150465

UNIVERSITY OF CALIFORNIA, SAN DIEGO
HUMAN RESEARCH PROTECTIONS PROGRAM

TO: Dr. Atul Malhotra

RE: Project #150465
The Effect of Melatonin on Sleep and Ventilatory Control in Obstructive Sleep Apnea

Dear Dr. Malhotra:

The above-referenced project was reviewed and approved by one of this institution's Institutional Review Boards in accordance with the requirements of the Code of Federal Regulations on the Protection of Human Subjects (45 CFR 46 and 21 CFR 50 and 56), including its relevant Subparts. This approval, based on the degree of risk, is for 365 days from the date of **IRB review and approval** unless otherwise stated in this letter. The regulations require that continuing review be conducted on or before the 1-year anniversary date of the IRB approval, even though the research activity may not begin until sometime after the IRB has given approval.

The IRB has determined the investigational device associated with this study is a Non-Significant Risk Device in that it does not meet the criteria for a Significant Risk Device per the criteria outlined in 21 CFR 812.3(m) including that the device does not present a potential for serious risk to the health, safety, or welfare of a subject. The study must follow all the abbreviated regulations at 21 CFR 812.2(b).

The IRB has also determined that this project presents no more than minimal risk to human subjects in that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.

Date of IRB review and approval:3/26/2015

On behalf of the UCSD Institutional Review Boards,

/mb

Anthony Magit, M.D.
Director
UCSD Human Research Protections Program
(858) 657-5100; hrpp@ucsd.edu

Note: IRB approval does not constitute funding **or other institutional required approvals.** Should your studies involve other review committees such as Office of Clinical Trials Administration (OCTA), Office of Coverage Analysis Administration (OCAA), Conflict of Interest (COI), Protocol Review Monitoring

Committee (PRMC), and committees under Environmental Health & Safety (EH&S) such as Institutional Biosafety Committee (IBC), Human Exposure Committee (HERC), and RSSC (Radiation Safety and Surveillance Committee), it is the researchers responsibility to ensure that all approvals are in place prior to conducting research involving human subjects or their related specimens.

Approval release date: 6/15/2015

UCSD HUMAN RESEARCH PROTECTIONS PROGRAM

GENERAL APPROVAL INFORMATION

The information below does not encompass all human subjects protections requirements, however, is intended to highlight those of significance to ensure awareness by researchers engaged in research involving human subjects or their related specimens and data.

Approval Letters and Consent Documents

Unless otherwise stated, approval letters will be accompanied by stamped, approved consents. Should a study be closed to accrual and no consent released as a result, this information will be documented on the approval letter. Also, any waivers will be documented in the approval letter (such as waiver of documented consent or waiver of authorization for use of PHI).

The PI must ensure approval is in place from other appropriate review boards (such as Radiation Safety, Institutional Biosafety Committee, Conflict of Interest, ESCRO Committee, etc.)

If other institutions are involved, the PI must ensure that IRB approvals (or other administrative approvals) from those sites are secured and forwarded for the study file. In addition, PI's must ensure that the clinical trial agreement, as applicable, or other funding (such as a grant) is appropriately in place prior to conducting any research activities. IRB approval does not constitute funding approval.

Duration of IRB approval

The IRB may grant approval up to 365 days. (See 45 CFR 46.109(d) (DHHS) and 21 CFR 56.109(d) (FDA)). However, for some studies the committee may grant approval for a lesser period or a specific number of subjects to allow for more frequent monitoring. The approval letter or related documentation will indicate this information.

Because IRB review of research studies must be completed at least annually, investigators should plan ahead to meet required continuing review dates. **Please submit complete continuing review documentation at least 45 days prior to the expiration date to guard against a lapse in IRB approval.** The signed continuing review facepages and any other required hard copies must be received by the HRPP office before the continuing review process can begin.

As a courtesy, automated continuing review reminders can be set-up by PIs at various intervals (75 days, 45 days, 30 days, for example) on the website at https://irb.ucsd.edu. However, as these are automated electronic messages based on data entered, and the HRPP cannot anticipate which type of software programs (such as spam-blockers or anti-virus software) may block receipt of the messages, **PI's are required to not rely upon notification, but have internal mechanisms which track continuing review submission times**. Ultimately, it is the PI's responsibility to initiate a continuing review application, allowing sufficient time for the review and re-approval process to be completed before the current approval expires.

Continuing review is required even if no changes are made, or if the only study activity is participant follow-up, and even if the only study activity is data analysis.

What happens if there is a lapse in IRB approval?

If the IRB has not reviewed and approved a research study by the study expiration date, **all research activities must stop**. This includes the following:

All research-related interventions or interactions with currently enrolled subjects (unless the IRB finds that it is in the best interests of the individual subjects to continue participating in the research interventions or interactions;*) recruitment and informed consent procedures; and continued collection and/or analysis of data/information.

**Exception:* Research-related interventions or interactions with enrolled subjects may continue if the IRB determines that stopping the research would jeopardize the rights or welfare of current subjects. The IRB will decide which subjects should continue receiving the intervention during the lapse in approval. A request for such an exception must be submitted in writing to the attention of the IRB Chair by the Principal Investigator.If any project activity—even activity required for participant safety—occurs or continues after the expiration date, the investigator is out of compliance with both federal regulations and university policy. Retrospective approval for work done after the expiration date cannot be granted.

Amendment/revision to an IRB approved study

IRB approval is required before implementing any changes in the approved research plan, consent documents, recruitment materials, or other study-related documents. Please see Amendment Fact Sheet at http://irb.ucsd.edu/amendmodchg.pdf for submission guidance.

Adverse Event and Unanticipated Problems Reporting

All problems having to do with subject safety must be reported to the IRB within ten working days. All deaths, whether or not they are directly related to study procedures, must be reported. For adverse events, please utilize the form found at https://irb.ucsd.edu/UPR_biomedical.doc. For deviations and other reports, a cover letter and any supplemental information appropriate to the review should be provided. Please see IRB Guidelines for more information at https://irb.ucsd.edu.

Changes in financial Interest or Conflict of Interest (COI) disclosure

Any changes in the financial relationship between the study sponsor and any of the investigators on the study and/or any new potential conflicts of interest must be reported immediately to the Independent Review Committee via the Conflict of Interest Office. If these changes affect the conduct of the study or result in a change in the required wording of the approved consent form, then these changes must also be submitted as an amendment request.

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UCSD Human Research Protections Program
New Biomedical Application
RESEARCH PLAN

Instructions for completing the Research Plan are available on the HRPP website.
The headings on this set of instructions correspond to the headings of the Research Plan.
General Instructions: Enter a response for all topic headings.
Enter "Not Applicable" rather than leaving an item blank if the item does not apply to this project.

Version date: 05/11/2011

1. PROJECT TITLE

The Effect of Melatonin on Sleep and Ventilatory Control in Obstructive Sleep Apnea

2. PRINCIPAL INVESTIGATOR

Atul Malhotra, MD

3. FACILITIES

UCSD, Clinical Teaching Facility – Sleep Laboratory

4. ESTIMATED DURATION OF THE STUDY

2-3 years

5. LAY LANGUAGE SUMMARY OR SYNOPSIS (no more than one paragraph)

Our hypothesis is that oxidative stress induced during repeated apneas in obstructive sleep apnea (OSA) patients alters the neural control of breathing which destabilizes ventilatory control and exacerbates OSA. Thus antioxidant treatment has the potential to reduce OSA severity. Melatonin is a hormone which regulates sleep patterns, but it is also a potent antioxidant. Melatonin production is suppressed when the eyes register light so people with healthy sleep exhibit a peak in blood serum levels around 2am which then decreases towards morning. OSA patients exhibit lower melatonin levels with a later peak around 6am which then extends later into the day. This abnormal pattern is thought to compound difficulty falling asleep and daytime mental fatigue. Therefore the potential benefits of melatonin treatment in OSA patients are two-fold: most importantly via its antioxidant actions melatonin may reduce chemoreflex sensitivity, stabilize ventilatory control and reduce OSA severity; by normalizing sleep phase melatonin may also allow patients to fall asleep easier and wake more refreshed.

6. SPECIFIC AIMS

In a randomized, double-blind placebo controlled trial, we plan to study the effects of 30 days melatonin treatment on oxidative stress, ventilatory control and sleep in two groups of matched, untreated OSA patients. We will determine whether melatonin treatment readjusts the pattern of melatonin blood serum levels and the effects on sleep and subjective sleepiness (AIM 1). The main aims of the study will test whether 30 days of melatonin treatment reduces blood oxidative stress markers in untreated OSA patients (AIM 2), and whether melatonin treatment alters chemoreflex control while awake and reduces OSA severity, measured as both a reduced apnea hypopnea index (AHI) and loop gain during sleep (Aim 3).

7. BACKGROUND AND SIGNIFICANCE

OSA is a common sleep disorder characterized by repeated collapse of the upper airway during sleep causing bouts of hypercapnia and hypoxia, typically followed by arousal, hyperventilation and a subsequent apnea. The resultant swings in blood gases and fragmented sleep have been associated with major neurocognitive and cardiovascular sequelae. Although the commonly cited statistics are that 4% of middle aged US men and 2% of US women have symptomatic OSA, these figures may be underestimates. However despite its prevalence and well recognized consequences, treatment of OSA remains unacceptable due to poor adherence to and variable efficacy of existing therapies, leading many to advocate for further research into underlying mechanisms to identify new therapeutic targets.

During sleep ventilatory control is dominated by the level of CO_2 and O_2 in the blood. Arterial CO_2 has the greatest influence, with increasing CO_2 stimulating an increase in ventilatory drive and vice versa. Ventilatory

drive not only determines the level of activity of the thoracic pump muscles, but also the upper airway dilator muscles. Consequently the upper airway is susceptible to collapse when CO_2, and therefore neural drive to the upper airway muscles, is low. Loop gain is an engineering method used to measure the stability of the negative feedback chemoreflex control system, calculated as the ratio of the ventilatory response to the disturbance which elicited the response. Higher LG equals less stable control, as a disproportionately large ventilatory response will result in a greater degree of hypocapnia and subsequent reduction in ventilatory drive. Thus high LG contributes to propagating apneas. Supporting this is evidence that OSA patients have higher LG than non-OSA and LG correlates with the apnea hypopnea index (AHI). Additionally, treatments such as supplemental oxygen and acetazolamide which reduce LG significantly reduce AHI. However these treatments essentially counteract high LG, rather than treating the cause of high LG.

LG includes “plant” (respiratory apparatus) and “controller” (chemoreflex) gain components. OSA patients exhibit abnormalities in chemoreflex control which increase the sensitivity to blood gases and thus increase controller and LG. These abnormalities normalize with continuous positive airway pressure (CPAP) treatment, indicating they are induced by OSA itself. Intermittent hypoxia, as occurs during repeated apneas, induces lasting changes in the neural control of breathing which induces the same abnormalities and increased controller gain as is seen in OSA. Therefore it is thought that the IH experienced in OSA contributes to worsening of OSA by inducing changes to the neural control of breathing which increase controller and LG. The cellular changes induced by IH are dependent on the formation of reactive oxygen species (ROS) and both animal and human studies have shown antioxidant treatment prior to IH experimentally blocks these neural changes. Thus antioxidants may be a suitable alternative treatment in OSA, by treating the actual cause of high LG. Indeed there have been two published studies showing vitamins and N-acetyl-cysteine reduced AHI in OSA patients.

Melatonin is a hormone produced in the pineal gland of the brain which is most well-known for its critical role in regulating sleep and the circadian clock. However it is also a potent antioxidant which not only acts as a direct free radical scavenger, but it also stimulates synthesis of other antioxidants. Unlike most antioxidants it also crosses all cell membranes easily, allowing it to cross the blood brain barrier and also enter intracellular compartments such as mitochondria where ROS production is highest. Owing to the fact that most antioxidants become reactive species themselves once they have been oxidized, many antioxidants become toxic and cause disease with prolonged high doses. For this reason most antioxidants are unsuitable as a long-term treatment option in OSA. However melatonin does not have this problem because as it is oxidized it converts into multiple different metabolites all of which have various antioxidant functions. This adds to its efficacy as it neutralizes an extremely wide range of radical species. Consequently melatonin is non-toxic at even extremely high doses and extended use. Indeed melatonin has been found effective in treating multiple diseases in humans associated with ROS and oxidative stress, such as diabetes, chronic obstructive pulmonary disease and multiple sclerosis. Of particular interest is that OSA is intimately associated with metabolic disorder, with the two conditions having compounding negative consequences on cardiovascular disease, and melatonin has been shown to improve hypertension, oxidative stress and blood lipid profiles in metabolic disorder. For these reasons, we propose that melatonin may be a safe long term treatment option in OSA patients that are unable to tolerate CPAP, which may block the neural changes induced by IH, thereby reducing controller and LG and therefore reducing AHI. Additionally, melatonin treatment may have added benefits associated with preventing ROS induced morbidities of both OSA and metabolic disorder.

Melatonin production is inhibited by light and normally peaks around 2am. However OSA exhibit much lower levels of melatonin with a lower peak at around 6am. OSA is associated with chronic systemic oxidative stress and endogenous levels of melatonin play a critical role in regulation of total antioxidant status. Therefore this abnormal pattern of melatonin production in OSA likely contributes to exacerbation of oxidative stress and also to sleep and cognitive deficits in OSA, as this would delay the circadian sleep phase, making it difficult for

patients to fall asleep and adding to mental fatigue during the morning. Therefore, in addition to its antioxidant activity, melatonin treatment in OSA may have additional benefits via normalizing sleep phase. Thus the primary focus of this study will be to investigate the effects of melatonin treatment in OSA on waking chemoreflex control and ventilatory stability as assessed via LG and AHI during sleep, but we also intend to assess if there are additional sleep and cardiovascular benefits of melatonin treatment, by assessing melatonin blood serum phase resetting effects, sleep quality, blood pressure and electrocardiogram (ECG).

8. PROGRESS REPORT

All experiments will be conducted here at UCSD.

9. RESEARCH DESIGN AND METHODS

This study is a physiological research study (Aims 2 and 3) and a small pilot study (Aim 1). There is currently no published data of melatonin treatment and the effects on sleep phase in OSA patients. Therefore participants will be enrolled to participate in Aim 1 to determine appropriate timing of melatonin treatment and effects on sleep prior to commencement of the main protocol consisting of Aims 2 and 3. Patients enrolled into Aim 1 will not undertake Aims 2 and 3.

All patients will be recruited either from the sleep clinic after diagnosis and approval from their treating physician, or via advertisement after medical screening. CPAP treatment reduces oxidative stress, normalizes chemoreflex control and reduces AHI. Therefore, so as not to interfere with the aims of the study, newly diagnosed patients consenting to take part in the study will have their normal course of treatment delayed until after the protocol is complete, after which their treatment will be continued as normal without affect from the study.

Aim 1 will involve a two week protocol. Both untreated OSA patients and matched healthy non-OSA control participants will be enrolled and undertake 1 week assessing natural circadian rhythm and endogenous melatonin blood serum profiles. OSA patients will then be randomly assigned to two groups (with different time of melatonin dosage). Each group will complete a second week to assess the effects of melatonin supplementation. Control participants will not undertake the second week. The last night of both week 1 and 2 will involve overnight assessments consisting of approximately 10 hours each.

A separate group of patients will be enrolled into the main protocol consisting of Aims 2 and 3. Matched patients will be randomly assigned to two groups (placebo or treatment). All patients will complete the same 31 day protocol, consisting of 2 afternoon experiments followed by an overnight assessment each approximately 14 hours long. The first day and night will consist of baseline measures of blood pressure, chemoreflex control, blood oxidative markers, and during sleep breathing parameters such as AHI and loop gain. After 30 days either placebo or melatonin treatment all patients will return to complete the second day and night, which will be a repeat of the measures taken at baseline.

Aim 1: Assessment of melatonin treatment on the pattern of melatonin blood serum levels and effects on sleep and subjective sleepiness. The first week will evaluate baseline sleep habits and overnight melatonin blood serum levels in untreated OSA patients and healthy non-OSA participants. OSA patients will then undertake a second week assessing the effects of melatonin treatment. At the baseline evaluation participants will complete the Horne-Ostberg morningness-eveningness questionnaire. During the first week all participants will wear actigraphy watches and complete a 7 day sleep diary to assess baseline sleep habits followed by an overnight polysomnography (PSG). On the night of the sleep study patients will complete an Epworth Sleepiness Scale (ESS) evaluation to assess daytime subjective sleepiness for the week prior. 10 mL of blood will be drawn every 2 hours from 10pm till 6am to assess melatonin blood serum levels. Details of measurements are

described below.

OSA patients will then be randomly assigned into two groups. Group A will take 10mg melatonin 2 hours before bed for 7 days at home; group B will take 10mg of melatonin 4 hours before bed for 7 days. During the week of melatonin treatment all patients will repeat a 7 day sleep diary while wearing actigraphy watches, followed by a repeat overnight sleep study, including ESS, PSG and blood serum melatonin tests. This will allow us to determine appropriate timing of melatonin dosage to effectively shift sleep phase, and to assess the effects of melatonin on subjective sleepiness and sleep architecture as determined from the overnight sleep study.

Aims 2 and 3: Eligible participants will be randomized to either the placebo or melatonin treatment group. All participants will complete the same protocol. Participants will complete a daytime experiment involving questionnaires, assessing lung function via spirometry, blood pressure and vascular reactivity via EndoPat followed by chemoreflex control tests. That night participants will undergo an overnight PSG (details below) to assess AHI, arousal threshold, genioglossal activity and LG during sleep. In the morning 50 mL of blood will be drawn to test for oxidative stress markers and other metabolic markers. Participants will then go home and take either placebo or melatonin for 30 days at the time determined from results of Aim 1, after which patients will return and repeat both the day and night experiments taken at baseline. This will allow assessment of the effects of melatonin on oxidative stress (Aim 2) and the effects on ventilatory control assessed by waking chemoreflex tests (detailed below), and overnight AHI and LG (Aim 3).

The Polysomongraphy equipment (EEG, EOG, EMG, EKG, pulse oximetry, thermistor, nasal cannula) is standard for diagnostic polysomnography and is not FDA approved as they all are considered class I devices. Actigraphy watches are a standard measurement for determining movement, it is not FDA approved as it is a class I device.

The 7 day sleep diaries, and ESS are standard questionnaires and equipment used in clinical practice for assessment of sleep habits and subjective sleepiness.

Melatonin blood serum tests: (Aim 1)
On the night of the sleep study participants will arrive at approximately 7pm after eating dinner at their normal time. Participants will be instructed not to drink alcohol for 24 hours prior to the overnight visit. Lights out will occur between 10pm and midnight, determined by the patient according to their normal behavior. Patients will sleep in a private sound proof dark room. At 8pm a peripheral I.V. with a saline flush will be inserted into the back of the non-dominant hand. The first blood sample will not be taken for 2 hours to avoid interference in accurate melatonin reading by potential rise in adrenergic levels due to venipuncture. At 10pm (light period) 10 mL of blood will be drawn (first 3ml will be discarded due to saline flush, and 7ml reserved for analysis) and every two hours thereafter until 6am (light period) with the use of a red light with minimal disturbance to the participants sleep to determine the pattern of melatonin serum levels and effects of melatonin treatment. The 10pm sample will be drawn before bed and the 6am sample drawn immediately upon waking and lights on period. A total of 50ml blood will be drawn per night. Blood will be drawn into vials, centrifuged to separate serum, which will be stored at -70 degrees F until serum melatonin levels can be tested using commercially available ELISA kits by the CTRI at UCSD.

Overnight study: Polysomnography (PSG).
This study will be performed at the CTF Sleep Laboratory. The same procedure will be used in Aim 2 and 3 (with some additional equipment stated below). Approximately 1 hour before the patient's normal bed time patients will be fitted with the equipment for PSG. Paste-on electrodes will be applied to the scalp, chin, chest and face. This allows monitoring of EEG, EMG, EKG and EOG to document wakefulness/sleep and follow

heart rhythm throughout the study. Paste-on EMG electrodes placed over the anterior tibialis muscles to detect leg movement activity and elasticized bands are secured around the abdomen and chest to detect respiratory motion. Respiration is further monitored by placing an oxygen cannula in the nose to detect nasal airflow, and a thermistor taped near the nose and mouth to detect oro/nasal airflow. A pulse oximeter probe will be clipped to the finger or earlobe for continuous oxygen monitoring. A microphone is attached to the suprasternal notch to detect snoring and a body position sensor attached to a thoracic belt to monitor body position.

This equipment (EEG, EOG, EMG, EKG, pulse oximetry, thermistor, nasal cannula) is standard for diagnostic polysomnography and should not be uncomfortable. Once all of this equipment has been comfortably and securely fastened, the subject will be allowed to fall asleep and data recording will begin. Subjects will be asked to remain in the supine position as much as possible. The study will end at approximately 6 am, at which time the monitoring equipment will be removed and the subject is free to leave. Apneas and hypopneas will be defined using the recently published American Academy of Sleep Medicine (AASM) guidelines for syndrome definition and measurement techniques.

If we see any life threatening abnormalities in any of the studies, we will discuss it with both the treating physicians and participants.

Experimental visits Aims 2 and 3
Before each overnight visit, a urine test will be given to any women of child-bearing age. If we find the subject is pregnant, they will be excluded from the study.

Participants will be instructed to have had lunch a minimum of 3 hours before arrival, avoid alcohol during the day of the study and night before, and avoid caffeine and strenuous exercise during the day of the experiment. Patients will arrive at the laboratory at 4 pm and undergo physical assessment (including height, weight, neck and abdominal circumference, supine abdominal height and spirometry). Participants will complete questionnaires to assess sleep quality (Pittsburgh sleep quality), general health (SF-36) and sleepiness (Epworth Sleepiness Scale). To assess concentration and reaction time participants will undergo a psychomotor vigilance test (PVT) which is a short (approx.. 5 minute) test run on a computer program. Endothelial function will be assessed using EndoPAT (described below). Blood pressure will be taken after EndoPAT to prevent altering vascular reactivity due to cuff occlusion. At 5 pm participants will undergo two tests to determine ventilatory responses to both hypercapnia and hypoxia using a modified rebreathing technique. At 6pm participants will have a break for dinner. At 8pm participants will be instrumented for the overnight PSG and allowed to sleep. Patients will be woken at 6am and a blood sample will be taken immediately to assess oxidative markers.

EndoPAT test: Endothelial vasodilator function will be assessed using the EndoPAT 2000 (Itamar Medical, Caesarea, Israel) device. This technique evaluates the percentage change of flow from baseline to the maximum flow during reactive hyperaemia following a five minute ischemia of the distal forearm (achieved by blowing up a blood pressure cuff around the arm). The advantage of this technique is that it is non-invasive, simple, and reproducible with less observer dependence compared to ultrasound based techniques. This test has been shown to be accurate, sensitive and reproducible and EndoScore™ calculated by the EndoPAT test has been shown to be highly predictive of traditional cardiovascular risk factors.

This test takes approximately 15 minutes. EndoPAT measures digital pulse amplitude with the probes placed on the tip of each index finger. The EndoPAT probe comprises a pneumatic plethysmograph that applies uniform pressure to the surface of the distal finger, allowing measurement of pulse volume changes in the finger. Digital pulse amplitude will be measured during three stages from both right and left index fingers. Arterial flow of one arm will be occluded for 5 minutes by a blood pressure cuff placed on a proximal forearm at a pressure of 60 mm Hg above the systolic blood pressure or 300 mm Hg (whichever is higher). The digital pulse amplitude of the non-occluded arm will be measured as the control baseline. The three stages of the test will be: 1) Before

occlusion (resting period) - digital pulse amplitude will be recorded for 5 min at rest; 2) During arterial flow occlusion- arterial flow occlusion will be applied in one arm and digital pulse amplitude will be recorded for another 5 min; 3) After occlusion- the cuff is rapidly deflated to reverse the occlusion and digital pulse amplitude recorded for 5 minutes to assess peripheral vasodilator response to reactive hyperemia.

Chemoreflex tests: At 5 pm participants will undergo two tests to determine ventilatory responses to both hypercapnia and hypoxia using a well published modified rebreathing technique. Stick on electrodes will be placed on the chest for continual monitoring of EKG and oxyheamaglobin will be measured by finger probe throughout. While in the supine position participants will be asked to breathe via a mouth piece with a nose clip on. The mouth piece will be connected to a bag containing either a hypoxic gas mixture of 50mmHg O_2, 42mmHg CO_2 in balance N_2 or a hyperoxic gas mixture of 150mmHg O_2, 42mmH CO_2 in N_2. The mouth piece will be connected to a pneumotachograph and carbon dioxide and oxygen will be sampled at the mouth piece continuously. The gas line used for sampling will feed back into the bag.

Initially participants will breathe room air for 5 minutes, followed by voluntary hyperventilation for 5 minutes while being coached to maintain end tidal CO_2 between 20 - 25mmHg. After end tidal CO_2 has been maintained at this level for 5 minutes, participants breathing will be switched over to the bag upon which they will be instructed to take three rapid deep breaths to allow equilibrium of CO_2 between the bag, lungs and blood stores. Rebreathing will then continue until end tidal CO_2 has reached 10mmHg above the CO_2 threshold (which is the point at which ventilation begins to increase with increasing CO_2). Oxygen in the bag will be manually bled into the bag to maintain the pre-test levels throughout.

PSG: In addition to the equipment described above, a snug fitting mask (either nasal or a full-face depending on mask fit and subject preference) will be fitted to allow measurement of respiratory parameters (breath-by-breath minute ventilation, inspiratory volume and carbon dioxide levels). The pressure recording catheter will be threaded through a sealable port on the mask.

Mask CO_2 will be monitored throughout using capnography and a peak picking algorithm employed to determine the end-tidal partial pressure of CO_2 ($P_{ET}CO_2$).

Genioglossal muscle activity (EMGgg) will be measured with a pair of unipolar intramuscular electrodes referenced to a single ground to provide a bipolar recording. We will use conventional techniques that have been in routine use in our laboratory for the last 10+ years. The technique involves inserting two sterile fine wire electrodes perorally via needles into the genioglossus muscle. The floor of the mouth will be topically anaesthetised by placing a lignocaine soaked cotton swab (containing ~4 ml of 4% lignocaine) under the tongue. Participants will be provided with a cup and instructed to spit and not to swallow their saliva until the cotton swab has been removed (~3 min) and their mouth rinsed with water. Two 25 gauge needles each containing a Teflon-coated stainless steel recording wire (<0.1 mm in diameter with ~1 mm at the tip bared of Teflon and bent to form a small hook) will then be placed 1.5-2 cm into the body of the genioglossus muscle. The needles will be inserted at right angles to the oral mucosa 3-4 mm either side of the frenulum and posterior to the salivary duct. Immediately after insertion the needles will be removed leaving the small recording wires in place. Our laboratory has performed this procedure on many occasions without incident. To prevent the wires from being accidentally removed by pulling, both wires will be firmly taped to the subject's cheeks. The wires are easily removed at the end of the experiment by gentle traction.

For the measurement of epiglottic pressure (Pepi) and calculation of arousal threshold, a thin solid state catheter will be introduced trans-nasally to the level of the epiglottis (positioned visually, 1 cm past the base of the tongue). This catheter will be placed after administration of a nasal decongestant (Oxymetazoline Hydrochloride 0.05% w/v, 2 sprays) and topical anaesthesia (lignocaine, 2 x 10 mg 4% lignocaine sprays plus

lignocaine gel (2%) applied to the catheter). The catheter will be secured at the nose with tape and connected to a pressure transducer.

Arousal threshold will be calculated as the average nadir epiglottic pressure immediately prior to cortical arousal (>3 sec of high-frequency activity on the EEG) from 20 randomly selected respiratory events. Sleep and ventilation will be scored from de-identified studies by a single experienced sleep technician blinded to treatment (MEL/placebo). Apneas and hypopneas will be defined using American Academy of Sleep Medicine (AASM) guidelines. To calculate LG breathing and sleep data will be exported and analyzed using the authors publicly available script using Matlab (MathWorks, Natick, MA, USA).

Posture has well known effects on lung volume and upper airway muscle control which have significant effects on AHI, while posture also directly influences ventilatory output via influence on the vestibular system. Therefore to limit confounding effects of posture on ventilatory control and AHI, throughout both overnight sleep studies for Aims 2 and 3 patients sleep will be restricted to the supine position. Participants are usually able to maintain supine position voluntarily even while sleeping, however if the participant changes position a technician will enter the room quietly with the lights left off and ask the participant to roll onto their back.

In some cases, the quality of sleep may not allow full data to be obtained. In these cases, the participant will be invited back for an additional night for recording the remaining data. However, the participant will certainly have no obligation to undergo additional recordings.

Oxidative markers blood test (Aim 2)
Immediately upon waking from the overnight sleep study (PSG), 50 mL of blood will be drawn from the forearm for analysis of blood serum Angiotensin II, total glutathione, oxidized/reduced glutathione ratio, glutathione peroxidase, super oxide dismutase, C-Reactive protein, catalase, HOMA and potentially other oxidative markers to be defined. Samples will be immediately processed to separate serum, which will be stored in darkness at -70 F to preserve until analysis can be conducted using commercially available ELISA kits by the CTRI at UCSD.

Biostatistical Analyses
During longitudinal biometric studies it is often inevitable that there will be some missed data. For this reason we will use linear mixed model analysis as this form of analysis is highly robust to data with missed or uneven data sets.

For Aim 1, we will use linear mixed model analysis and Pearson's correlation to compare groups (melatonin 2hrs vs 4hrs before bed) and outcomes (blood serum melatonin, ESS scores and sleep parameters such as sleep onset latency, sleep stages and total sleep time) to determine the effect of melatonin dosage on phase shifting, subjective sleepiness and sleep and to determine potential correlation between outcomes.

For Aim 2 we will also use linear mixed model analysis to compare treatment (placebo vs melatonin) and time effects (baseline measure vs the repeat measure after 30 days treatment) on outcomes of blood serum oxidative markers.

For Aim 3 we will also use linear mixed model analysis to compare treatment (placebo vs melatonin) and time effects (baseline vs the repeat measure after 30 days treatment) on outcomes of waking chemoreflex control and during sleep LG, AHI, minimum oxygen saturation, time spent below 90% oxygen saturation, apnea duration, apnea to hypopnea ratio and obstructive to central event ratio. The primary outcomes of Aim 3 will be AHI and

LG. Previous studies have shown that patients with high AHI have a high LG. Therefore, based on previously reported data of between subject variability in AHI and LG in patients with severe OSA (AHI of approximately 63), with 40 patients we would be able to detect with 80% power a difference in AHI of 30.9 events per hour, with a standard deviation of 34 and two tailed significance of 0.05. This is a clinically relevant reduction in AHI, which would reduce AHI toward the mild to moderate range. Using the same previously reported data, with 40 patients we would be able to detect with 80% power a difference in LG of 0.16, with a standard deviation of 0.18 and two tailed significance of 0.05. This would be considered a small effect, as previously reported effects of oxygen therapy found LG halved from 0.69 ± 0.18 to 0.34 ± 0.04. This reduction in LG reduced AHI by 53 events per hour. Consequently with 40 patients it would be unlikely we would miss a moderate or large effect on LG which would be expected to have a clinically relevant effect on AHI.

We will also compare results from Aim 2 and 3 to determine potential interactions between outcomes of blood oxidative markers, AHI, LG and waking chemoreflex control. Using both LG and AHI as the dependent variable, we will compare other outcomes as covariates.

10. HUMAN SUBJECTS

We will recruit 60 newly diagnosed and/or untreated OSA patients from the sleep clinic following initial diagnosis and also via advertisement. We will also recruit 20 healthy non-OSA participants either through the sleep clinic after a sleep study confirming lack of OSA, or via advertisement followed by a home sleep test. Estrogen is a ventilatory stimulant and alters ventilatory control. Consequently in order to include women in the study women will be asked their menstrual cycle history to schedule test days during the follicular phase. Pre-menopausal women will also be asked to provide a urine sample for a pregnancy test to confirm they are not pregnant and able to participate.

Aim 1: We will compare circadian rhythm and endogenous melatonin blood serum profiles in 20 untreated OSA patients and 20 non-OSA controls. We will then determine the effects of different time of dosage of melatonin on sleep phase, subjective sleepiness and sleep in the 20 untreated OSA patients.
Aim 2: We will study 40 untreated OSA patients before and after 30 days of melatonin or placebo treatment to determine the effects of melatonin on blood oxidative markers.
Aim 3: We will study the same 40 untreated OSA patients before and after 30 days of melatonin or placebo treatment to determine the effects of melatonin on ventilatory control both awake and during sleep.

Inclusion Criteria:

- Ages 18-70 years
- No other sleep disorders
- Severe OSA (≥30 AHI) and confirmed no OSA

Exclusion Criteria:

- Pregnant females
- Smokers (quit ≥ 1 year ago acceptable)
- Abnormal lung function (FEV1 and FVC < 80% predicted)
- Any known cardiac (apart from treated hypertension with acceptable drugs, see below), pulmonary (including asthma), renal, neurologic (including epilepsy), neuromuscular, hepatic disease, or patients with diabetes.
- History of driving or other accidents due to sleepiness or an ESS > 18.
- Prior or current use of melatonin.
- Use of serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, ACE inhibitors or any drugs which interfere with the renin-Angiotensin system, Losartan (or other Angiotensin II type 1 receptor

antagonists), calcium channel blockers, anti-inflammatories, anticoagulants, immunosuppressants, vitamins or any antioxidants.
- Use of any medications that may affect sleep or breathing.
- A psychiatric disorder, other than mild depression; e.g. schizophrenia, bipolar disorder, major depression, panic or anxiety disorders.
- Substantial alcohol (>3oz/day) or use of illicit drugs.
- More than 10 cups of beverages with caffeine (coffee, tea, soda/pop) per day.

11. RECRUITMENT

Untreated OSA patients will be recruited by research staff from the CTF Sleep Laboratory. Subjects will be recruited either from the sleep clinic after initial diagnosis, via ResearchMatch or after they initiate contact in response to IRB approved standard posters, newspaper advertisements, and/or flyers.

Subjects who initiate first contact or who are identified via ResearchMatch will be followed up with a telephone screening to ensure they qualify for participation in the study. After passing the phone screening signed informed consent and HIPAA authorization will be obtained by a research staff member before any study procedures are conducted. Following consent, participants will then undergo a home sleep test to verify if they have sleep apnea and to rule out any other sleep disorders. The home sleep test is a standard PSG using the same equipment as during the main experiment detailed above.

A HIPAA waiver form has been submitted to authorize a waiver of HIPAA and waiver of consent for recruitment purposes. Following approval, clinic patient files will be screened by an authorized research coordinator to identify patients that have completed a diagnostic sleep study and meet criteria to participate in the study. The patient will then be contacted directly by a research coordinator and be provided with a brief summary of the research and asked if they are interested in participating. If they agree they would be provided with full details of the study and would be enrolled into the study with fully informed consent. Patients would be informed that research staff are not permitted to disclose their diagnosis to them. Patients will be informed that their diagnosis will be provided by their physician as normal and participation in the study will not alter their course of treatment. The treating physician of patients that agree to be enrolled in the study will be informed and provided with a description of the protocol.

All participants will have an “opt-out” provision to avoid any possibility of coercion. ResearchMatch will be used to contact prospective participants with IRB approved recruitment message. Advertisement for this study will also be placed in appropriate locations. Recruitment advertisement text previously provided will be utilized for all modes of recruitment mentioned previously. If the provided text is to be changed, each recruitment item will be submitted to IRB for review and reapproval.

Slicer Dicer will be used to identify both Summary & Patient Level Requests:

Investigators plan to use UC San Diego Health’s Epic SlicerDicer, a self-service cohort discovery tool. Investigators will have access to direct summary and patient level data. Using SlicerDicer, investigators will:

Review the charts at a patient level to identify potential patients for recruitment.

We will be identifying patients with untreated Obstructive Sleep Apnea, ages 18-70 years, non-smokers, non-diabetic, and the without the following diseases: cardiovascular other than well controlled hypertension, pulmonary other than well controlled asthma, renal, neurologic, neuromuscular, and hepatic. We will create a call list with the patient’s name, phone number, and email address. This call list will be stored digitally under

password protection on a UCSD secured drive shared between the research team. Once identified, a member of the study team will contact the patient to ask if are interested in hearing about sleep medicine research study opportunities. If yes, the study team member will provide full details about the study. If no, the patient's name will be removed from the call list. All Epic Slicer Dicer users will have access and security provided by UCSD's Epic access team.

Prospective subjects will also be recruited from the community after individuals have initiated contact with the research staff. Permission to distribute IRB approved messages and flyers is being requested for the following sites and locations.

UCSD Listserve, UCSD Hospital Bulletin Board, Facebook, Twitter, Craigslist, RedCap Survey Form
IRB approved flyers will be digitally posted on the UCSD Listserve, Facebook, Twitter, and Craigslist. The flyers will also be placed on UCSD Hospital Bulletin Boards by the Community Engagement Manager for the Clinical Transitional Research Institute (CTRI).

Facebook Page Welcome Message
We are requesting permission to have this message posted on a Facebook page created for recruitment purposes.

> Welcome to the UCSD Pulmonary and Sleep Medicine Research group's Facebook page. We have ongoing research studies, primarily investigating obstructive sleep apnea. If you or someone you know has obstructive sleep apnea, please see our IRB approved flyers below. Occasionally, we are looking for healthy individuals without sleep disorders. Please regularly check our page for flyers describing these research opportunities.

Department of Motor Vehicles Booth
The Research Team will set up a booth between the hours of 8AM-5PM outside of the Clairemont Department of Motor Vehicles (DMV) at 4375 Derrick Drive, San Diego, CA 92117 to distribute IRB approved flyers. A permit application for Activity on State Property will be submitted to the California Highway Patrol for approval at least 30 days before the booth is prepared.

Radio
We are requesting permission to have the following message broadcasted on iHeartMedia San Diego radio stations--Star 94.1, 101.5 KGB, Channel 93.3, Rock 105.3, XTRA 1360 Fox Sports, KOGO: 600 AM, JAM'N 95.7--KPBS 89.5 FM, Jazz 88.3 FM, KFSD 1450 AM, KURS 1040 AM, and AM 1170 The Answer:

> UCSD Pulmonary and Sleep Medicine Research is offering research study opportunities, primarily for people with sleep apnea. If you or someone you know suffers from obstructive sleep apnea, contact us at (858) 246-2154 or sleepresearch@ucsd.edu for more information.

When this message is IRB approved, it will be submitted only to the radio stations listed above. We will request permission for any additional radio broadcasting stations.

Research Group Website
UCSD Pulmonary and Sleep Medicine Research group has a webpage hosted by UCSD health sciences. Wording from IRB approved flyers will be posted there.

UCSD CTRI REDCap Survey
Prospective subjects can voluntarily choose to fill out a UCSD CTRI REDCap Survey for this protocol.

Information will be securely collected and stored via REDCap and will be used to determine if the prospective subject is eligible for the study.

12. INFORMED CONSENT

Individuals interested in participating in this study will be given detailed explanation of the procedures, potential benefits, risks and discomforts of the study by the study researchers. A copy of the consent form will be mailed a minimum of 48 hours in advance to the individuals interested in participating in the study. The principal investigator will obtain informed, written consent on the day of the study. Subjects unable to give their own consent will not be included in the study. Subjects who have given a written consent, will be given a copy of the signed consent form. The original consent will be kept in the subjects research file in a locked cabinet.

They will also be informed that participation in the research is completely voluntary and will not directly impact the care that they receive at UCSD. Informed consent will be obtained in English for all English speakers. For patients who are not English speakers, consent procedures will be assisted by a certified interpreter (in person or over the telephone). Documents will be used that are in the subject's primary language. Patients will be told that continued, qualified interpretative services to the participant will be provided. Subjects unable to give their own consent will not be included in the study.

13. ALTERNATIVES TO STUDY PARTICIPATION

Other treatments for OSA include lifestyle changes, positional therapy, medicine, oral appliances, continuous positive airway pressure (CPAP), or surgery.

14. POTENTIAL RISKS

We believe that the risks associated with the study of blood tests, polysomnography, LG, hypercapnic and hypoxic chemoreflex tests to be small, having performed them in hundreds of subjects over the last 10 years. However, they will be individually addressed below.

Polysomnography: Polysomnography is a standard clinical test without true medical risk. However, as addressed above, the required instrumentation may lead to poor quality sleep and therefore sleepiness the next day.

Of note, due to the unfamiliar environment and equipment patients will likely have disrupted sleep during this recording. Thus, these patients may not sleep well. Therefore, they may become sleepy during the day. During this time the patients will be instructed to not drive or to drive as little as possible. They also should not operate machinery or participate in any other dangerous activity. These patients will be followed carefully during this time.

We do not believe there is any cardiovascular risk associated with briefly withholding CPAP in OSA, particularly because we are not studying patients with known cardiovascular disease.

Waking chemoreflex tests: This is a well published protocol which is routinely used to measure chemoreflex control in many laboratories both within the US and internationally. There should be no serious risks associated, although hypoxia may make some people feel light headed or disoriented and both hypercapnia and hypoxia are expected to increase heart rate and blood pressure. As we will not be recruiting patients with cardiac or respiratory disease there should be no risks associated with this procedure. However, patients will be informed at the beginning of the trial to terminate the protocol by coming off the mouth piece if they feel significant discomfort at any time, at which point, resumption of room air breathing would cause any discomfort to dissipate immediately. Additionally, heart rate, EKG, blood oxygen saturation and both inspired and expired CO_2 and O_2 will be monitored continuously throughout the protocol to ensure all are within safe limits.

EndoPat: This test is non-invasive and similar to having blood pressure tested which may cause mild pain during the 5 minutes of occlusion.

Epiglottic catheter: Inserting the pressure catheter through the nostril and into the pharynx may cause discomfort and gagging. However discomfort is usually mild, only during insertion and the catheter is well tolerated once positioned. The use of anesthetic (lidocaine) prior to insertion will significantly reduce this risk.

Genioglossal EMG: Electrode insertion is likely to be mildly painful, however this will be minimized with the use of topical anaesthesia. There is also the potential for infection or hematomas to occur, however the needle and mouth will be sterilized and the procedure will be conducted by trained staff who have several years of experience conducting the procedure with no adverse events.

Venipuncture for both melatonin and oxidative markers: Needle insertion is likely to be mildly painful although most people are familiar with venipuncture and therefore tolerate it well. There is the potential that small hematomas could develop, although we consider this unlikely. There is the possibility infection could occur as a result of the needle, although both the needle and skin surface will be sterile. We have, however, never encountered such a problem or heard of it happening elsewhere. As venipuncture will be conducted by trained and qualified staff, we believe the risks to be minimal.

Determination of Ventilation and Loop Gain During Sleep: The electrodes (EEG, EMG, and EOG), mask and oximeter used to monitor sleep and breathing may be mildly uncomfortable and could interfere with normal sleep. However, apart from the possibility of poor quality of sleep, no other important risk is anticipated.

Melatonin Administration: Melatonin is not considered a drug in the USA, but a food substance which is sold over the counter. Melatonin is a mildly hypnotic agent commonly used to treat insomnia, jet lag and sleep dysregulation, with the main potential side effect being sleepiness/grogginess the following day. Other reported side effects include headaches and dizziness. In very rare cases side effects of abdominal discomfort, mild anxiety, irritability, confusion and short-lasting feelings of depression have been reported. In addition, melatonin supplements can interact with various medications, including: blood-thinning medications (anticoagulants), medications that suppress the immune system (immunosuppressants), diabetes medications, and birth control pills. However we will not be recruiting women or patients taking any of these medications, therefore these interactions will not be of consequence in this study. Melatonin is considered non-toxic with no upper dosage limit, although for sleep phase effects dosage varies between 0.3mg to 5mg, with no reported difference in the effects on blood serum levels, phase shifting or core body temperature with doses over 1mg. Additionally, melatonin has a rapid half-life and fast clearance rate, with plasma half-life for doses up to 5mg reported to be less than 1 hr from time of dosage. Thus negative side effects of daytime sleepiness/grogginess are usually reported following use of slow release formulas, which we will not be using in this study. Additionally daily doses of 10mg melatonin have been used in several human trials investigating antioxidant effects of melatonin in treatment of inflammatory and chronic systemic oxidative stress induced disease, with rarely sited and only mild negative side effects. Consequently there is ample evidence that 10mg daily dosage of melatonin is safe with minimal to no risk. Due to melatonin's high safety profile it is sold over the counter in 10mg tablets. Therefore the proposed investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of melatonin. However subjects will be warned on these potential side effects and they will be advised to contact staff immediately regarding any negative side effects. Participants will be warned of the potential risk of driving or other potentially dangerous activities following melatonin therapy. For all overnight studies subjects/patients will be allowed unlimited sleep until they feel and they demonstrate to a physician their readiness to go home. They will be offered taxi fare or public transportation to travel home or else will be accompanied home by a responsible

adult.

There is a risk of loss of subject confidentiality. We will protect all subject information to the best of our ability so this will not happen.

15. RISK MANAGEMENT PROCEDURES AND ADEQUACY OF RESOURCES

As we anticipate minimal risk from our studies, we doubt any subject will need to be dropped. We do administer melatonin for each Aim of the study, but the dosage of 10mg utilized in this study has been used in several previously published human trials before, with none reporting any serious adverse effects. However participants will be closely monitored throughout the protocol and advised to contact the research staff immediately if any adverse side effects should present. If a subject were to experience worsening of their sleep apnea (e.g. repetitive desaturations below 70% lasting >30s), nasal CPAP would be administered and the experiment would be terminated.
We believe the risks of our research are quite minimal. All recordings will be performed in the closely monitored environment of our research laboratory in the CTF. Emergency equipment and personnel are readily available in the unlikely event of a serious complication.

16. PRIVACY AND CONFIDENTIALITY CONSIDERATIONS INCLUDING DATA ACCESS AND MANAGEMENT

We will make every effort to maintain patient privacy and confidentiality both during and following the study, including during initial screening and consenting. Electronic study data will be de-identified by substitution of codes for names and hospital identifiers and will be stored on a secure disk for access by Investigator and study staff only and a hard copy will be stored in a locked cabinet. All research staff are CITI certified and have had impressed upon them the importance of confidentiality. This study does not involve the collection of sensitive personal information from subjects. Data will be stored only at UCSD sites and will use will be confined to that specified in this protocol and its approved amendments.

17. POTENTIAL BENEFITS

There are no direct benefits to participation. Society in general may benefit as this study will help achieve a more complete understanding of the upper airway. Only by achieving a more complete understanding of the upper airway dilator muscles can better therapies be developed to treat obstructive sleep apnea.

18. RISK/BENEFIT RATIO

The investigators feel risks associated with these studies are outweighed by the benefits. We have greater than 10 years of experience doing this type of research and have never had a serious adverse event.
We anticipate furthering of our knowledge on our understanding of sleep apnea as a result of this research. We will be gathering more information about their sleep and be happy to discuss it with the treating physicians and participants upon request.

19. EXPENSE TO PARTICIPANT

There will be no cost to the subject for participating in this study. All of the tests and procedures that will be done for this research will be paid for by study funds. We will pay for any sleep studies done for research purposes.

Costs for any ongoing or routine medical care subjects would receive apart from this study will be billed to them or to their insurance company in the usual way. The subject will be responsible for any deductibles or co-payments required by your insurer.

20. COMPENSATION FOR PARTICIPATION

The participant will be compensated $150 per overnight study and an additional $25 for the time required to

complete each week of wearing the actigraphy watch and completing the sleep diaries for Aim 1. For Aim 3 participants will also be compensated $50 for both of the daytime chemoreflex tests. Therefore participants may receive up to $350 for being in this study. They will be paid as follows if they are enrolled in the study, receive study drug, and complete all parts of the study.

- Screening phone call/visit $0
- Aim 1 (week 1) $175
- Aim 1 (week 2) $175
- Aim 2 & 3 $350
- If data is inconclusive and needs to be retested, $150 per extra overnight.

Parking will be available free of charge and participants will also be reimbursed for minor out of pocket expenses including meal vouchers, public transportation or taxi vouchers.

If the subject terminates the study early, they will receive an amount based on the visits that have been completed. If any of the visits are missed, the subject will not be compensated for those visits.

21. PRIVILEGES/CERTIFICATIONS/LICENSES AND RESEARCH TEAM RESPONSIBILITIES

Dr. Atul Malhotra is a licensed physician and Principal Investigator of this study. Dr. Malhotra has medical privileges at UCSD and UC Medical Centers to perform sleep studies. Dr Malhotra will oversee the study and be involved in data analysis and interpretation of the study results.

Naomi Deacon is a PhD candidate who will be involved in recruiting participants, the consent process, conducting experiments, data analysis, interpretation of results and final report writing processes. Naa-Oye Bosompra is a research assistant who will also be involved in recruiting participants and the consent process.

Dr. Rachel Jen, Dr. Robert Owens, and Dr. Yanru Sylvia Li will be involved in the consent process and general medical assessment prior to study commencement.

Angelica Moore, Dillon Gilbertson and Janelle Fine are research assistants who will be involved in conducting experiments and data analysis.

Pam DeYoung is a certified Registered Polysomnographic Technologists and will perform placement of sleep electrodes (EEG, EOG, and all respiratory measurements as necessary for study protocol) at UCSD Clinical Teaching Facility. Pam DeYoung will also be responsible for recruitment, scheduling, data analysis and interpretation of the study results.
All personnel are employed by UCSD and have completed the appropriate CITI training.

22. BIBLIOGRAPHY

1. Wellman, A., A. Malhotra, et al. (2008). "Effect of oxygen in obstructive sleep apnea: role of loop gain." Respir Physiol Neurobiol **162**(2): 144-151.
2. Prabhakar, N. R. (2011). "Sensory plasticity of the carotid body: role of reactive oxygen species and physiological significance." Respiratory physiology & neurobiology 178(3): 375-380.
3. Chowdhuri, S., I. Shanidze, et al. (2010). "Effect of episodic hypoxia on the susceptibility to hypocapnic central apnea during NREM sleep." J Appl Physiol 108(2): 369-377.

4. Sadasivam, K., K. Patial, et al. (2011). "Anti-oxidant treatment in obstructive sleep apnoea syndrome." Indian J Chest Dis Allied Sci 53(3): 153-162.
5. Reiter, R. J., D. X. Tan, et al. (2003). "Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans." Acta Biochim Pol 50(4): 1129-1146.
6. Hernandez, C., J. Abreu, et al. (2007). "Nocturnal melatonin plasma levels in patients with OSAS: the effect of CPAP." Eur Respir J 30(3): 496-500.
7. Terrill, P. I., B. A. Edwards, et al. (2014). "Quantifying the ventilatory control contribution to sleep apnoea using polysomnography." Eur Respir J.(in press).
8. Kozirog, M., A. R. Poliwczak, et al. (2011). "Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome." Journal of pineal research 50(3): 261-266.

23. FUNDING SUPPORT FOR THIS STUDY

NIH RO1 HL085188
Total Project Period From: 03/01/2015 To: 03/01/2018
This grant will be going through UCSD.

24. BIOLOGICAL MATERIALS TRANSFER AGREEMENT

N/A. Not being transferred outside UCSD

25. INVESTIGATIONAL DRUG FACT SHEET AND IND/IDE HOLDER

N/A

26. IMPACT ON STAFF

N/A

27. CONFLICT OF INTEREST

The PI and any key personnel associated with this study do not have any financial interests or other "conflicts" related to this study.

28. SUPPLEMENTAL INSTRUCTIONS FOR CANCER-RELATED STUDIES

N/A

29. OTHER APPROVALS/REGULATED MATERIALS

N/A

30. PROCEDURES FOR SURROGATE CONSENT AND/OR DECISIONAL CAPACITY ASSESSMENT

N/A