.
119. Sweitzer NK, Shenoy M, Stein JH, Keles S, Palta M, LeCaire T and Mitchell GF. Increases in central aortic impedance precede alterations in arterial stiffness measures in type 1 diabetes. *Diabetes Care* 2007;30:2886-2891.
120. Rechtschaffen A and Kales A. *A manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects*. Washington, DC: US Government Printing Office, 1968.
121. Young T, Finn L, Peppard PE, Szklo-Coxe M, Austin D, Nieto FJ, Stubbs R and Hla KM. Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort. *Sleep* 2008;31:1071-1078.
122. Young T, Palta M, Dempsey J, Skatrud J, Weber S and Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;328:1230-1235.
123. Gold AR, Marcus CL, Dipalo F and Gold MS. Upper airway collapsibility during sleep in upper airway resistance syndrome. *Chest* 2002;121:1531-1540.
124. Kirkness JP, Schwartz AR, Schneider H, Punjabi NM, Maly JJ, Laffan AM, McGinley BM, Magnuson T, Schweitzer M, Smith PL and Patil SP. Contribution of male sex, age, and obesity to mechanical instability of the upper airway during sleep. *J Appl Physiol* 2008;104:1618-1624.
125. Patil SP, Punjabi NM, Schneider H, O'Donnell CP, Smith PL and Schwartz AR. A simplified method for measuring critical pressures during sleep in the clinical setting. *Am J Respir Crit Care Med* 2004;170:86-93.
126. Xie A, Skatrud JB and Dempsey JA. Effect of hypoxia on the hypopnoeic and apnoeic threshold for CO(2) in sleeping humans. *J Physiol* 2001;535:269-278.
127. Nakayama H, Smith CA, Rodman JR, Skatrud JB and Dempsey JA. Carotid body denervation eliminates apnea in response to transient hypocapnia. *J Appl Physiol* 2003;94:155-164.

128. Wilson CR, Satoh M, Skatrud JB and Dempsey JA. Non-chemical inhibition of respiratory motor output during mechanical ventilation in sleeping humans. *J Physiol* 1999;518 (Pt 2):605-618.
129. Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. *Sleep* 1992;15:376-381.
130. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG and Roccella EJ. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension* 2005;45:142-161.
131. Hla KM, Young T, Finn L, Peppard PE, Szklo-Coxe M and Stubbs M. Longitudinal association of sleep-disordered breathing and nondipping of nocturnal blood pressure in the Wisconsin Sleep Cohort Study. *Sleep* 2008;31: 795-800.
132. Hla KM, Young TB, Bidwell T, Palta M, Skatrud JB and Dempsey J. Sleep apnea and hypertension. A population-based study. *Ann Intern Med* 1994;120: 382-388.
133. Stickland MK, Morgan BJ, Dempsey JA. Carotid chemoreceptor modulation of sympathetic vasoconstrictor outflow during exercise in healthy humans. *J Physiol.* 2008;586:1743-1754.
134. Product Information: Alloprim™, allopurinol. Nabi, Boca Raton, FL, 2003.
135. Murrell GAC, Rapeport WG. Clinical pharmacokinetics of allopurinol. *Clin Pharm.* 1986;11:343–353.
136. Day RO, Graham S, Wong H, et al. Pharmacodynamics of oxypurinol after administration of allopurinol to healthy subjects. *Br J Clin Pharmacol.* 1996;41:299–304.
137. Day RO, Miners J, Birkett DJ, et al. Relationship between plasma oxipurinol concentrations and xanthine oxidase activity in volunteers dosed with allopurinol. *Br J Clin Pharmacol.* 1988;26:429–434.
138. Breithaupt H, Tittel M. Kinetics of allopurinol after single intravenous and oral doses. *Eur J Clin Pharmacol.* 1982;22:77–84.
139. Sasaki M, Fujimura A, Harada K, et al: Clinical pharmacology of multiple-dose losartan, and angiotensin II receptor antagonist, in patients with essential hypertension. *J Clin Pharmacol* 1996;36:403-408.
140. Munafo A, Christen Y, Nussberger J, Shum LY, Borland RB, Lee RJ, Waeber B, Biollaz J, Brunner HR. Drug concentration response relationships in normal volunteers after oral administration of losartan, an angiotensin II receptor antagonist. *Clin Pharmacol Ther* 1992;51:513-521.
141. Ohtawa M, Takayama F, Saitoh K, et al: Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans. *Br J Clin Pharmacol* 1993;35:290-297.
142. Ebert TJ, Morgan BJ, Barney JA, Denahan T, and Smith JJ. Effects of aging on baroreflex regulation of sympathetic activity in humans. *Am J Physiol* 1992;263: H798-803.
143. Jacobsen TN, Morgan BJ, Scherrer U, Vissing SF, Lange RA, Johnson N, Ring WS, Rahko PS, Hanson P, and Victor RG. Relative contributions of cardiopulmonary and sinoaortic baroreflexes in causing sympathetic activation in the human skeletal muscle circulation during orthostatic stress. *Circ Res* 1993;73:367-378.
144. St Croix CM, Satoh M, Morgan BJ, Skatrud JB, and Dempsey JA. Role of respiratory motor output in within-breath modulation of muscle sympathetic nerve activity in humans. *Circ Res* 1999;85:457-469.

14 Appendices

- Appendix 1 - Descriptions of Study Procedures
- Appendix 2 - Table of Study Procedures and Study Visits
- Appendix 3 – Exclusionary Medication List