Mild Intermittent Hypoxia and CPAP: A Multi-Pronged Approach to Treat Sleep Apnea in Intact and Spinal Cord Injured Humans

Record # 1 R56HL142757-01

Date: November 5th 2018

Experimental Protocol

Specific Aim 1. To determine if IH coupled with CPAP mitigates autonomic, cardiovascular, neurocognitive and metabolic measures both directly and indirectly by improving CPAP compliance as a consequence of LTF of upper airway muscle activity initiated by exposure to IH.

a. Rationale. Many studies have reported that IH directly mitigates co-morbidities typically associated with OSA. In addition, exposure to IH may increase CPAP compliance by initiating LTF of upper airway muscle activity that could enhance upper airway patency leading to a reduction in therapeutic CPAP. Reduced pressures could result in improved compliance and outcome measure.

b. Recruitment. The participants with OSA may be recruited from Sleep Disorders Centers affiliated with Wayne State University and John D. Dingell Veterans Administration Medical Center. The total number of patients studied in these laboratories per year is approximately 4500 and about 40 % of the participants will meet the inclusion criteria. We will also target hypertension clinics at John D. Dingell VA Medical Center and the Detroit Medical Center as additional sites to recruit participants with coincident hypertension and OSA; since up to 30 % of individuals diagnosed with hypertension also have been diagnosed with OSA. Advertisements will also be placed in the local newspaper (i.e. Metro Times) and on various websites (i.e. Craigslist, Facebook and Wayne State University Pipeline). During recently completed studies approximately 100 individuals were screened in order to recruit 20 participants that met the inclusion criteria. In the present proposal we believe this rate of recruitment will improve because our inclusion criteria is less stringent than in the past (i.e. previously we recruited individuals with OSA that fell within a narrow age range and did not suffer from other co-morbid conditions). Based on past experience, once participants meet the inclusion criteria and have successfully completed the screening sleep study and practice trial few drop out beyond this point. Nonetheless we have taken into account that 2 participants from each group (see Participants below) may drop out beyond the point of the screening and practice trials, so an additional 4 participants may be enrolled if required.

c. Participants. To complete the R56 proposal, Hypoxia group will be comprised of 4 participants with OSA that will be treated with IH and CPAP. Sham group will be comprised of 4 participants with OSA that will be exposed to a sham protocol and CPAP. Participants will be randomized to Hypoxia or Sham group. Participants will be male or female of any race, 18-60 years of age with a BMI of less than 40 kg/m2 and pure or predominantly (i.e. comprised of both a central and obstructive component) OSA (AHI ≤ 80 events per hour and an average oxygen desaturation level of 85 % or greater). Participants will not have received CPAP treatment previously. Participants' will be diagnosed with Stage 1 to Stage 2 hypertension as categorized by the 2018 American Heart Association guidelines. Systolic and diastolic pressure will range from 130 - 159 mmHg and 80 - 99 mmHg, respectively. Participants with a systolic pressure greater than 159 mmHg or a diastolic pressure greater than 99 mmHg will be excluded from the study. Based on the characteristics of this group the short exposure to mild IH that we will induce does not pose a risk beyond what exists for participants without OSA based on previous studies we have completed. Moreover, the nocturnal oxygen desaturation routinely experienced by the recruited participants will be mild, which will reduce the possibility of central nervous system hypoxic depression. Thus, we believe that initiation of various forms of plasticity following exposure to IH may manifest themselves more clearly in these individuals. The participants will be free of any other known cardiovascular disease. The participants will not be on any medications used to treat

cardiovascular disorders or any sleep promoting supplements including melatonin. All participants will have normal lung function with no or minimal alcohol consumption (< 2 oz of alcohol/night). Females in the Hypoxia and Sham groups will be studied at similar points in their menstrual cycle. The participants will not be night shift workers or recently travelled across time zones. These criteria will be confirmed by the completion of health, lifestyle, and sleep questionnaires, from pulmonary function tests, a physical examination and a nocturnal polysomnography examination that will be completed at John D. Dingell Veterans Administration Medical Center. During the physical examination blood pressure will be measured and a baseline EKG will be obtained. Assessment of cricomental space, pharyngeal grade, overbite, tonsillar grade, palatal position and tongue size will be completed. Completing this assessment will allow us to ensure that upper airway dimensions are similar between OSA participants that are being treated with CPAP and IH, compared to the group that will be only be treated with CPAP. A pregnancy test will be completed on female participants to avoid fetal exposure to IH.

d. Protocol and Measurements. Visit 1: Health, lifestyle and sleep questionnaires (i.e. SF-36 Health survey and Epworth Sleepiness Scale) along with pulmonary function tests, blood pressure measurements, a 12-lead electrocardiogram and a physical examination will be completed. Visit 2: A sleep study beginning at approximately 10 pm will be completed. The study will be used to confirm the presence or absence of OSA. Visit 3: A sleep study will be completed to determine the therapeutic positive airway pressure required to eliminate flow limitation. In addition, the pressure associated with airway collapsibility will be determined (i.e. the critical closing pressure - PCRIT). Upon awakening participants in Hypoxia group will undergo a trial exposure to IH (two 2-minute episodes of 8% oxygen separated by two-minute normoxic intervals, with an accompanied 2 mmHg increase in carbon dioxide levels) while sham group will be exposed to room air in order to acclimate to the equipment and accompanying sensations. Visit 4: Following the sleep study, participants will be given a blood pressure holter monitor (ABPM-05, Meditech Inc. Framingham, MA) to measure blood pressure and pulse rate at home over a 24-hour period. Data collection will begin at 6 am on a weekend day. Blood pressure values will be obtained every 20 minutes. Participants will be given a diary and an actigraph watch to document activities completed over the 24-hour period. Thereafter data will be standardized relative to activity level (i.e. quiet and active wakefulness and sleep). Participants will be encouraged to adhere to a daily routine that will be replicated following completion of the IH protocol. These measures are being made to monitor the changes in blood pressure associated with activities of daily living. Visits 5 - 19: Following the completion of visits 1-4, participants will visit the lab 15 times over a 3-week period during the day. The participants will visit the laboratory each week day for 3 weeks. If a week day visit is missed it will be replaced by a visit on a week end day. During each visit, participants in Hypoxia group will be exposed to 12 two-minute episodes of 8% oxygen combined with a 2 mmHg increase in carbon dioxide separated by two minute normoxic intervals. The IH protocol will be comprised of a 20-minute baseline period followed by exposure to twelve - two minute episodes of hypoxia [partial pressure of end-tidal oxygen ($P_{ET}O_2$) = 50 mmHg]. Each episode will be interspersed with a 2-minute recovery period under normoxic conditions. The $P_{ET}CO_2$ will be sustained 2 mmHg above baseline values for the last ten minutes of baseline and throughout the remainder of the protocol. In addition to the IH protocol, a group of participants will also be exposed to a sham protocol in addition to being treated with CPAP during sleep. The sham protocol will be administered during wakefulness for a minimum of 15 days over a 3-week period. During the sham protocol the participants will be exposed to atmospheric levels of oxygen and carbon dioxide for the duration of the IH protocol. During the IH and sham protocol respiratory

(ventilation, PETCO2, PETO2, oxygen saturation) and cardiovascular (heart rate and EKG) parameters will be monitored continuously throughout each visit. Likewise, with the exception of visits 5, 12 and 19 (see immediately below) automated and auscultatory blood pressure measurements will be obtained prior to the onset of the protocol, during hypoxic episodes 1, 6 and 12, during recovery from these episodes, as well as, at the midpoint and completion of the end recovery period of the IH or sham protocol. On each day of the protocol participants will continue to be treated with CPAP each night while they sleep in their home environment with the exception of visits 12 and 19 (see Table 1 below). They will be instructed to use CPAP each night for as long as possible.

	Consent & Screening	Diagnostic Sleep Study	CPAP Titration & PCRIT	IH or Sham	Blood Draw	Beat to Beat Blood Pressure	Automated Blood Pressure	Neurocognitive Testing
Visit 1	✓						✓	
Visit 2		✓					✓	
Visit 3			✓				✓	
Visit 4							✓	
Visit 5				✓	✓	✓	✓	✓
Visit 6				✓			✓	
Visit 7				✓			✓	
Visit 8				✓			✓	
Visit 9				✓			✓	
Visit 10				✓			✓	
Visit 11				✓			✓	
Visit 12			✓	✓	✓	✓	✓	✓
Visit 13				✓			✓	
Visit 14				✓			✓	
Visit 15				✓			✓	
Visit 16				✓			✓	
Visit 17				✓			✓	
Visit 18				✓			✓	
Visit 19			✓	✓	✓	✓	✓	✓
Visit 20							✓	

Table 1. Planned measures for each visit to the laboratory (Aim 1). CPAP – continuous positive airway pressure; IH – intermittent hypoxia; P_{CRIT} –critical closing pressure

Visits 5, 12 & 19: On visits 5, 12 & 19 additional autonomic, cardiovascular, neurocognitive and metabolic measures will be obtained. Upon entering the laboratory participants will complete a series of questionnaires and tasks to assess vigilance and neurocognitive function. The Pathfinder Number Test and Psychomotor Vigilance Task will assess attention and psychomotor function. The Buschke Selective Reminding Test will assess learning and memory. The Epworth Sleepiness Scale will assess day time sleepiness. The Mini Mental State Examination will be used

to assess five areas of cognition function (i.e. orientation, registration attention, calculation, recall and language). Thereafter, 15 ml of blood will be sampled prior to exposure to the IH or sham protocol to identify potential metabolic (i.e. lipid profile, hemoglobin A1C), inflammatory (i.e. asymmetric dimethylarginine and high sensitivity C-reactive protein) and angiogenic/vasculogenic (hypoxia inducible factor 1α and vascular endothelial growth factor) biomarkers that might reflect the efficacy of exposure to IH. Thereafter, on visits 5 & 19 beat to beat measures of blood pressure will be obtained throughout the IH or sham protocol. Beat to beat measures of blood pressure will also be obtained on visit 12. On visits 12 & 19 daytime exposure to the IH or sham protocol will be followed by a sleep study completed in the laboratory on the evening following the day time visit. Measures obtained during these sleep studies will be similar to the measures described for Visit 3. Visit 20: The protocol outlined for Visit 4 will be repeated on Visit 20.

Data and Statistical Analysis.

Research staff that will not administer the gases will be assigned to complete a blind analysis of all measures. The staff assigned to analyze data (e.g. ventilation and blood pressure) collected during the direct application of hypoxia might be able to determine the applied stimulus based on the respiratory or cardiovascular response. However, the criteria used to analyze the data is rigorous and bias is unlikely. The Shaprio-Wilk test will be used to test if the data is normally distributed. Assuming that the data will be normally distributed, a two-way analysis of variance with repeated measures in conjunction with Student-Newman Keuls post-hoc test will be used to determine if differences in 24 hour blood pressure measures are evident before and after exposure to IH or the sham protocol. The two factors in the design will "Group (Hypoxia vs. sham)" and "time point (Visit 4 vs. Visit 20)." A similar analysis will be used to determine if primary (i.e. blood pressure, autonomic nervous system activity, therapeutic CPAP, critical closing pressure, limb motor function tests) and secondary (vigilance and neurocognitive measures and blood biomarkers) outcome measures differ before, midway and after completion of the IH or sham protocol. The two factors in the design will be "Group (Hypoxia vs. sham)" and "time point (Visit 5 vs. Visit 12 vs. Visit 19)". Primary outcome variables collected during IH or the sham protocol (i.e. blood pressure, autonomic nervous system activity, minute ventilation, tidal volume, breathing frequency, PETCO2, PETO2) will be analyzed using a three-way analysis of variance with repeated measures in conjunction with Student-Newman Keuls post hoc. The three factors in the design will be "Group (Hypoxia vs. sham)", "Visit Number (Visit 5 vs. Visit 12 vs. Visit 19)" and "Time Point (Baseline vs. Initial hypoxic episode vs. Final hypoxic episode vs recovery of IH or sham protocol)." A Pearson product-moment correlation and regression analysis will be used to examine the relationship between CPAP adherence (e.g. percentage of days CPAP used over 4 or 6 hours) during the last half of the protocol with changes in the primary and secondary outcome measures for hypoxia and sham groups. If CPAP adherence impacts on the primary and/or secondary outcome measures we expect the slope of the relationship will be greater in Hypoxia group compared to sham group (i.e. for a given level of adherence modification to the primary or secondary outcome variable will be greater in the CPAP and IH group compared to the CPAP and sham group).

Potential Problems & Solutions. (i) The use of a parallel design could increase the burden of recruiting participants. However, we have extensive experience in recruiting and retaining participants enrolled in parallel design protocols requiring multiple visits. This statement is further supported by the participants that completed the protocol during collection of our preliminary data. Use of a cross-over design would make blinding of the participants difficult and would extend the

IH protocol from 3 to at least 10 weeks, since a wash-out period would be required between IH and sham. Given concerns that the protocol could be too long, we believe a parallel design is appropriate. (ii) Exposure to mild IH during sleep in untreated participants might result in increased breathing instability. This potential problem will not be an issue in the present study. Participants will be exposed to IH while awake during daytime hours. Likewise, participants will be treated with CPAP during sleep and we anticipate that therapeutic CPAP will be reduced while sleep apnea is effectively treated as the protocol progresses. Nonetheless, the CPAP instrument that we utilize monitors the AHI each night. If there is an unexpected increase in apnea severity the participant will be asked to withdraw from the study. (iii) We anticipate that sympathetic nervous system activity and blood pressure will be reduced in response to mild forms of IH coupled with CPAP. However, if progressive increases in blood pressure are evident over 3 visits that equate to sustained increases in systolic and diastolic blood pressure of greater than 10 mmHg the participants will be withdrawn from the study. Because the participants are coming to the laboratory each day changes in health status will be monitored day to day. (iv) The instrumentation and methods employed during sleep on Visits 3, 5, 12 and 19 could lead to disruption of sleep and the inability to collect the planned measures. Given the population we are employing this is typically not an issue when the participants are treated with CPAP. Nonetheless, all measurements will be obtained during deep N2 or N3 of non-rapid eye movement sleep and a sleep efficiency of at least 75 % will be required. If these criteria are not obtained a repeat sleep study will be completed on the following day. (v) Participants will be blinded to the composition of the gas mixture. However, they may recognize increases in heart rate and breathing during IH exposure. During the consenting process we will describe potential responses that might be experienced during each visit to all participants, without linking the responses to IH exposure. Based on past experience naïve participants often do not directly associate increases in breathing and heart rate with IH. Nevertheless, at least one of our outcome measures will allow us to differentiate if knowledge of the gas mixture, or other factors, influenced our results. More specifically, measures of the critical closing pressure during sleep should not be influenced by knowledge of the gas mixture. If CPAP compliance improves without a coincident improvement in the critical closing pressure we would question if knowledge of the gas mixture, or other factors, are influencing our results.