# THE EFFECTS OF INDUCED SLEEP FRAGMENTATION ON CARDIAC SYMPATHOVAGAL BALANCE

by

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B.S., University of Pittsburgh, 2003

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2009

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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Abstract

Obstructive Sleep Apnea Hypopnea (OSAH) is a prevalent disorder that occurs in about 5% of the middle-aged adult population. Comprised of repetitive episodes of complete and/or partial upper airway obstruction during sleep, OSAH results in cyclic oxyhemoglobin desaturation-resaturation (e.g. intermittent hypoxia) and arousal from sleep (sleep fragmentation). Consequences of OSAH include increased risk of cardiovascular morbidity and mortality and elevated sympathetic activity (SA) during daytime wakefulness as well as sleep.

This has potential clinical relevance because heightened SA is thought to be one mechanism explaining the association between OSAH and cardiovascular disease. Although OSAH is associated with increased sympathetic contribution to cardiac sympatho-vagal balance (SVB), the pathway mediating this effect (intermittent hypoxia, sleep fragmentation (SF) or both) is unclear. Because obstructive upper airway events in OSAH patients precipitate both physiologic phenomena in a generally concomitant manner, it has been difficult to sort the individual contributions of each in clinical populations.

The aim of this study was to investigate the relationship between SVB and experimentally induced SF including examination of the possible interaction with being overweight in a healthy non-OSAH population.

Twenty-nine subjects entered into a 4 night / 3 day sleep study to evaluate the effect of experimentally induced SF. Subjects provided a spectrum of body mass index (BMI) ranging from normal to overweight. Subjects experienced two nights of undisturbed sleep followed by two nights of fragmented sleep. Awake SVB reflected by heart rate variability was measured during wakefulness before and after a night of undisturbed sleep and a night of fragmented sleep. Sleep duration and architecture were assessed under both sleep conditions.

SVB was decreased by transient changes from awake to sleep. SVB was affected by SF on an undisturbed night, but not a disturbed night. BMI had no effect. The public health significance of this study was that both

OSAH and increased SVB have increased risk of cardiovascular disease; through improved understanding of the relationship between particular components of OSAH (SF or oxygenation-reoxygenation cycle) and increased SVB could lead to improved treatment of OSAH and the reduction of cardiovascular disease in the population.

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List of Abbreviations

AHI – Apnea / Hypopnea Index

ANOVA – Analysis of Variance

BMI – Body Mass Index

CVD — Cardiovascular disease

EEG – Electroencephalogram

EKG — Electrocardiogram

EMG – Electromyogram

EOG — Electrooculogram

GLM – Generalized Linear Model

LF – Low Frequency

LHR – Low to High Frequency Ratio

HF – High Frequency

HRV – Heart rate variability

MSNA – Muscle sympathetic nerve activity

OSAH – Obstructive Sleep Apnea / Hypopnea

OSAMSOS – Obstructive Sleep Apnea and Metabolic Syndrome: Role of Oxidative Stress

PSG – Polysomnography

SA – Sympathetic Activity

SFI – Sleep Fragmentation Index

$SpO_2$ – Percent of Oxygen Saturation in blood

SVB –Sympathovagal Balance

TRT – Total Recording Time

TST – Total Sleep Time

# 1. Introduction

The intermittent complete and partial upper airway obstructions (apnea and hypopnea, respectively) during sleep that characterize obstructive sleep apnea hypopnea (OSAH) brings about two fundamental physiologic consequences: episodic, intermittent hypoxia reflecting the oxyhemoglobin desaturation during the apneas / hypopneas with subsequent resaturation when the airway reopens on termination of the event; as well as arousal from sleep which is usually temporally linked to termination of the events. The specific contribution of each of these consequences to abnormal cardiac sympathovagal balance (SVB) and adverse clinical outcome is uncertain. This study is relevant since previous research has shown that OSAH patients have increased sympathetic activation during wakefulness and sleep and that this is independent of obesity which is a prevalent condition in this patient population.

Due to the physiologic and temporal linkage within apnea and hypopnea events, it is difficult to separately assess the effects of intermittent hypoxia and sleep fragmentation (arousal) on SVB in OSAH patients. Because OSAH therapy simultaneously alleviates intermittent hypoxemia and sleep fragmentation, it is not useful in determining the independent physiological impact of each phenomenon. OSAH patients tend to have various other co-morbidities that could affect SVB. Consequently a model of sleep fragmentation was used in otherwise normal human subjects (non-OSAH) to assess the specific impact of sleep disruption on SVB.

It was hypothesized that overnight sleep fragmentation would alter SVB as reflected by spectral analysis of heart rate variability (HRV) during wakefulness in the next morning such that: 1) Sympathetic activity, measured by HRV shortly after awakening in the morning, would increase with experimentally induced fragmented sleep compared to undisturbed sleep over the preceding night and, 2) the increase would be exaggerated by obesity after controlling for other covariates known to affect HRV and OSAH.

Specifically, this thesis evaluates the effect of sleep on HRV. First the effect of transient sleep intervals on HRV recorded during periods of behaviorally assessed wakefulness ("awake" HRV recordings) will be assessed and the outcome of this assessment will be the measure used in subsequent analyses. Univariate statistical comparisons will be made comparing the HRV measured at different time points. Statistically significant comparisons will be further evaluated through assessment of multivariate regression models, specifically looking for the effect of sleep fragmentation and for the effect of being overweight on HRV.

The second aspect of this thesis is the design, implementation and maintenance of a data management system for the Obstructive Sleep Apnea and Metabolic Syndrome: Role of Oxidative Stress (OSAMSOS) project. The OSAMSOS project is the parent study from which the data for these analyses are derived. The management system was designed around a user friendly point and click interface that was specifically developed to address the specific needs of the project. The database holds all of the study data and integrates information from the patient surveys, sleep studies, laboratory assays, and heart rate information. The system was developed using MS Access.

# 2. Background

## 2.1 Obstructive Sleep Apnea Hypopnea

Over the last three decades, the association between severe OSAH and cardiovascular morbidity and mortality has been increasingly recognized. Several large community studies have examined the prevalence of OSAH; Sleep Heart Health Study [1], Wisconsin Sleep Cohort [2], and Penn State cohort [3]. As such, OSAH is believed to be common in the community (Table 1) and largely under-diagnosed. Summarizing the data from 7 studies (5 international), Punjabi estimated prevalence of OSAH at 3 - 7 % of adult males and 2 – 5% of adult females are affected [4]. Young et. al. estimated that mild or asymptomatic OSAH occurs in 20% of the adult population (30 to 60 years of age) and that symptomatic OSAH (Table 2) occurs in 5% of adult population.[5] Moreover, because the prevalence of OSAH increases with age until the 7$^{th}$ decade, this disorder will be increasingly prevalent as the population ages. There is some evidence suggesting that OSAH prevalence changes at age 65 and the characteristics of those with OSAH may be different.[5] The plateau in prevalence may be due to a survivor bias (that is, people with the more severe OSAH have not survived to the 7$^{th}$ decade leaving those who are more resistant to the effects and/or those with less severe disorder).

Untreated severe OSAH is associated with an approximate three to five fold increase in cardiovascular mortality[6, 7]. There also appears to be a dose response between increasing severity of OSAH and increased incidence of hypertension.[8] Patients also have increased risk

of cardiovascular morbidity [6, 9] including congestive heart failure and acute coronary syndrome [10]. One possible mechanism for the adverse cardiovascular outcomes could be from elevated sympathetic nervous activity that is sustained into wakefulness after the overnight sleep period in these patients.[11] Increased cardiac SA independant of OSAH is associated with increased mortality in patients with cardiovascular disease (CVD) and [12, 13] increase risk of myocardial ischemia and cardiovascular death[14].

In patients with OSAH, the upper airway becomes completely occluded or partially obstructed during sleep which results in complete cessation (obstructive apnea) or reduced airflow (obstructive hypopnea), respectively. Other apnea patterns include central apneas or mixed apneas. Obstructive apneas and hypopneas are identified when airflow ceases or is markedly reduced despite the patient's persistent respiratory efforts. Central apneas are identified when airflow is absent because the patient has not made respiratory efforts. Whereas central apneas often considered a consequence of cardiovascular or cerebrovascular disease [15], at least severe OSAH is believed likely to contribute to risk for cardiac and cerebrovascular disease. Mixed apneas reflect an event in which initially there is absent airflow due to absent respiratory effort but airflow remains absent when efforts resume. Mixed Apneas are usually grouped with obstructive apneas when analyzing breathing during sleep.

Inspiratory efforts against an occluded or partially obstructed upper airway may result in large intra-thoracic pressure swings characterized by generation of abnormal degrees of negative intrathoracic pressure. The excessively negative intra-thoracic pressure may influence the physiology and function of both the right and left ventricles. While increasing venous return to the right ventricle, the negative intra-thoracic pressure impairs left ventricular function by increasing after-load.[16] During the initial portion of the obstructive apnea or hypopnea there

is a reduction in cardiac output and blood pressure. These events also result in reduction in blood oxygen level (oxyhemoglobin desaturation or hypoxemia) as shown in Figure 1. With progression of the event, the reduced cardiac output, blood pressure and hypoxemia elicits compensatory augmentation of sympathetic nervous system activity to maintain homeostatic conditions [17]. Termination of apneas and hypopneas are associated with arousal from sleep which results in activation of the upper airway dilator muscles, restoration of airway patency and ability to generate airflow with inspiratory effort. Arousal is also associated with a transient surge in SA [18] which results in further increase in peripheral vascular resistance, an overshoot of systemic blood pressure and reduction in cardiac output for a brief interval following termination of an obstructive apnea event.

## 2.2 Obstructive Sleep Apnea Hypopnea and Sympathetic Activity

OSAH patients have increased sympathetic nervous system activation during both daytime wakefulness and overnight sleep as reflected by muscle sympathetic nerve activity (MSNA) (assessed by microneurography), catecholamine levels (circulating in blood or excreted through the urine), and HRV.

Microneurography is a method that records and permits quantification of efferent nerve traffic MSNA. Assessment of MSNA to gain insight regarding cardiac sympathetic nerve activity has typically involved insertion of a microprobe into the peroneal nerve posterior to the fibular head. These studies have demonstrated increased sympathetic nervous traffic during daytime wakefulness that appears to be correlated with the severity of OSAH. [19-21]

Circulating and urinary catecholamines have also been employed as a measure of systemic sympathetic activity. Catecholamines (epinephrine and norepinephrine) are elevated in normotensive OSAH patients.[21, 22] While potentially informative, by its nature, determinations from urine collections provide samples may be influenced by all conditions experienced over a relatively protracted time interval (e.g. collection during wakefulness to assess the effect of events during the previous sleep period is likely to be influenced by extraneous factors such as exertion and changing posture) and blood sampling is invasive and inherently stressful. On the other hand, assessment of HRV requires a brief period data collection from an electrocardiogram thereby providing a non-invasive, non-stressful snapshot of cardiac sympathovagal activity.

HRV is a measure of the temporal variability between heart beats (ventricular contractions) reflected on an electrocardiogram (EKG) signal as the "R" wave of the "QRS" complex (Figure 2). The power spectrum of the inter-beat variability may be used as an indirect measure of cardiac autonomic regulation. [23]

While observational studies have demonstrated the association between OSAH and heightened cardiac sympathetic activity during wakefulness and sleep, therapeutic interventional studies have provided compelling evidence of causality. After 6-12 months of continuous positive airway pressure (CPAP) therapy SA can be returned to levels that are comparable to those recorded in non-OSAH individuals [22, 24]**.** CPAP therapy introduces air under relatively low levels of pressure into the upper airway and acts as splint to support upper airway patency, prevent oxyhemoglobin desaturation and normalize sleep continuity. Short-term CPAP tends to decrease epinephrine levels, suggesting a decrease in SA as suggested by urine catecholamine change from day to night.[22] Six months or more of CPAP therapy has been shown to

normalize sympathetic activity during wakefulness as reflected by MSNA.[24-26] HRV analysis has suggested that long term CPAP use is associated with decreased cardiac SA.[27]

**Table 1: Prevalence of Obstructive Sleep Apnea Hypopnea**

| | | | Prevalence | |
|---|---|---|---|---|
| First Author (Reference) | N | Diagnostic method | Male | Female |
| Young [2] | 602 | Polysomnography | 4.0% | 2.0% |
| Bixler [3] | 1741 | Polysomnography | 3.9% | 1.2% |
| Punjabi [4] | | Summary of 7 studies | 3 - 7% | 2 - 5% |

**Table 2: Symptoms of Obstructive Sleep Apnea / Hypopnea**

- Excessive Daytime Sleepiness
- Habitual Loud Snoring
- Nocturnal Breathing Pauses, Choking, and Gasping


**Figure 1: Polysomnography demonstrating an Obstructive Apnea Event with associtated desatureation / resaturation**

20 second PSG epoch showing an obstructive apnea event (in pink) with associated oxyhemoglobin desaturation (in yellow). C4-A1A2: electroencephalogram (central lead EEG); EOG1-A1A2 and EOG2-A1A2: bilateral electrooculograms; EMG1: submental electromyogram; C3-A1A2 (electroencephalogram (central lead EEG); EKG2: electrocardiogram; TIBS: anterior tibialis (limb) electromyogram; RESP: oral airflow; RESPRS: nasal airflow; THORACIC: chest wall movement indicating breathing effort; ABDO: abdominal wall movement indicating breathing effort; OXIM: oximetry

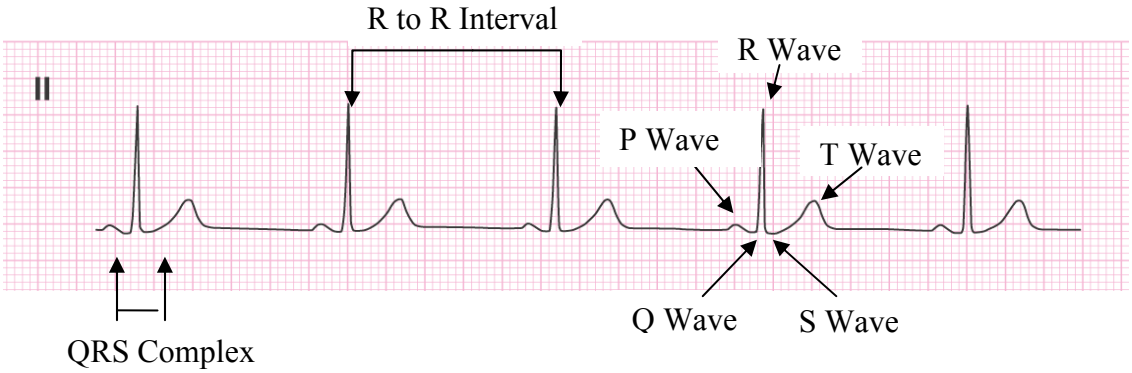



**Figure 2: Electrocardiogram**

# 3. Methodology

## 3.1 Study Design

A parallel study was designed to assess the effect of moderate overnight sleep fragmentation on SVB and a secondary analysis to assess the modifying effect of overweight/obesity (see Figure 3 for a summary of the study design). This analysis was a sub-study of a larger project to distinguish the effect of sleep fragmentation on various biomarkers and metabolic parameters. The current report focuses on the effect of sleep fragmentation on SVB. The 3-day/4night protocol design consisted of observing sleep over a four consecutive 24 nights.

On Night 1 subjects had a screening/acclimatization sleep study (polysomnogram, PSG). Night 1 screened the subjects for undiagnosed sleep disorders. This night served a dual purpose of acclimatization to the study environment (first night effect) [28], and screening for exclusionary OSAH. First night effect means that unfamiliarity with the environment may influence sleep quantity architecture. Without evidence of exclusionary degrees of OSAH or evidence or other abnormal sleep patterns during the acclimation night, the subject continued in the protocol. Subjects arrived at the Neuroscience Clinical and Translational Research Center (N-CTRC) in Western Psychiatric Institute and Clinic around 7:30pm. PSG monitoring sensors (see section 3.3 Polysomnography below for more details) were placed by trained technicians at approximately 8:30pm. For every night of the protocol, the aim of the sleep technicians was to

have the subjects start their sleep period by 11 pm. The morning after night 1 and night 3, at 7:00 am if not already awake, the subject was awakened by the sleep technician. PSG sensors were removed from the subject and permitted to shower prior to breakfast. During the day, subjects were visually monitored by a member of the research team to ensure maintenance of wakefulness. Subjects were permitted to watch television, access the internet, and have visitors. Subjects were on a weight maintaining, low anti-oxidant diet provided by the N-CTRC dietician. The diet did not permit caffeine. Subjects were also asked to refrain from caffeine for at least 24 hours prior to starting the study protocol. The screening/acclimatization night PSG (Night 1) and baseline data-collection PSG (Night 2) were recorded during undisturbed sleep (no experimentally induced sleep fragmentation). Night 3 and Night 4 reflected nights during which there was experimentally-induced sleep fragmentation.

Ten minute recordings of a single-channel awake EKG signal were made at four different time points: evening before night 2 (EBN-2); morning-after-night 2 (MAN-2); evening-before-night 4 (EBN-4); and, morning-after-night 4 (MAN-4). The evening EKG recordings were done after the monitoring sensors were applied and the subject had rested in bed undisturbed for as long as the N-CTRC protocol/orders said (10 minutes). Ten minute EKG recordings were obtained while the subjects were lying quietly in the supine posture and assessed to be behaviorally awake (by a N-CTRC technician) along with sleep staging based on PSG. If there was a need, subjects were given the opportunity to void prior to EKG data collections for HRV analysis. The morning EKG recordings were performed after being awakened by a sleep technician at 7:00 am. Subjects were then asked to lay quietly supine, but awake for 10 minutes. During the EKG recording, sleep technicians monitored the PSG in real time. If the technician

thought the subject was beginning to doze, they would verbally encourage the subject to remain awake.

After lunch on the day following the baseline data-collection PSG (Night 2), subjects were allowed to leave the study area. Prior to the subject leaving, the study coordinator provided reminders of what they should not do [including but not limited to napping, refraining from caffeine, snacking.]. Subjects were counseled to return to the N-CTRC by 5 pm, prior to dinner. The study coordinator inquired about study violations. If no violation, then the subjects were allowed to continue with the study.

During nights 3 and 4 sleep fragmentation (arousals) was experimentally induced by the technicians attending the PSG. Efforts to induce arousal began after the subject had experienced 2 minutes of Stage 2 sleep or a deeper sleep stage as determined by real-time visual monitoring of the PSG recording by a trained sleep technician. To induce experimental sleep fragmentation the sequence of arousal methods were:

1. Tones were sounded through a speaker near the subject's head utilizing an increasing tone (in 10 decibel increments) in until an arousal was recorded or 105 decibels were reached. If initially unsuccessful in eliciting arousal, a given tone intensity was applied once again prior to increasing the decibels.
2. Clicking sounds were generated through the speaker by turning the microphone on and off.
3. Technician knocked on the door
4. Technician lightly touched the subject on the leg or shoulder.

Subsequent arousal attempts were initiated at the last successful tone intensity level, or 105 decibels whichever was lower.

## 3.2 Study Population

Adult subjects (≥ 18 but < 65 years old) were recruited from the greater Pittsburgh, Pennsylvania community area via advertisements: flyers, local newspaper, campus newspaper, brochures, direct mailings, and word of mouth. The population selected for this paper was a subset of the larger parent study. Respondents were pre-screened for major exclusionary features during a telephone interview during which time an outline of the study and requirements for participation were also explained. Inclusion criteria are listed in Table 3. The study was approved by the Institutional Review Board (IRB) of the University of Pittsburgh.

Subjects who passed the telephone screening and who were willing to continue with the screening process met with a study investigator for a screening medical interview and physical examination. Height and weight were obtained to calculate Body Mass Index (BMI = kilograms / meters$^2$). Study groups were determined based on BMI: Group 1's BMI was < 25 and Group 2's includes those individuals who are overweight (BMI between 25 and 30) and obese (BMI >30). Age was calculated in years based on date of the in person screening.

## 3.3 Polysomnography

The gold-standard test for assessing sleep and breathing is polysomnography (PSG), which represents simultaneous recording of multiple variables reflecting sleep duration, architecture (e.g. sleep stages) and continuity (e.g. arousal frequency) as well as parameters of breathing and oxygenation. PSG was recorded during sleep, and during the awake EKG on EBN-2, MAN-2, EBN-4, and MAN-4. The PSG montage include simultaneous recording of bilateral central and occipital - electroencephalogram (EEG), electrooculogram (EOG), submentalis electromyogram (EMG), lead $V_2$ electrocardiogram (EKG) lead, nasal air flow was recorded using a nasal pressure sensor, and arterial oxyhemoglobin saturation was recorded by finger pulse oximetry ($SpO_2$). PSG signals were digitally recorded and stored as well as displayed on a video monitor in real-time.

Standard PSG was scored according to the criteria described by Rechtschaffen and Kales [29]. Sleep was scored in 20 second epochs. Apneas were defined as complete cessation of airflow lasting $\geq$10 seconds and hypopneas were defined by $\geq$30% reduction in airflow from baseline that lasted $\geq$10 seconds and associated with $\geq$4% oxyhemoglobin desaturation.[30] The Apnea-Hypopnea index (AHI) was calculated by dividing the sum of the total number of apneas and hypopneas by the number of hours slept. Arousals were identified by criteria of the American Sleep Disorders Association (now the American Academy of Sleep Medicine) [31, 32] The Sleep Fragmentation Index (SFI) was calculated by dividing the sum of the total number of arousals (which would include spontaneous arousals, respiratory arousals, and experimentally

induced arousals) and awakenings divided by total hours of sleep. Average $SpO_2$ during overnight sleep readings was measured during sleep. The percentage of total sleep time (TST in minutes) spent with $SpO_2$ <90% (%TST with $SpO_2$ < 90%) was also calculated. Overnight duration of SWS was calculated by the time spent in minutes in Stages 3 and 4 sleep.

### 3.4 Heart Rate Variability Analysis

SVB was estimated by examining the autoregressive spectra of the inter-beat-interval (IBI) of the EKG, obtained from a modified chest lead using standard surface electrodes [33]. Interest was focused on the high frequency (HF) band (0.15-0.4 Hertz, Hz) and the low frequency (LF) band (0.04-0.15 Hz) [34]. The high frequency band is reported to reflect parasympathetic activity; the low frequency band reflected sympathetic and parasympathetic activity.[33, 35] The ratio of low to high frequency bands (LHR = LF / HF) is an index of sympathovagal balance. An increase in LHR is thought to be associated with an increase in sympathetic activity. During the four time periods (EBN-2, MAN-2, EBN-4, and MAN-4), simultaneous recording of EKG and standard PSG occurred.

The EKG, was sampled at 1024 Hz and an automated, commercially available algorithm extracted the IBI sequence from the EKG signal (Mindware Heart Rate Variability Scoring Module, version 2.16; Mindware Technologies Ltd., Columbus, OH). The EKG signal was evaluated by a trained technician for artifacts and edited for arrhythmias and ectopic beats. Corrections were made by interpolating preceding/successive beats [36]. Two-minute IBI epochs were evaluated and the autoregressive (AR) spectrum for each epoch was estimated using the

Burg algorithm. The order of each AR model in the range of $12 \leq P \leq 20$ were selected using Akaike's final prediction error [37]. The spectrum was decomposed utilizing the residuals theorem. [38] This allows for heart rate variability to be accumulated in two bands (0.04-0.15 Hz Eq and 0.15-0.4 Hz Eq.). Commercially available software was utilized for an automated IBI extraction algorithm.

| | Undisturbed Sleep | | | Fragmented Sleep | | | |
|---|---|---|---|---|---|---|---|
| **Night 1**<br><br>**Acclimate Night**<br>PSG | | **Night 2**<br><br>**Baseline Night**<br>PSG | | **Night 3**<br><br>**Experimental Night 1**<br>PSG | | **Night 4**<br><br>**Experimental Night 2**<br>PSG | |
| | **Evening Before Night 2**<br>10 minute awake EKG with PSG | | **Morning After Night 2**<br>10 minute awake EKG with PSG | | **Evening Before Night 4**<br>10 minute awake EKG with PSG | | **Morning After Night 4**<br>10 minute awake EKG with PSG |

PSG = Polysomnography; EKG = Electrocardiogram

**Figure 3: Study Design Paradigm**

**Table 3: Inclusion Criterion**

- Adults ≥ 18 and < 65 years of age
- Non-smoker for at least 6 months
- No historical or physical examination evidence of active cardiopulmonary, neurologic, renal, hepatic or thyroid disease (individuals on a stable thyroid replacement were included)
- No history of movement disorder during sleep, or circadian rhythm disorders
- No excessive daytime sleepiness, Epworth Sleepiness Scale Score < 9 [39, 40]
- No history of chronic insomnia, mood or affective disorders or other psychiatric disorders as assessed by the Patient Health Questionnaire, and Pittsburgh Sleep Quality Index [41]
- Subjects with a history of Depression who report having no hospitalizations for Depression within the previous 3 years and was on a stable regimen of anti-depressants for 3 months
- Subjects must report a regular sleep-wake pattern with an estimated sleep time between 6.5 and 10.0 hours per night
- Not taking medications known to affect heme metabolism
- Ability and willingess to undergo a weight maintaining low antioxidant diet provided by study dietician
- Willingness to avoid cafeinnated beverages and food starting day prior to the start of the study periord and during the study period
- Subjects report consumption of an average of $\leq$ 1 alcoholic beverage / day

**Table 3: Inclusion Criterion**, **continued**

| |
|---|
| • Ability to understand the study and sign informed consent |
| • Women with child-bearing potential must test negative via commercially available urine pregnancy test |
| • Lived within the greater Pittsburgh, Pennsylvannia area |
| • No uncontrolled hypertension (blood pressure: systolic >150/ diastolic >100) |

# 4. Data Management

For the parent study a Microsoft Access database was created with the intention to track, enter, and store information about each subject and their test results. Information about each subject would arrive at various times depending on when sample analyses took place (e.g. for some analyses samples had to be batched). To facilitate data management a point and click interface was created (Figure 4).

Data arrived from the labs in either an electronic format or a hardcopy format. When appropriate, the electronic information was copied and pasted directly into the appropriate table. Most of the information received from the various labs was in a hardcopy format. Being that data entry was highly susceptible to keystroke errors, electronic forms were created. The forms were setup to mimic the paper version. In addition, the form also had a double entry feature built in to help minimize the possibility of keystroke errors. Double entry is a method setup to compare one entry to another entry made a later time. If both entries were the same, then the original entry was considered valid. If entries were discrepant then the reason for the difference can be checked and the correct value entered. Besides the double entry, validation rules were created inside each form to aid in ensuring the entered value was appropriate, for example: TST had to be less than the total recording time; AHI value could not be negative; and, total hypopnea had to equal the sum of obstructive, central and mixed hypopneas, etc. Figure 5 shows part of the form that was designed for entering sleep reports from the N-CTRC which includes a double entry feature.

Not all subjects had complete ascertainment, for example: some subjects' blood samples could not be obtained due to that poor venous access; or, subjects did not complete all four nights in the study for some reason. A study completion form was created to track the amount of information collected on each subject. This allowed the study coordinator to track what information / samples were obtained about the subject. The database was able to generate reports regarding samples. Reports included information such as what samples were available from which subject that still needed to be delivered to the lab(s) and what sample is awaiting results from the lab.

The Access database for the parent study provided the information for this thesis. The information stored in the database tables were directly imported into analytical statistical packages. SAS programs were written to directly retrieve information from the database.

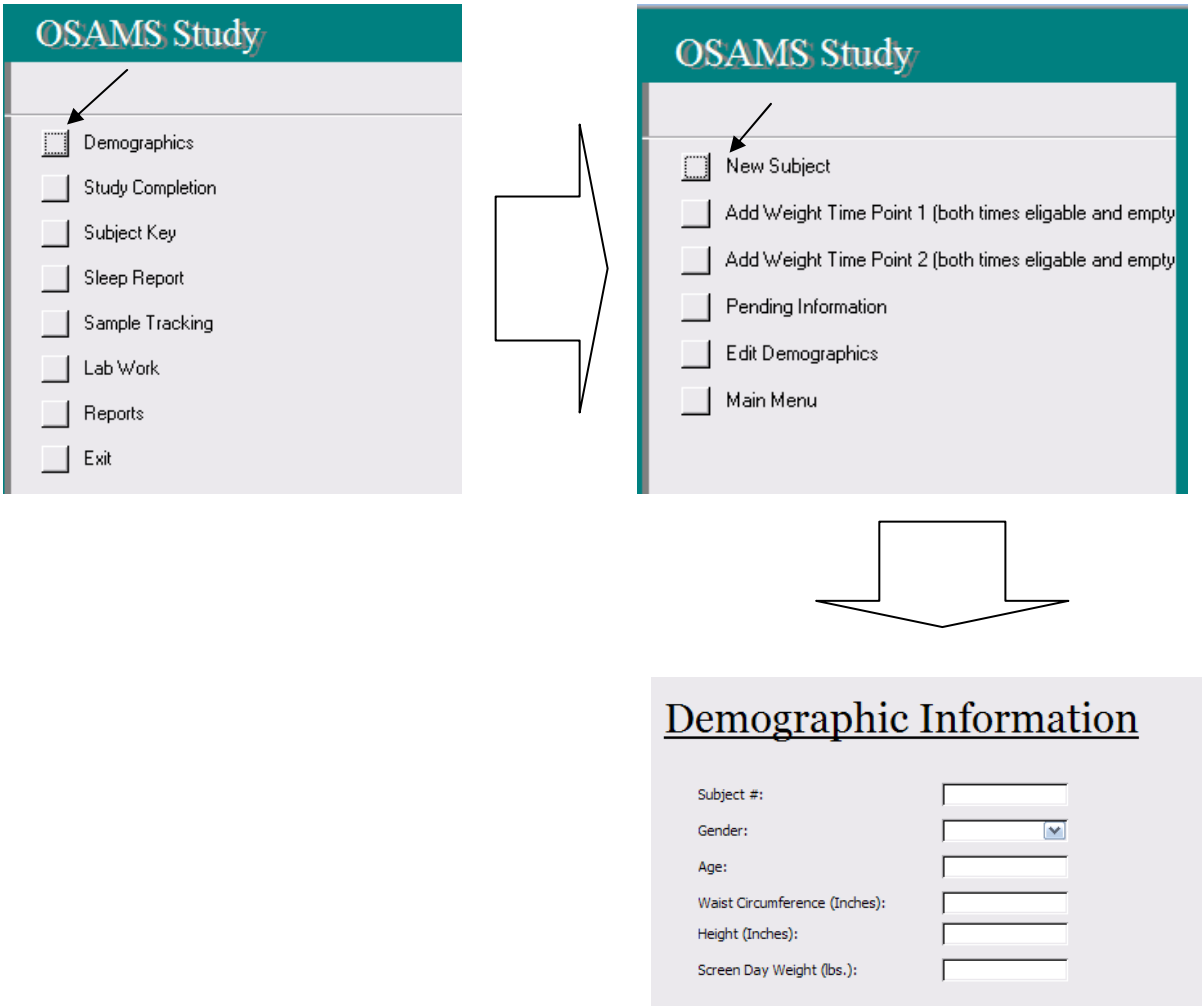



**Figure 4: Example of clickable interface flow in study database**

Example of clickable interface leading to the Demographic Information data entry form. Black arrows point to where the user would click to get to the Demographic Information Form.

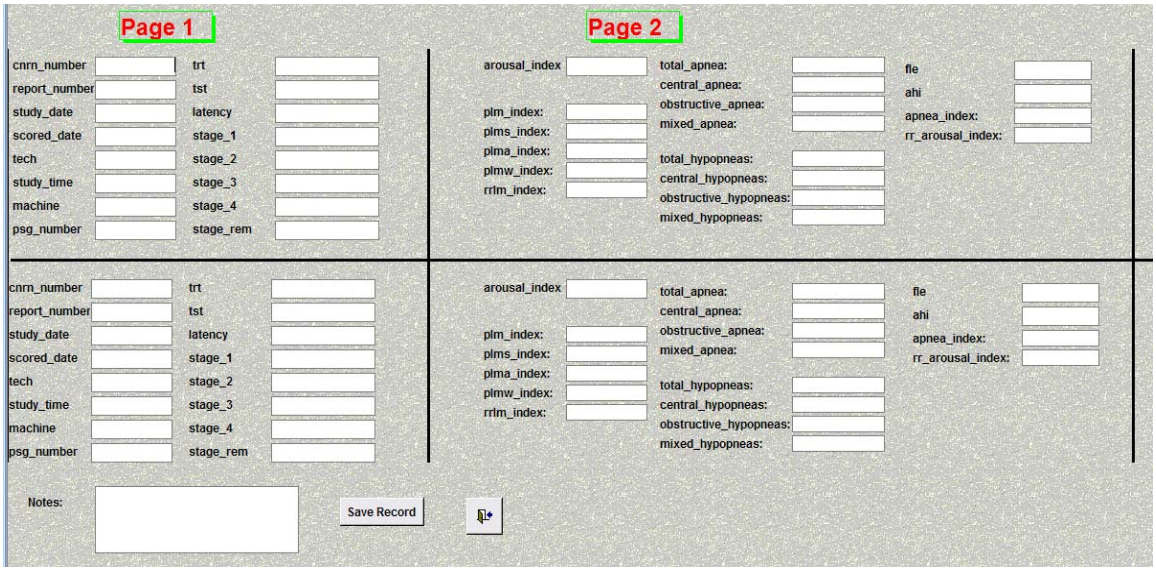



**Figure 5: Form for entering sleep information**

Sleep report data entry form which also includes the setup for double entry.

# 5. Statistical Considerations

## 5.1 Variable Creation and Consolidation

The awake EKG was recorded for 10 minutes and from this five 2-minute LHR epochs were derived. These epochs were combined by averaging so that each person had one averaged reading for each time point. It was noted that there were some subjects that had transient changes between sleep / awake state during the "awake" EKG recording. The variation in sleep / awake could affect the HRV recording, and the impact of this was unknown. For each of the four time periods, LHR was calculated three different ways: based on all epochs ($LHR_{ALL}$), based on non-sleep epochs ($LHR_{AWAKE}$), and based on sleep epochs ($LHR_{SLEEP}$). Thus, each 2-minute LHR epoch had six 20-second epochs of sleep staging evaluated. If any of the epochs of sleep staging were scored as any stage of sleep, then the entire 2-minute LHR epoch was removed prior to generating the averaged $LHR_{AWAKE}$. LHR was also generated by averaging values from epochs scored only as a stage of sleep ($LHR_{SLEEP}$). Overnight change in LHR was calculated as averaged LHR morning minus averaged LHR night as assessed at night 2 (undisturbed sleep) and night 4 (fragmented sleep).

SFI, AHI, average $SpO_2$ during sleep, percent TST with $SpO_2 < 90\%$, SWS, and TST information were all collected from the overnight PSG assessed on undisturbed and fragmented sleep and treated as continuous variables. Age was also treated in a continuous fashion. BMI

was treated as a categorical variable as defined by BMI < 25 kg / $m^2$ as the baseline. Gender was defined as 1 for females and 0 for males (baseline). Fragmented sleep, treated as a categorical variable, was defined as 1 and undisturbed seep was defined as 0 (baseline).

## 5.2 Statistical Methods

The data was analyzed using Statistical Analysis System (SAS) software version 9.2. Continuous data were summarized using basic descriptive statistics (mean, standard deviation, minimum, and maximum). PROC UNIVARIATE procedures were used to generate descriptive statistics for the continuous variables; Kolmogorov-Smirnov test (K/S) and quantile-quantile (QQPLOT) plot of the residuals where used to assess the normality of variables. When appropriate, log transformations were performed and normality reassessed. LHR in the power domain is usually skewed and log transformation of LHR is generally done in the literature for parametric analyses.[42] Values are reported as mean (standard deviation) (minimum: maximum). Categorical data was assessed using frequency counts and percentages using a PROC FREQ procedure.

When appropriate a generalized linear model using PROC GLM clustering on the subject level was used for comparisons with attention directed towards repeated measures ANOVA.

Repeated measures multiple linear regression using the PROC MIXED procedure was used to analyze the relationship between outcome and predictor variables. PROC MIXED options used were: Method was maximum likelihood estimation (METHOD = ml); subject

option was selected as the study identification variable called id (SUBJECT = id); covariance structure was unstructured (TYPE = un). Significance was selected with an alpha < 0.05.

Model building was then done by a stepwise selection process. Variables entered with a $p < 0.2$ and then were removed if $p > 0.1$. Regardless of their significance, grouped BMI and Sleep Fragmentation indicator variables would remain in the model. Model comparisons were made via -2 log likelihood comparisons utilizing a $\chi^2$ distribution. Residual and fit diagnostics were then evaluated and addressed. Statistical significance of covariates was considered at an alpha < 0.05.

### 5.2.1 Statistical Comparisons

To select the appropriate outcome variable ($LHR_{ALL,}$ or $LHR_{AWAKE}$) those individuals that had evidence of sleep during the recording of their LHR were assessed. This was only done for participants who had both evidence of sleep and evidence of awake during the same time point. A generalized linear model clustering on the subject level was used to assess if there is a significant difference between $LHR_{SLEEP}$ compared to $LHR_{AWAKE}$. A repeated measures ANOVA using the PROC GLM procedure was used for the analysis. If there was no significant difference between the awake and sleep recorded LHR epochs then the preference would be to select the $LHR_{ALL}$ as the outcome variable otherwise the $LHR_{AWAKE}$ would be used.

The effect of sleep fragmentation on the individual sleep variables (AHI, SWS, SFI, average SPO2, % TST with SPO2 < 90%, and TST) was assessed via a paired t-test. Those significant sleep variables were further evaluated in model building as covariates.

Outcome variables comparisons were made of the LHR using a paired t-test (PROC TTEST) between the time points to determine if there were differences between time points: EBN-2 vs. EBN-4; EBN-2 vs. MAN-2; EBN-4 vs. MAN-4; EBN-2 vs. MAN-4; and, overnight change on undisturbed night vs. overnight change on fragmented night. Those with significant differences were further evaluated via model building using linear regression, or a mixed models as described below.

The magnitude of the overnight change of LHR was individually evaluated over undisturbed sleep and over fragmented sleep separately. The association of variables of sleep and demographic information were individually evaluated by a linear regression model via PROC REG procedure to assess if any predictor of interest modulates LHR on any individual night.

Comparisons were then made of the differences between the magnitudes of overnight change of LHR using a repeated measures linear regression (PROC MIXED). Evening before undisturbed sleep and morning after fragmented sleep was evaluated using the same procedures described for the comparison of the magnitude of overnight change of LHR.

# 6. Results

Overall synopsis: Twenty-nine subjects (18 female) completed the four night / three day protocol with all four awake EKG recordings. The demographic characteristics are summarized in Table 4. An evaluation of the effect of transient sleep on "awake" LHR recoding was found to show significant differences. As a result, the two subjects that had evidence of sleep in all LHR epochs during a given recording period were removed from all subsequent analyses. Paired t-tests were used to assess if there were significant differences between the recording periods. Those statistically significant comparisons were further explored utilizing multivariate regression models with particular interest in evaluating the effect of sleep fragmentation on LHR while controlling for overweight.

## 6.1 Outcome Variable Selection

SVB is known to differ between wakefulness and sleep in normal individuals and after a night sleep fragmentation there was concern about inadvertent, brief sleep episodes that were not detected by the technician collecting the EKG data. Thus to be more assured that differences between SVB before and after sleep fragmentation were not confounded by undetected sleep the

following exploration was conducted. An assessment was made to determine if sleep occurring during the HRV recordings alters the outcome measure of LHR. As the assessment was made, there were two subjects that showed evidence of sleep during at least some portion of all five two minute HRV analysis segments (in other words, there were no sleep-free HRV analysis segments during a given time point). Those two subjects (Table 5) were removed from further analysis. To determine if sleep affects LHR, $LHR_{AWAKE}$ was compared to $LHR_{SLEEP}$ using repeated measures ANOVA. Only those subjects that had evidence of sleep and awake during a given time point (i.e. EBN-2) were assessed.

Twenty-three of 27 subjects had epochs with EEG evidence of sleep during their "awake" EKG recording for HRV analysis. For those 23 subjects, there were a total of 45 out of a possible 92 time points affected by sleep and had evidence of awake. For all time points the mean $LHR_{AWAKE}$ 2.30 (sd: 2.12) was compared to a mean $LHR_{SLEEP}$ 1.91 (sd: 1.63). Normality was assessed and found that the $LHR_{AWAKE}$ and $LHR_{SLEEP}$ were skewed and not normally distributed based on K/S statistics and QQ plots and were thus log transformed. Reassessment indicated that the transformed values were normally distributed. Log transformed, $LHR_{AWAKE}$ and $LHR_{SLEEP}$ were used in the subsequent analyses. Repeated measures ANOVA had a significant overall F statistic ($p = 0.0017$) suggesting that Log $LHR_{AWAKE}$ was significantly different from Log $LHR_{SLEEP}$. Thus is was decided to use log $LHR_{AWAKE}$ for the remaining analyses. As a consequence of using the $LHR_{AWAKE}$, the two subjects that had a missing time point would not be included in the subsequent analyses. Demographics for the remaining 27 subjects are summarized in Table 6, Figure 6, and Figure 7.

## 6.2 Sleep Parameters

Parameters of sleep architecture, continuity and breathing during sleep for the 27 subjects were summarized in Table 7. In addition, Table 7 also reports the p value for the paired t-test comparing undisturbed sleep and fragmented sleep. The sleep variables (AHI, SFI, SWS, average SPO2, % TST below SPO2 90%, and TST) were all significantly affected by experimentally-induced sleep fragmentation to warrant further evaluation in model building. Figure 8 through Figure 11 displays the graphic representation of the sleep variables described in Table 7. It was noted that there were subjects that did not follow the same pattern as the rest of the study population or had values that appeared to possibly influential. Efforts were made to identify those subject(s) to see if there was a particular subject or subject characteristic that could explain the pattern.

Appendix lists the subjects that were identified with the value of interest underlined. No subject nor any specific pattern were identified among those subjects listed.

## 6.3 The Effect of Transient Sleep Intervals on Low to High Frequency Ratio

$LHR_{AWAKE}$ for the subpopulation (n=27) being assessed is summarized in Table 9. To assess if Log $LHR_{AWAKE}$ was different at different time points a paired t-test was used (Table 10). There was a significant overnight increase of Log $LHR_{AWAKE}$ from the evening to morning $LHR_{AWAKE}$ recording on the undisturbed night of sleep (EBN-2 vs. MAN-2) ($p = 0.0398$) suggesting an increase in SVB. In contrast, there was no statistically significant overnight change of Log $LHR_{AWAKE}$ during the second night of experimentally-induced sleep fragmentation (EBN-4 vs. MAN-4). There was a statistically significant increase comparing the EBN-2 and EBN-4 ($P = 0.0269$) Log $LHR_{AWAKE}$ recordings. The comparison of the morning values revealed no significant difference. The EBN-2 value compared to the MAN-4 sleep was not significant ($P=0.0933$).

The magnitude of overnight change on the undisturbed night vs. the second fragmented night was also compared. The overnight change as determined by taking the morning Log $LHR_{AWAKE}$ subtracted from evening Log $LHR_{AWAKE}$ values are listed in Table 11. The overnight change on undisturbed night sleep verses overnight change on fragmented night sleep was borderline significant ($P = 0.063$) compared to the overnight change on fragmented sleep.

### 6.3.1 Linear Regression Comparing Morning Log Low to High Frequency Ratio While Awake to Evening Log Low to High Frequency Ratio While Awake during each Sleep Condition (Undisturbed or 2nd Fragmented Sleep) Separately

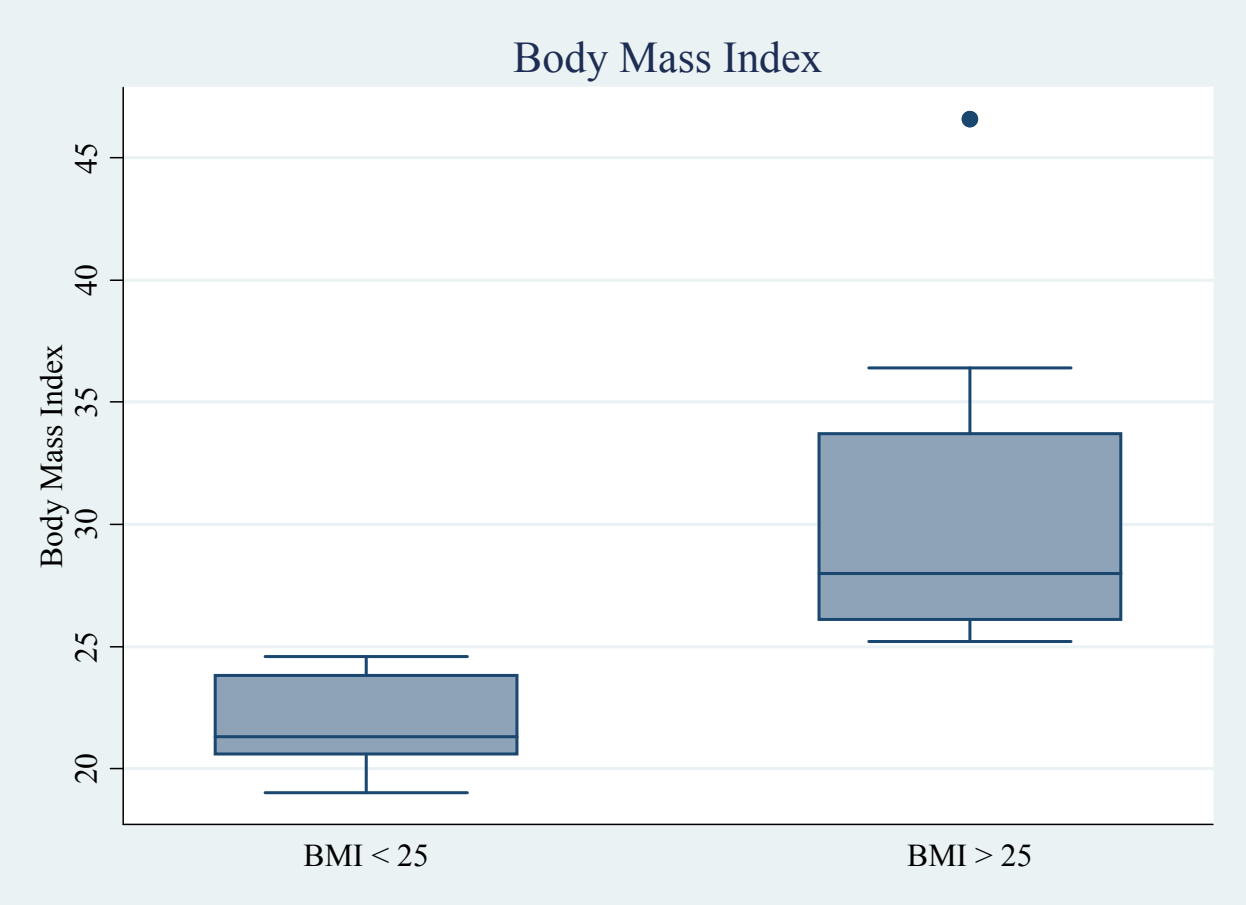
Linear regression using PROC REG procedure was used to assess if overnight change was associated with any available covariate. Table 12 shows that the overnight change on the second fragmented night sleep was not affected by any of the covariates assessed. To further evaluate those significant covariates for the overnight change on an undisturbed night a stepwise model building process was utilized. Model 1 shows the final model where the covariates selected had a p value < 0.2 in the univariate analysis as listed in Table 12. BMI group was forced to remain in the model and the other covariates were permitted to leave. The final model suggests that as SFI can affect Log LHR values whereas there was no significant change due to BMI group.

### 6.3.2 Overnight Change of Log Low to High Frequency Ratio While Awake across an Undisturbed Night of Sleep verses Overnight Change of Log Low to High Frequency Ratio While Awake across a 2nd night of Fragmented Night

The difference of the magnitude of the overnight change of $LHR_{AWAKE}$ change between undisturbed and second night of fragmented sleep [(MAN-2 - EBN-2) vs. (MAN-4 - EBN-4)]

was assessed using a repeated measures multiple linear regression. A PROC MIXED procedure was used to assess the relationship. Table 13 lists the univariate results for the individual predictors on the outcome variable of Log $LHR_{AWAKE}$ overnight change. Significant covariates were further evaluated in a stepwise model building process. The variables assessed were grouped BMI, fragmented sleep indicator, averaged $SpO_2$ during sleep and %time < SPO2 90% during sleep. None of the covariates assessed remained in the final model. Tested interactions provided an nonsignificant improvement in the model's fit.

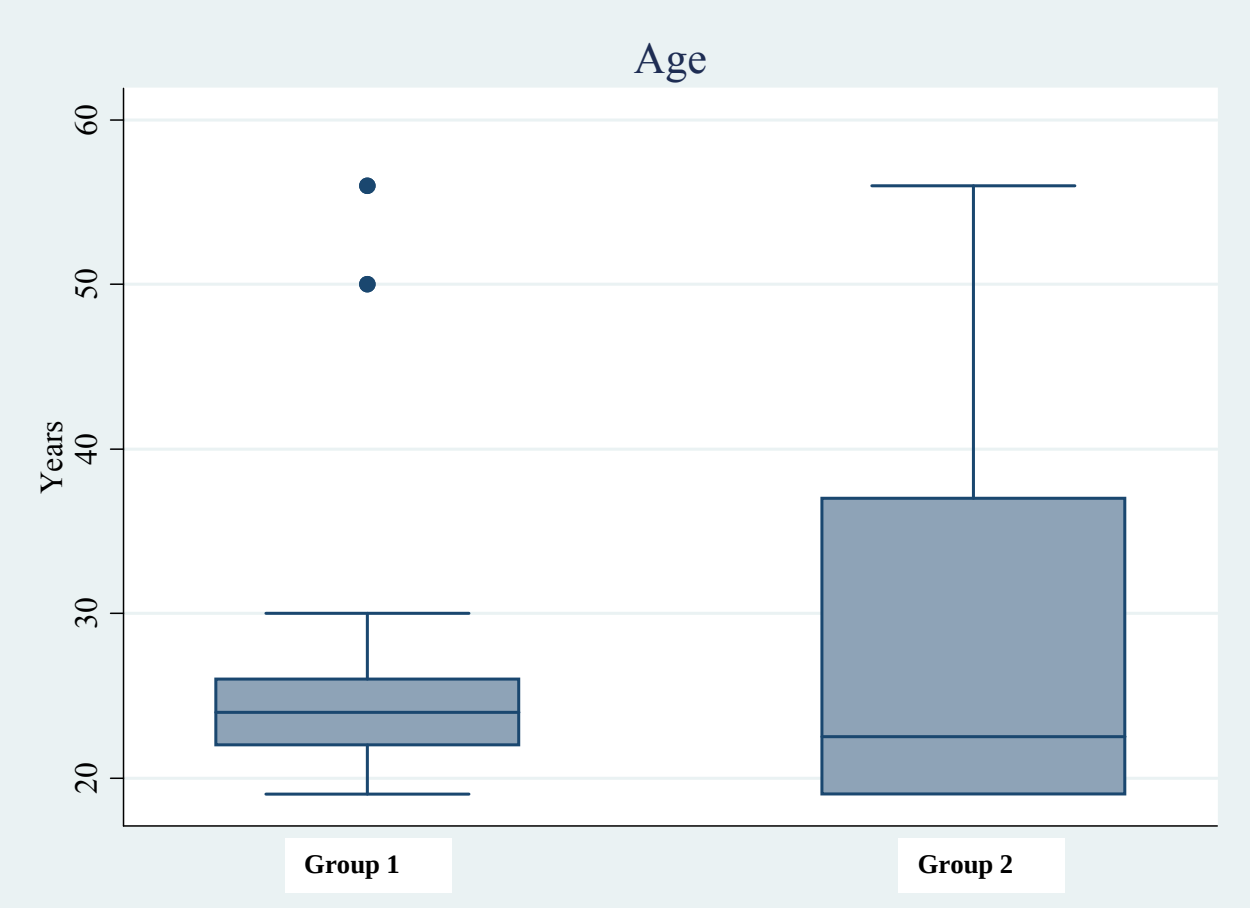
An additional analysis (Model 2) was performed to assess whether induced sleep fragmentation was related to the magnitude of overnight change while taking into account the other covariates. The results suggest that there was no effect of induced sleep fragmentation or grouped BMI on the difference of overnight change of log $LHR_{AWAKE}$ between N2 and N4 after accounting for average SpO2 and % TST<SPO2<90%.




There was a significant difference in BMI between the groups ($P < 0.001$).

Group 1: BMI < 25 kg / $m^2$. Group 2: BMI > 25 kg / $m^2$.

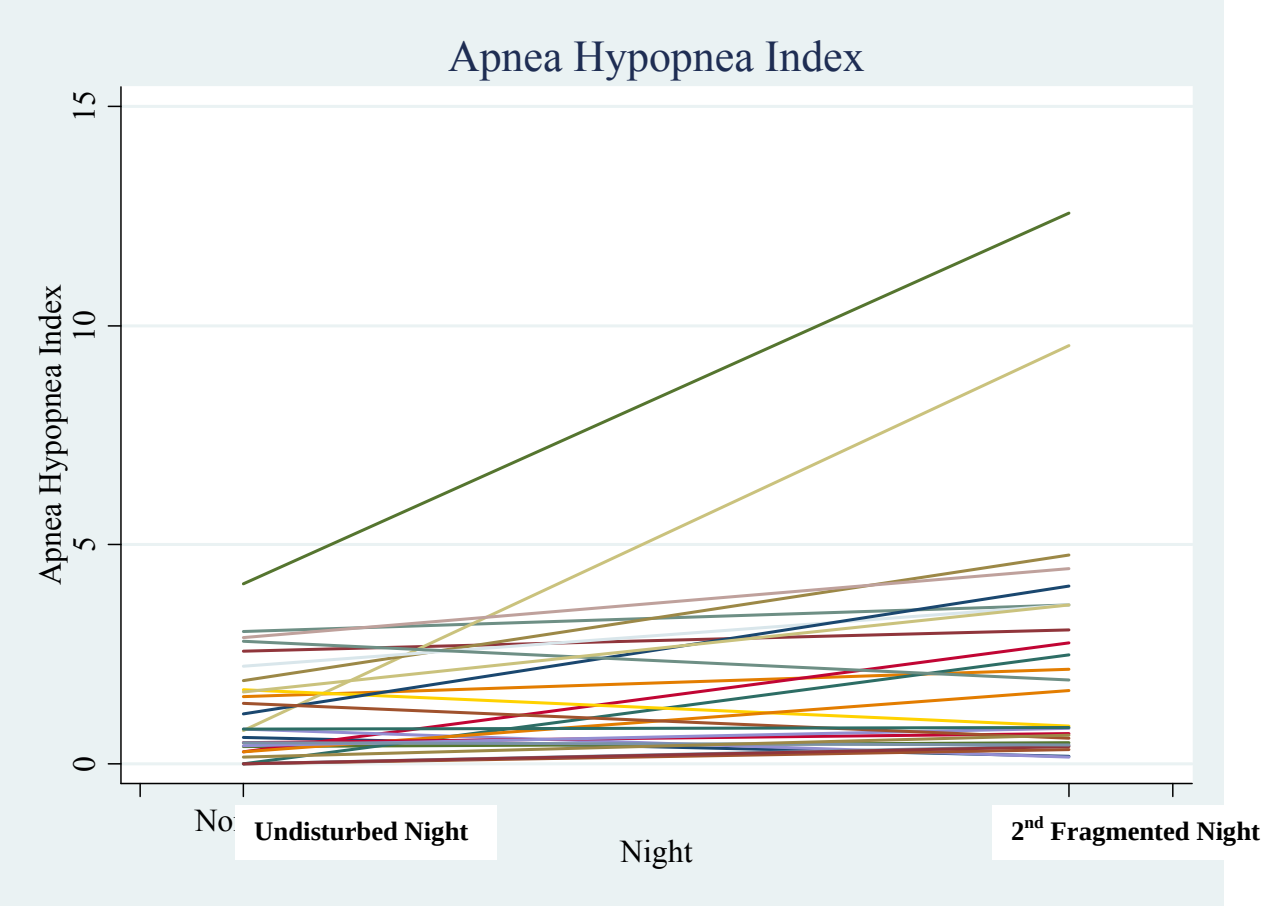
**Figure 6: Body Mass Index by Study Group (N=27)**




There was not a significant difference in age between groups. (P > 0.2)

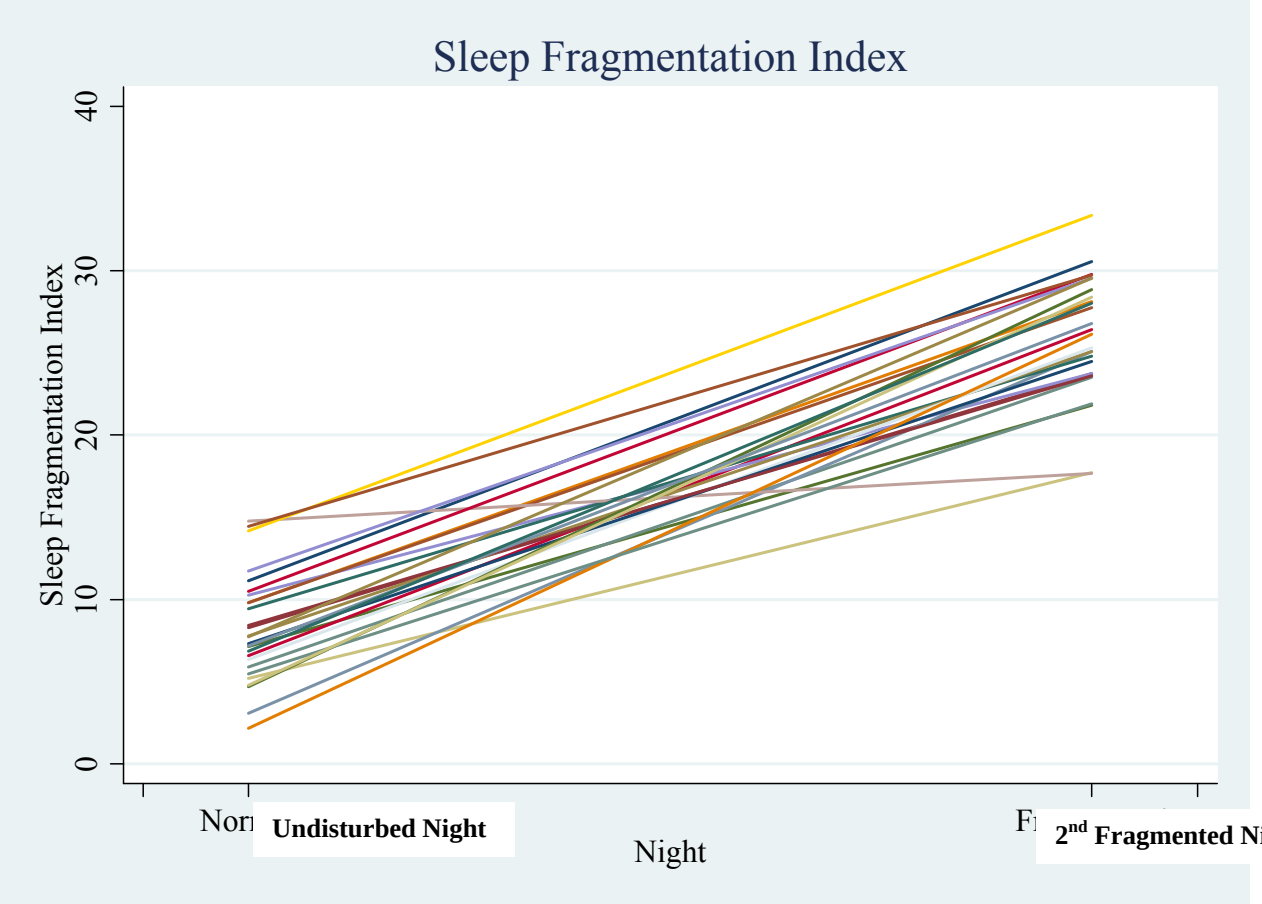
Group 1: BMI < 25 kg / $m^2$. Group 2: BMI > 25 kg / $m^2$.

**Figure 7: Age by Study Group (N=27)**



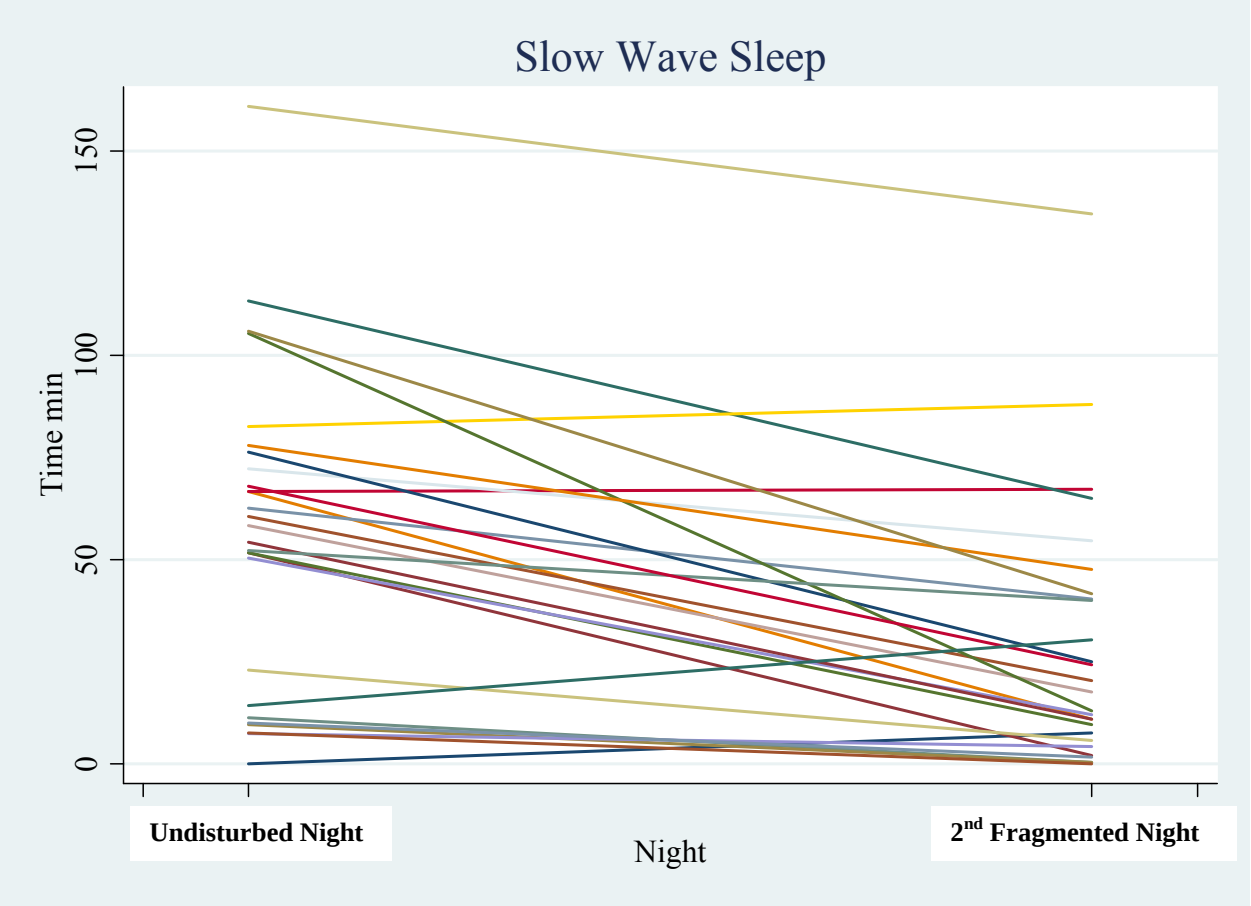

There was a statistically significant difference in AHI between nights (P > 0.0094), where AHI is higher on the second night of experimentally induced sleep fragmentation.

**Figure 8: Apnea Hypopnea Index (N=27)**



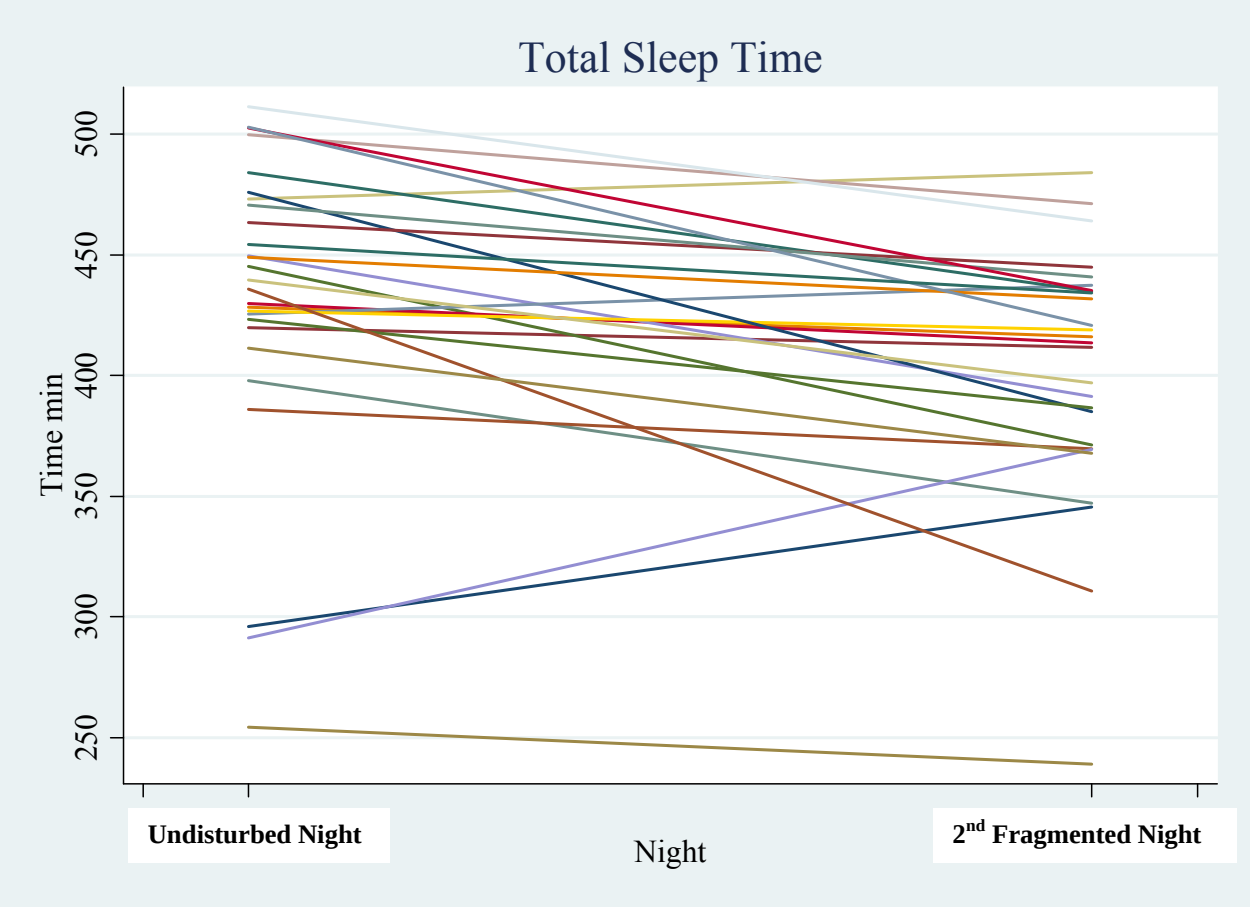

There was a statistically significant difference in SFI between nights ($P < 0.0001$), where SFI is higher on the second night of experimentally induced sleep fragmentation.

**Figure 9: Sleep Fragmentation Index (N=27)**



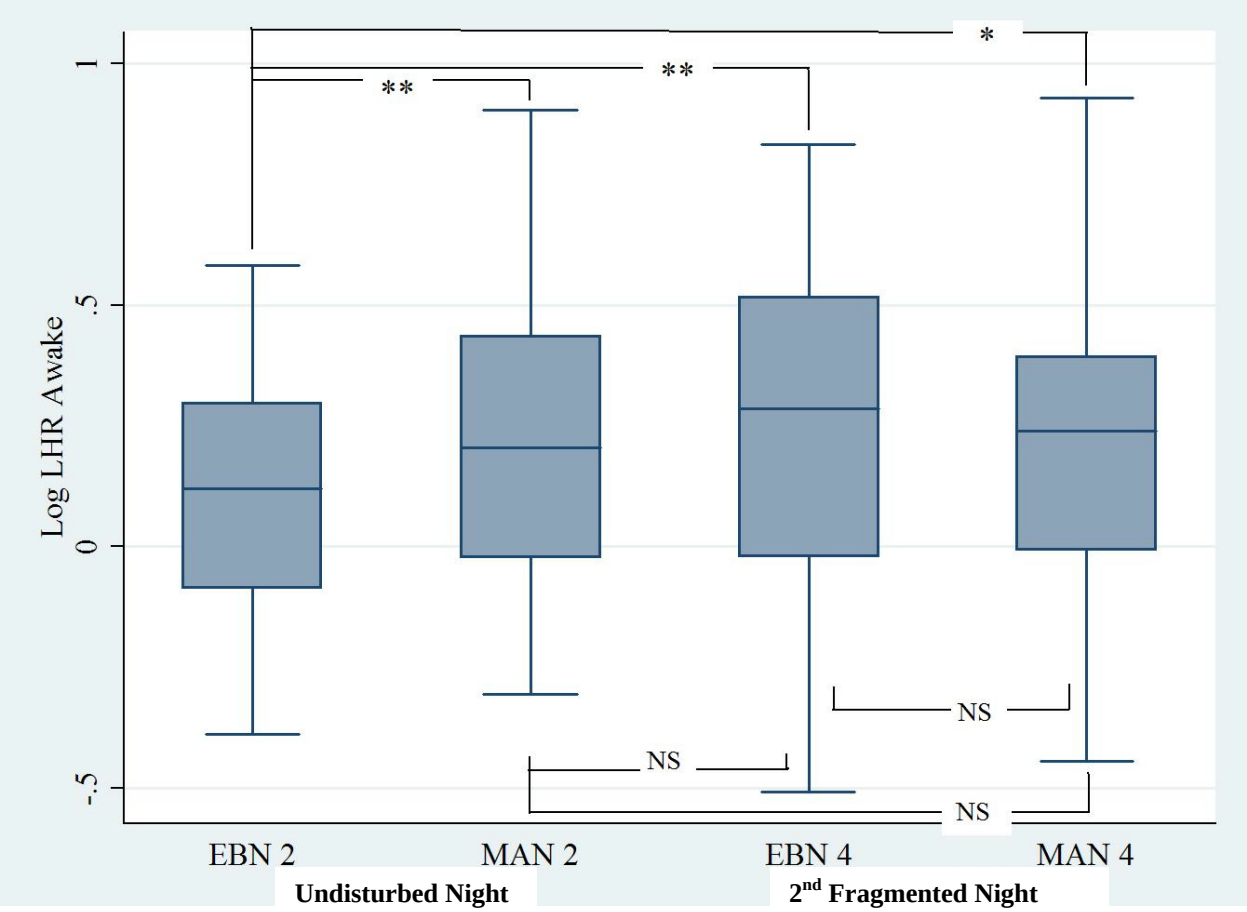

There was a statistically significant difference in slow wave sleep time between Undisturbed and Second Night of Fragmented Sleep ($P < 0.0001$) where the second Fragmented night had less time in slow wave sleep.

**Figure 10: Slow Wave Sleep (N=27)**



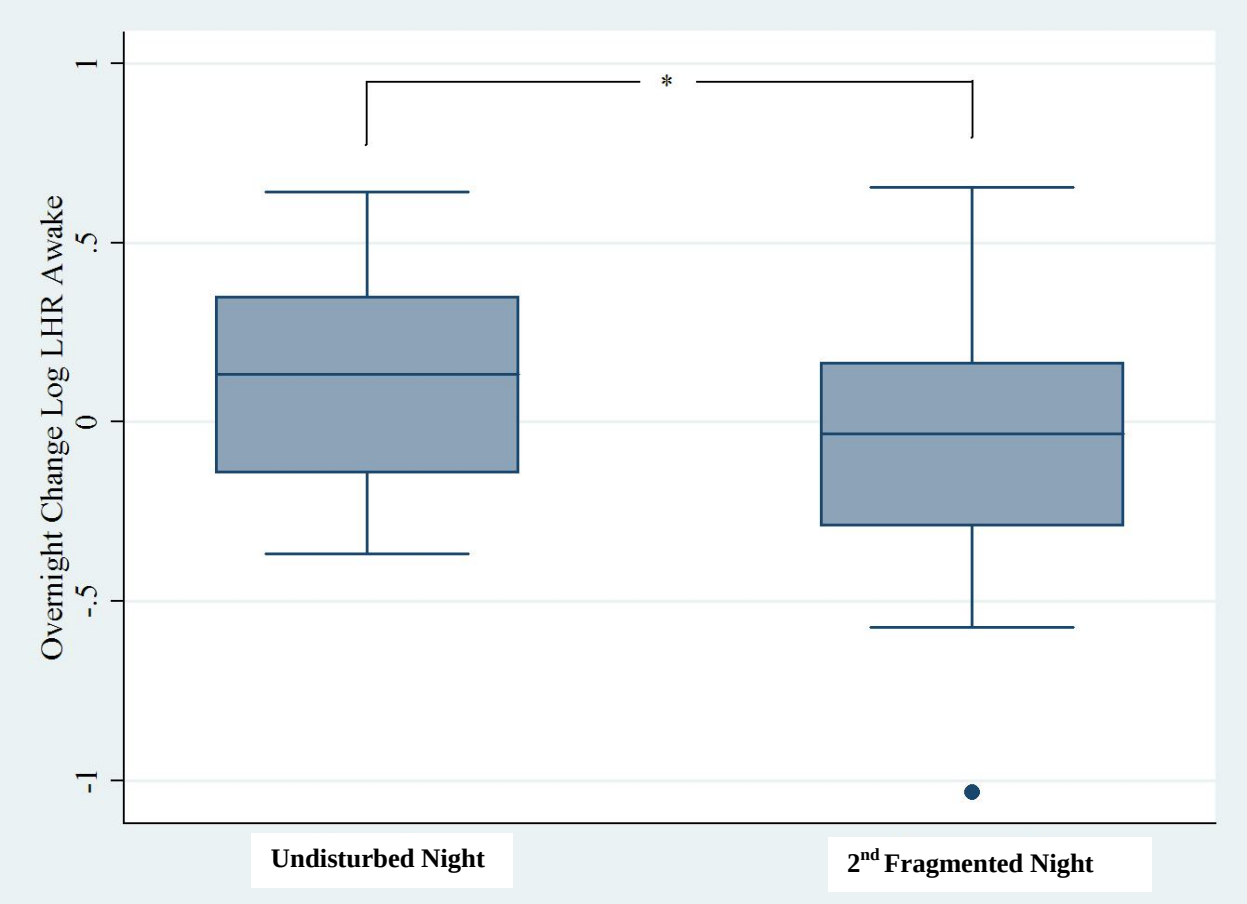

There was a statistically significant difference in total sleep time between Undisturbed and the Second Night of Fragmented Sleep (P = 0.0009) where the Second Night of Fragmented night had less total sleep time.

**Figure 11: Total Sleep Time (N=27)**




NS = P > 0.2; * = 0.1 > P > 0.05; ** = 0.05 > P > 0.01

**Figure 12: Log Low to High Frequency Ratio at Each Time Point with Significance Based On Table 9 and Table 10**




* = P = 0.0603

**Figure 13: Overnight Change of Log Low to High Frequency Ratio based on Table 11**

**Table 4: Study Demographics, Entire Population**

| N = 29 | Population | Group 1 | Group 2 |
|---|---|---|---|
| Female | 19 (65.5 %) | 8 (57.1%) | 11 (73.3 %) |
| Male | 10 (34.5 %) | 6 (42.9 %) | 4 (26.7 %) |
| BMI mean (std dev) | 26.4 (6.1) | 22.0 (2.0) | 30.4 (5.8) |
| (min : max) | (19.0 : 46.6) | (19.0:24.6) | (25.2:46.6) |
| Age, years | | | |
| mean (std dev) | 27.7 (11.4) | 27.2(11.4) | 28.2 (11.9) |
| (min : max) | (19 : 56) | (19 : 56) | (19 : 56) |

NS = P > 0.2

**Table 5: Two Subjects Removed due to Missing Time Points due to Sleep-Contaminated Heart Rate Variability Analysis Segments**

| Subject ID | 65 | | | | 51 | | | |
|---|---|---|---|---|---|---|---|---|
| Group | 1 | | | | 2 | | | |
| Gender | Male | | | | Female | | | |
| Age, yrs | 19 | | | | 21 | | | |
| BMI | 24.5 | | | | 27.8 | | | |
| Night | Undisturbed | | Fragmented | | Undisturbed | | Fragmented | |
| SFI | 9.46 | | 26.29 | | 13.22 | | 25.43 | |
| AHI | 1.47 | | 14.22 | | 1.28 | | 1.77 | |
| EKG time point | Evening | Morning | Evening | Morning | Evening | Morning | Evening | Morning |
| $LHR_{ALL}$ | 1.14 | 2.56 | 2.19 | 2.66 | 3.28 | 0.817 | 3.87 | 1.1 |
| $LHR_{AWAKE}$ | 1.14 | 2.31 | - | 1.54 | 3.21 | 0.65 | 4.70 | - |
| $LHR_{SLEEP}$ | - | 3.66 | 2.19 | 2.94 | - | - | 2.18 | 1.1 |

BMI = Body Mass Index, SFI = sleep fragmentation index, AHI = apnea hypopnea index

**Table 6: Subject Demographics without Epochs Scored as Sleep during Heart Rate Variability Data Collection (N=27)**

| | Study Population N = 27 | Group | | P value |
|---|---|---|---|---|
| | | 1 (BMI < 25) | 2 (BMI > 25) | |
| Female<br>Male | 18 (66.7 %)<br>9 (33.3 %) | 8 (61.5 %)<br>5 (38.5 %) | 10 (71.4 %)<br>4 (28.6 %) | NS |
| BMI<br>mean (sd)<br>(min : max) | 28.3 (11.6)<br>(19.0:46.6) | 21.9 (1.9)<br>(19.0:24.6) | 30.6 (6.0)<br>(25.2:46.6) | 0.000 |
| Age, years<br>mean (sd)<br>(min : max) | 26.4 (6.3)<br>(19:56) | 27.8 (11.6)<br>(19:56) | 28.7 (12.1)<br>(19:56) | NS |

**Table 7: Sleep Variables Compared on Undisturbed and 2nd Fragmented Night Using a Paired T-Test (N=27)**

| N= 27 | Undisturbed Night | 2nd Fragmented Night | P Value |
|---|---|---|---|
| Apnea Hypopnea Index | | | |
| mean (std dev) | 1.2 (1.1) | 2.5 (2.9) | 0.0094 |
| (min : max) | (0 : 4.1) | (0.2 : 12.6) | |
| Sleep Fragmentation Index | | | |
| mean (std dev) | 8.2 (3.3) | 26.0 (3.7) | < 0.0001 |
| (min : max) | (2.1 : 14.8) | (17.7 : 33.4) | |
| Slow Wave Sleep, min. | | | |
| mean (std dev) | 56.6 (38.5) | 28.7 (31.7) | < 0.0001 |
| (min : max) | (0 : 161.0) | (0 : 134.7) | |
| Average $SpO_2$ during sleep | | | |
| mean (std dev) | 96.9 (0.8) | 97.2 (0.9) | 0.0431 |
| (min : max) | (95 : 98) | (94 : 99) | |
| % TST SPO2 < 90% | | | |
| mean (std dev) | 0 (0) | 0.02 (0.06) | 0.0961 |
| (min : max) | (0 : 0) | (0 : 0.2) | |
| Total Sleep Time, min. | | | |
| mean (std dev) | 431.4 (63.5) | 401.5 (52.3) | 0.0009 |
| (min : max) | (254.3 : 511.3) | (239.0 : 484.0) | |

NS = Not Significant p > 0.2

**Table 8: Percent Total Sleep Time below Pulse Oximetry 90%**

| % TST below $SpO_2$ 90% | Undisturbed Night | 2nd Fragmented Night |
|---|---|---|
| 0 | 27 | 24 |
| 0.1 | 0 | 1 |
| 0.2 | 0 | 2 |
| Total | 27 | 27 |

**Table 9: Low to High Frequency Ratio While Awake Summarized at Each Time Point (N = 27)**

| $LHR_{AWAKE}$ | Mean | Std Dev | Minimum | Maximum |
|---|---|---|---|---|
| Evening Before Undisturbed Sleep | 1.51 | 0.93 | 0.41 | 3.83 |
| Morning After Undisturbed Sleep | 2.16 | 1.77 | 0.50 | 8.04 |
| Evening Before Fragmented Sleep | 2.37 | 1.69 | 0.31 | 6.79 |
| Morning After Fragmented Sleep | 2.16 | 1.93 | 0.36 | 8.47 |

**Table 10: Compared Log Low to High Frequency Ratio while Awake at Various Time Points Utilizing a Paired T-Test**

| N = 27 | Evening Before Normal Sleep | Morning after Normal Sleep | Evening Before 2nd Night of Fragmented Sleep |
|---|---|---|---|
| Morning After Undisturbed Sleep | P = 0.0398 | - | P = 0.0269 |
| Evening Before 2nd Night of Fragmented Sleep | P = 0.0269 | NS | - |
| Morning After 2nd Night of Fragmented Sleep | P = 0.0933 | NS | NS |

NS = non-significant p >0.2

**Table 11: Log Transformed Difference of Overnight Change of Log Low to High Frequency Ratio While Awake (N=27)**

Total Population

| Variable | Night | Mean | Std Dev | Minimum | Maximum |
|---|---|---|---|---|---|
| Log Overnight Change | Undisturbed | 0.276 | 0.663 | -0.850 | 1.476 |
| Log Overnight Change | Fragmented | 0.118 | 0.886 | -2.380 | 1.505 |

Log Overnight Change = Log morning - Log night

**Table 12: Each Row Represents a Single Univariate Regression: Linear Regression of Log of Overnight Change While Awake Individually Assessed on Undisturbed Sleep and Fragmented Sleep**

| Overnight Change (Log Morning − Log Night) | | | | | | |
|---|---|---|---|---|---|---|
| N = 27 | Undisturbed Sleep | | | 2nd Night ofFragmented Sleep | | |
| Variable | β | $R^2$ | P | β | $R^2$ | P |
| Intercept Only | 0.276 | 0.0000 | 0.0398 | -0.118 | 0.0000 | NS |
| Age | -0.0108 | 0.0359 | NS | -0.0134 | 0.0310 | NS |
| AHI | 0.064 | 0.0119 | NS | 0.060 | 0.0140 | NS |
| Female | -0.158 | 0.0131 | NS | -0.375 | 0.0414 | NS |
| Overweight | 0.287 | 0.0484 | NS | 0.016 | 0.0001 | NS |
| SFI | -0.074 | 0.1351 | 0.0593 | 0.027 | 0.0124 | NS |
| Avg SPO2 | -0.249 | 0.0859 | 0.1380 | -0.138 | 0.0174 | NS |
| Time <SPO2 90% | - | - | - | -2.862 | 0.0324 | NS |
| SWS | 0.0001 | 0.0001 | NS | -0.0022 | 0.0064 | NS |
| TST | -0.0004 | 0.0012 | NS | -0.0016 | 0.0085 | NS |

NS = non-significant p >0.2. SFI was treated as a continuous variable. Overweight is an indicator variable with BMI < 25 kg/$m^2$ as the baseline.

**Table 13: Each Row Represents a Single Univariate Regression: Repeated Measures Linear Regression of the Comparison of Overnight Change of Log Low to High Frequency Ratio While Awake**

| N = 27 | Overnight Change (Log Morning - Log Night) | | | |
|---|---|---|---|---|
| Variable | β | -2 Log Likelihood | AIC | P |
| Intercept Only | 0.1416 | 125.9 | 133.9 | 0.1918 |
| Age | -0.0117 | 124.2 | 134.2 | NS |
| AHI | 0.0066 | 125.8 | 135.8 | NS |
| Female | -0.235 | 124.7 | 134.7 | NS |
| Fragmented Sleep | -0.394 | 122.1 | 132.1 | 0.0558 |
| Overweight | 0.198 | 125.0 | 135.0 | NS |
| Avg SPO2 | -0.245 | 122.2 | 132.2 | 0.0617 |
| % TST < SPO2 90% | -4.380 | 123.8 | 133.8 | 0.1529 |
| SWS | 0.0013 | 125.6 | 135.6 | NS |
| TST | 0.0002 | 125.8 | 135.8 | NS |

NS = Not significant p > 0.2 Fragmented Sleep is an indicator variable with undisturbed sleep as the baseline. Overweight is an indicator variable with BMI < 25 kg/m$^2$ as the baseline.

**Model 1: Linear Regression of Log Low to High Frequency Ratio While Awake on Undisturbed Night Sleep**

Log $LHR_{AWAKE}$ Change on Undisturbed Night Sleep

| Parameter Label | DF | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | 1 | 0.73922 | 0.35445 | 2.09 | 0.0478 |
| SFI | 1 | -0.07440 | 0.03762 | -1.98 | 0.0596 |
| Overweight | 1 | 0.28158 | 0.24060 | 1.17 | NS |

Log Overnight change = Log MAN-2 vs. Log EBN-2
Overweight is an indicator variable with BMI < 25 kg/m2 as the baseline
NS = Not significant p > 0.2

**Model 2: Overnight Change of Log Low to High Frequency Ratio While Awake Compared on Undisturbed Night verses 2nd Fragmented Night**

| Effect | Estimate | Standard Error | DF | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | 26.3662 | 12.8053 | 25 | 2.06 | 0.0501 |
| Overweight | 0.1828 | 0.2021 | 25 | 0.90 | NS |
| Fragmented Sleep | 0.2083 | 0.2042 | 25 | -1.02 | NS |
| Avg $SpO_2$ during sleep | -0.2702 | 0.1320 | 25 | -2.05 | 0.0513 |
| % TST < SPO2 90% | -5.7128 | 3.0880 | 25 | -1.85 | 0.0762 |

Fragmented Sleep is an indicator variable with Undisturbed sleep as the baseline
Overweight is an indicator variable with BMI < 25 kg/m2 as the baseline
Log Overnight change = Log Morning - Log Night
NS = Not significant p > 0.2

# 7. Discussion

OSAH patients have increased sympathetic activity during sleep that sustains through daytime wakefulness.[43-45] Which, if any of the specific components of OSAH, intermittent hypoxemia or sleep fragmentation contributes to elevated sympathetic activity is uncertain. This analysis, which is part of a larger parent study, was designed to evaluate the effect of sleep fragmentation on cardiac SVB while adjusting for obesity in a non-OSAH population. The main conclusions of this analysis were as follows:

1. Transient sleep intervals during a 10 minute recording period during which the subject is behaviorally assessed to be awake can significantly alter LHR. This highlights the need to objectively confirm wake-sleep state during assessment of awake SVB.
2. SVB is different in the morning following undisturbed sleep compared with the previous evening.
3. One night of sleep fragmentation is associated with altered SVB that is sustained at least until the following evening.

## 7.1. Sleep Parameters

Compared with a night without induced sleep interruption, the second night during which there was experimentally-induced sleep fragmentation was associated with statistically significant change in AHI, SWS, average SPO2, and TST decreased (Table 7). Percent TST below $SpO_2$ 90% showed a borderline significant increase due to sleep fragmentation. Increased SFI suggests that goal of inducing sleep fragmentation was obtained. The decreased SWS and decreased TST was a consequence attributed to the induced sleep fragmentation. Our goal was to arouse, but not awaken subjects from clearly established sleep (i.e. stage 2 or deeper). If subjects uniformly did not awaken and remain awake for periods of time it would have been expected that TST would not have decreased, but time spent in deeper stages of sleep SWS may have nonetheless been decreased. There was a mean reduction of TST by 29.91 (standard error 7.99) minutes from undisturbed to the second night of fragmented sleep. Although it is statistically significant, the biological and clinical significance of this difference is uncertain. It should be noted that in contrast to our study, Tasali et al. employed a similar sleep fragmentation protocol that did not observe a reduction in TST but like us did reduce SWS time. [46] This difference between the two studies may be explained by differences in the study populations with that of Tasali et al. reflected by a smaller, younger and "lean" study sample (n=9 subjects, 20-31 years of age, BMI: 19-24); so, it appears that ours was much more diverse and representative of the community.

There were potentially important difference between our protocol for recording HRV and that of Tasali et al. In contrast to our protocol which recorded HRV data just prior to bed and just after awakening, Tasali and coworkers collected data at midday, between 11:00 and 13:00 and the authors did not report activity levels prior to this collection. Our subjects were requested to have an empty bladder and lay motionless for 10 minutes prior to EKG recording, as these factors could affect SVB. The differences in posture during recording (Tasali's et al. subjects were sitting up, our subjects were supine) could also cause differences in SVB.

## 7.2 The Effect of Transient Sleep Intervals on Low to High Frequency Ratio

The effect of transient sleep on awake LHR was evaluated using a spectral analysis of heart beat-to-beat intervals. LHR based on data containing epochs "contaminated" by EEG evidence of sleep was statistically different from LHR based exclusively on epochs identified by EEG as awake. As such only epochs without sleep were used in these analyses. This finding contradicts Burgess et. al. that used pre-ejection period to measure cardiac sympathetic activity that did not show sympathetic activity change with transient change in the sleep/wake state.[47] LHR with sleep staging considered would be used for all further analyses of the cardiac sympathetic activity in this paper.

LHR in the evening prior to the baseline night of undisturbed sleep (EBN-2) was significantly lower from than in the evening prior to the second night of experimental sleep fragmentation (EBN-4). In this regard it is important to note that overnight conditions to which

subjects were exposed prior to the baseline undisturbed night (reflecting a night without experimental sleep disruption) differed from that on the night prior to the second experimentally-induced sleep fragmentation night (during which by definition, subjects' sleep was disrupted). These findings are consistent with the posit that one night of fragmented sleep is associated with sustained elevation of SVB that lasts at least until the following evening. The morning after sleep LHR values were not significantly different between sleep conditions.

### 7.2.1 Linear Regression Comparing Morning Log Low to High Frequency Ratio While Awake to Evening Log Low to High Frequency Ratio While Awake during Undisturbed Sleep

Although borderline significant, multivariate analysis of overnight change of Log $LHR_{AWAKE}$ on the undisturbed night's sleep was suggestive of an inverse association with SFI. This suggests that SFI could modulate LHR overnight change on an undisturbed night sleep. A similar analysis for fragmented night sleep resulted in no significant association for any of the covariates assessed.

### 7.2.2 Overnight Change of Log Low to High Frequency Ratio While Awake across an Undisturbed Night of Sleep verses Overnight Change of Log Low to High Frequency Ratio While Awake across a 2nd night of Fragmented Night

Comparison of the overnight change using a paired t-test resulted in a borderline significant decrease of the magnitude of the overnight change due to fragmented sleep. Further multivariate analysis resulted in non significant contributions of grouped weight or fragmented sleep. Measures of oxygenation (% TST below $SpO_2$ 90% during sleep and average $SpO_2$ during sleep) were borderline significant and had a negative effect on the magnitude of the change. That is to say that as the subjects experienced increasing % TST below $SpO_2$ 90% and increasing average $SpO_2$ during sleep, the magnitude of overnight change on the second fragmented night decreased.

## 7.3 Conclusion

There is an increase in awake cardiac SA from the evening before to the morning after a night of undisturbed sleep. The results of this study suggest that a night of fragmented sleep significantly increases awake cardiac sympathetic activity on the following evening to levels compared to the evening values prior to undisturbed sleep and not significantly different from the morning values after undisturbed sleep. Multivariate regression models suggest that fragmented

sleep while adjusting for obesity could not explain the observed differences in the magnitude of overnight change of the different sleep conditions. Being overweight or obese did not significantly change cardiac sympathovagal tone. This supports a similar finding by Narkiewicz et. al. where MSNA was not increased due to obesity in non-OSAH subjects.[48]

A possible explanation could be a plateau effect of the amount of SFI. After a night of moderately induced sleep fragmentation, the SVB cannot increase any further (a ceiling effect). Another possible explanation could be that SFI can only elevate the evening LHR recording (carryover effect) as suggested by the evening values being statically different, and the morning values being statically similar. Further changes in LHR could be due to the deoxygenation-reoxygenation cycle of the obstructive events. The idea of the plateau effect could be supported by a measure of oxygenation (Average $SpO_2$, or % time below $SpO_2$ 90%) remained in the final model assessing the differences in the magnitude of the overnight change. Moderately induced sleep fragmentation could not explain the differences observed in this population due to induced sleep fragmentation.

## 7.4 Study Limitations

Our conclusions are qualified by the limitation of the parent study design. We do not have LHR readings for evening before the first night of undisturbed sleep, morning after first night of sleep, evening before first fragmented night, and the morning after the first fragmented night. LHR recording associated with the acclimatization night would be highly susceptible to unknown sleep conditions (evening recording) and the first night effect (morning recording).

The addition of another baseline night and the addition of another fragmented sleep night, we could have provided a better understanding whether the changes are due to within subject variation or due to experimental conditions. In addition, we recognize that OSAH is a chronic disorder and that assessing SVB after only two nights of sleep fragmentation may not be truly representative of what happens in OSAH.

The order of sleep experienced was not randomized (e.g. undisturbed sleepalways preceded fragmented sleep). With the assumption that there would need at least one undisturbed night (and maybe more) between both conditions, this would have increased the total time spent in the study environment to 6 nights (acclimation / screening night, sleep condition 1 for two nights, washout night, sleep condition 2 for two nights). Logistic limitations associated with subjects staying in a carefully controlled environment and participant burden required a compromise of a 4 night protocol.

The elevated sympathetic activity could be in part due to an unequal parasympathetic withdrawal instead of an increase in sympathetic activation. Also conceivable is that the morning LHR reading could be artificially increased due to a technician possibly stimulating the subject by awakening her/him for the morning EKG reading. Changes in breathing rate could also have an impact on the LHR reading, breathing rate data was not available during the awake EKG recording.[45]

This was a sub study from a larger study. The parent study was not optimized (e.g. statistical power) for assessing differences in LHR (Appendix B: Statistical Power Calculations).

Appendix A: Subjects with Unexpected Values

Subjects with unexpected values based on graphic representations of their recordings were further assessed to determine if there was a pattern or a small number of subjects that would explain the unexpected values observed (Table 14).

**Table 14: Subjects with Unexpected Values Underlined**

| Subject ID | 40 | | | | 78 | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 23 | | | | 24 | | | |
| Gender | Male | | | | Female | | | |
| BMI | 26.70 | | | | 19.50 | | | |
| Study Group | 2 | | | | 1 | | | |
| Night | Undisturbed | | Fragmented | | Undisturbed | | Fragmented | |
| TST | 423.33 | | 386.67 | | **473** | | **484** | |
| SFI | 4.68 | | 28.86 | | 5.2 | | 17.73 | |
| SWS | 105.33 | | 13 | | 161 | | 134.67 | |
| AHI | **4.11** | | **12.57** | | **0.76** | | **9.55** | |
| Avg SPO2 | 97 | | 97 | | 97 | | 97 | |
| % Time Below SPO2 90% | 0 | | 0 | | 0 | | 0 | |
| Time Point | EBN-2 | MAN-2 | EBN-4 | MAN-4 | EBN-2 | MAN-2 | EBN-4 | MAN-4 |
| $LHR_{Awake}$ | 1.663 | 2.724 | 2.985 | 7.453 | 2.863 | 5.166 | 1.787 | 2.503 |
| $LHR_{SLEEP}$ | - | - | - | 4.370 | - | - | 1.010 | - |

**Table 14: Subjects with Unexpected Values , continued**

| Subject ID | 8 | | | | 10 | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 21 | | | | 23 | | | |
| Gender | Male | | | | Male | | | |
| BMI | 26.10 | | | | 20.10 | | | |
| Study Group | 2 | | | | 1 | | | |
| Night | Undisturbed | | Fragmented | | Undisturbed | | Fragmented | |
| TST, min. | 426.67 | | 419 | | 454.33 | | 434.33 | |
| SFI | 14.2 | | 33.37 | | 6.87 | | 28.04 | |
| SWS, min. | **82.66** | | **88** | | **14.33** | | **30.33** | |
| AHI | 1.69 | | 0.86 | | 0.79 | | 0.83 | |
| Avg SPO2, % | 97 | | 97 | | 97 | | 98 | |
| % TST Below $SpO_2$ 90% | **0** | | **0.2** | | 0 | | 0 | |
| Time Point | EBN-2 | MAN-2 | EBN-4 | MAN-4 | EBN-2 | MAN-2 | EBN-4 | MAN-4 |
| $LHR_{Awake}$ | 3.826 | 3.258 | 3.900 | 1.265 | 0.533 | 1.190 | 0.512 | 2.306 |
| $LHR_{SLEEP}$ | 1.250 | - | 4.240 | 1.930 | 0.380 | - | - | 0.880 |

**Table 14: Subjects with Unexpected Values , continued**

| Subject ID | 70 | | | | 20 | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 56 | | | | 22 | | | |
| Gender | Female | | | | Female | | | |
| BMI | 20.60 | | | | 35.20 | | | |
| Study Group | 2 | | | | 2 | | | |
| Night | Undisturbed | | Fragmented | | Undisturbed | | Fragmented | |
| TST, min. | **296** | | **345.67** | | 430 | | 413.67 | |
| SFI | 11.15 | | 30.55 | | 6.56 | | 26.4 | |
| SWS, min. | **0** | | **7.67** | | **66.66** | | **67.33** | |
| AHI | 0.61 | | 0.17 | | 0.28 | | 2.76 | |
| Avg SPO2, % | 98 | | 97 | | 96 | | 97 | |
| % TST Below $SpO_2$ 90% | 0 | | 0 | | 0 | | 0 | |
| Time Point | EBN-2 | MAN-2 | EBN-4 | MAN-4 | EBN-2 | MAN-2 | EBN-4 | MAN-4 |
| $LHR_{Awake}$ | 1.984 | 0.848 | 6.790 | 1.815 | 0.735 | 1.520 | 1.703 | 0.660 |
| $LHR_{SLEEP}$ | - | 0.830 | 5.560 | - | - | - | - | - |

**Table 14: Subjects with Unexpected Values , continued**

| Subject ID | 34 | | | | 140 | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 19 | | | | 47 | | | |
| Gender | Female | | | | Male | | | |
| BMI | 33.70 | | | | 25.20 | | | |
| Study Group | 2 | | | | 2 | | | |
| Night | Undisturbed | | Fragmented | | Undisturbed | | Fragmented | |
| TST, min. | 499.67 | | 471.33 | | 254.33 | | 239 | |
| SFI | **14.77** | | **17.69** | | 7.79 | | 25.1 | |
| SWS, min. | 58.33 | | 17.67 | | 9.67 | | 0.33 | |
| AHI | 2.88 | | 4.46 | | 1.89 | | 4.77 | |
| Avg SPO2, % | 97 | | 97 | | 95 | | 94 | |
| % TST Below $SpO_2$ 90% | **0** | | **0.1** | | **0** | | **0.2** | |
| Time Point | EBN-2 | EBN-2 | EBN 4 | MAN 4 | EBN-2 | MAN-2 | EBN-4 | MAN-4 |
| $LHR_{Awake}$ | 1.320 | 1.320 | **3.555** | **1.268** | 1.323 | 5.786 | 3.783 | 4.580 |
| $LHR_{SLEEP}$ | 1.500 | 1.500 | 1.767 | - | - | - | - | - |

**Table 14: Subjects with Unexpected Values , continued**

| Subject ID | 86 | | | | 87 | | | |
|---|---|---|---|---|---|---|---|---|
| Age | 31 | | | | 26 | | | |
| Gender | Female | | | | Female | | | |
| BMI | 31.30 | | | | 27.80 | | | |
| Study Group | 2 | | | | 2 | | | |
| Night | Fragmented | | Fragmented | | Undisturbed | | Fragmented | |
| TST, min. | **291.33** | | **369.333** | | **425.33** | | **437.33** | |
| SFI | 11.74 | | 29.57 | | 3.1 | | 25.11 | |
| SWS, min. | 7.33 | | 4.33 | | 62.67 | | 40.33 | |
| AHI | 0.41 | | 0.81 | | 0 | | 0.41 | |
| Avg SPO2, % | 97 | | 97 | | 97 | | 98 | |
| % TST Below $SpO_2$ 90% | 0 | | 0 | | 0 | | 0 | |
| Time Point | EBN-4 | MAN-4 | EBN-4 | MAN-4 | EBN-2 | MAN-2 | EBN-4 | MAN-4 |
| $LHR_{Awake}$ | 1.678 | 1.320 | 0.310 | 1.040 | 0.535 | 1.158 | 1.125 | 1.042 |
| $LHR_{SLEEP}$ | - | - | 0.550 | - | - | - | - | - |

Appendix B: Statistical Power Calculations

Statistical power calculations were performed to determine how under powered the sub-study was (Table 15) based on the observed difference of the means and observed standard deviation. Additional calculations were done to determine the sample size needed to detect a difference based on the observed difference and standard deviation (Table 16). Also calculated was the minimum statistically significant difference of the means based on the available sample size and observed standard deviations (Table 17).

**Table 15: Power Calculation for Detecting a Difference of the Observed Means and Observed Standard Deviation Using a Two-Sided Paired T-Test for a Sample Size of 27 with a Significance Level (Alpha) of 0.05**

| N = 27<br>Alpha = 0.05 | Power | Effect size | Difference of the means | Standard deviation |
|---|---|---|---|---|
| Change on undisturbed night (MAN-2 – EBN 2) | 55% | 0.417 | 0.28 | 0.66 |
| Change on fragmented night (MAN-4 – EBN-4) | 10% | 0.133 | 0.12 | 0.89 |
| Difference of the changes [(MAN-4 – EBN-4) vs. (MAN-2 – EBN-2)] | 50% | 0.378 | 0.39 | 1.04 |

**Table 16: Sample Size Calculation for Detecting a Difference of the Observed Means and Observed Standard Deviation using a Two-Sided Paired T-Test for Power of 80% with a Significance Level (Alpha) of 0.05**

| Power = 80%<br>Alpha = 0.05 | Sample size | Effect size | Difference of the means | Standard deviation |
|---|---|---|---|---|
| Change on undisturbed night (MAN-2 – EBN 2) | 48 | 0.417 | 0.28 | 0.66 |
| Change on fragmented night (MAN-4 – EBN-4) | 444 | 0.133 | 0.12 | 0.89 |
| Difference of the changes [(MAN-4 – EBN-4) vs. (MAN-2 – EBN-2)] | 55 | 0.378 | 0.39 | 1.04 |

**Table 17: Minimum Detectable Difference of the Means Based on Observed Standard Deviations using a Two-Sided Paired T-Test for Power of 80% with a Significance Level (Alpha) of 0.05 and a Sample Size of 27**

| Power = 80%<br>Alpha = 0.05<br>N = 27 | Minimum Detectable Difference of the means | Standard deviation | Effect size |
|---|---|---|---|
| Change on undisturbed night (MAN-2 – EBN 2) | 0.4 | 0.66 | 0.539 |
| Change on fragmented night (MAN-4 – EBN-4) | 0.5 | 0.89 | 0.539 |
| Difference of the changes [(MAN-4 – EBN-4) vs. (MAN-2 – EBN-2)] | 0.6 | 1.04 | 0.539 |

Bibliography


1. **Quan, S.F., et al., The Sleep Heart Health Study: design, rationale, and methods. Sleep, 1997. 20(12): p. 1077- 1085.**

2. **Young, T., et al., The Occurrence of Sleep-Disordered Breathing among Middle-Aged Adults. N Engl J Med, 1993. 328(17): p. 1230-1235.**

3. **Bixler, E.O., et al., Excessive Daytime Sleepiness in a General Population Sample: The Role of Sleep Apnea, Age, Obesity, Diabetes, and Depression. J Clin Endocrinol Metab, 2005. 90(8): p. 4510-4515.**

4. **Punjabi, N.M., The Epidemiology of Adult Obstructive Sleep Apnea. Proc Am Thorac Soc, 2008. 5(2): p. 136-143.**

5. **Young, T., P.E. Peppard, and D.J. Gottlieb, Epidemiology of Obstructive Sleep Apnea: A Population Health Perspective. Am. J. Respir. Crit. Care Med., 2002. 165(9): p. 1217-1239.**

6. **Marin JM, 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: p. 1046–1053.**

7. **Young, T., et al., Sleep disordered breathing and mortality: eighteen-year follow-up of the wisconsin sleep cohort. . Sleep, 2008. 31(8): p. 1071-1078.**

8. **Peppard PE, et al., Prospective study of the association between sleep-disordered breathing and hypertension. N. Engl. J. Med., 2000. 342: p. 1378-1384.**

9. **Peker Y, et al., Increased incidence of cardiovascular disease in middleaged men with obstructive sleep apnea. Am J Respir Cir Care Med 2002. 166: p. 159–165.**

10. **Takama N and Kurabayashi M, Possibility of close relationship between sleep disorder breathing and acute coronary syndrome. J Cardiol, 2007. 49(4): p. 171 - 177.**

11. **Somers, V.K., et al., Sympathetic Neural Mechanisms in Obstructive Sleep Apnea. J. Clin. Invest., 1995. 96(4): p. 1897-1904.**

12. **Bigger JT Jr, et al., Components of heart rate variability measured during healing of acute myocardial infarction. Am J Cardiol, 1988. 61: p. 208-15.**

13. **Kleiger RE, et al., Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987. 59: p. 256-62.**

14. **Durmaz, T., et al., Heart Rate Variability in Patients With Stable Coronary Artery Disease and Aspirin Resistance. International Heart Journal, 2008. 49(4): p. 413-422.**

15. **Solin, P., et al., Peripheral and Central Ventilatory Responses in Central Sleep Apnea with and without Congestive Heart Failure. Am. J. Respir. Crit. Care Med., 2000. 162(6): p. 2194-2200.**

16. **Karam, M., et al., Mechanism of decreased left ventricular stroke volume during inspiration in man. Circulation, 1984. 69(5): p. 866-873.**

17. **Garpestad, E., et al., Stroke volume and cardiac output decrease at termination of obstructive apneas. J Appl Physiol, 1992. 73(5): p. 1743-1748.**

18. **Somers, V.K., et al., Sympathetic-nerve activity during sleep in normal subjects. N Engl J Med, 1993. 328: p. 303-307.**

19. **Leuenberger, U., et al., Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. J Appl Physiol, 1995. 79(2): p. 581-588.**

20. **Leuenberger, U.A., et al., Effects of intermittent hypoxia on sympathetic activity and blood pressure in humans. Autonomic Neuroscience, 2005. 121(1-2): p. 87-93.**

21. **Carlson, J., et al., Augmented resting sympathetic activity in awake patients with obtructive sleep apnea. Chest, 1993. 103: p. 1763-1768.**

22. **Marrone, O., et al., Catecholamines and blood pressure in obstructive sleep apnea syndrome. Chest, 1993. 103(3): p. 722-727.**

23. **Electrophysiology Task Force of the European Society of Cardiology the North American Society of Pacing, Heart Rate Variability : Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation, 1996. 93(5): p. 1043-1065.**

24. **Donadio, V., et al., Parallel changes in resting muscle sympathetic nerve activity and blood pressure in a hypertensive OSAS patient demonstrate treatment efficacy Clin Auton Res, 2006. 16(3): p. 235-239.**

25. **Narkiewicz, K., et al., Nocturnal Continuous Positive Airway Pressure Decreases Daytime Sympathetic Traffic in Obstructive Sleep Apnea. Circulation, 1999. 100: p. 2332-2335.**

26. **Waradekar, N.V., et al., Influence of treatment on muscle sympathetic nerve activity in sleep apnea. Am J Respir Crit Care Med. , 1996. 153(4 Pt 1): p. 1333-8.**

27. **Khoo, M.C.K., et al., Cardiac Autonomic Control in Obstructive Sleep Apnea Effects of Long-term CPAP Therapy. Am J Respir Crit Care Med, 2001. 164: p. 807–812.**

28. **Agnew, H.W.J., W.B. Webb, and R.L. Williams, The first night effect: an EEG study of sleep. Psychophysiology, 1966. 2(3): p. 263 - 266.**

29. **Rechtschaffen A and Kales A, A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stages of Human Subjects. 1968, Brain Information Service/Brain Research Institute, UCLA: Los Angeles.**

30. **Kushida, C.A., et al., Practice Parameters for the Indications for Polysomnography and Related Procedures: An Update for 2005. Sleep, 2005. 28(4): p. 499 - 519.**

31. **Anonymous, EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. Sleep, 1992. 15(2): p. 173-84.**

32. **Bonnet, M., D. Carley, and the Atlas Task Force., ASDA Report. EEG arousals: Scoring rules and examples. . Sleep, 1992. 15: p. 173-184.**

**33.** **Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, Heart rate variability-standards of measurement, physiological interpretation, and clinical use. Circulation, 1996. 93: p. 1043-1065.**

**34.** **Pagani M, et al., Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. Circ. Res., 1986. 59: p. 178-193.**

**35.** **Malliani, A., et al., Cardiovascular neural regulation explored in the frequency domain. Circulation, 1991. 84: p. 482-492.**

**36.** **Kamath MV and Fallen EL, Correction of the heart rate variablity signal for ectopics & missing beats., in Heart Rate Variability, Malik M and Camm AJ, Editors. 1995, Futura: Armonk, NY. p. 75-85.**

**37.** **Akaike H, Statistical predictor identification. Am. Int. Stat. Math., 1970. 22: p. 203-217.**

**38.** **Zetterberg LH, A mathematical model for analysis of EEG. Electroencephalogr Clin Neurophysiol, 1969. 26: p. 338.**

**39.** **Johns M, Daytime sleepiness, snoring, and obstructive sleep apnea: the Epworth Sleepiness Scale. Chest, 1993. 103: p. 30-36.**

**40.** **Johns MW, A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep, 1991. 14(6): p. 540-545.**

**41.** **Buysse, D.J., et al., The Pittsburgh Sleep Quality Index: A New Instrument for Psychiatric Practice and Research. Psychiatry Research, 1989. 28: p. 193-213.**

**42.** **Kleiger RE, Stein PK, and Bigger JT Jr, Heart Rate Variability: Measurement and Clinical Untility. ANE, 2005. 10(1): p. 88-101.**

**43.** **Guilleminault, C., et al., Heart rate variability, sympathetic and vagal balance and EEG arousals in upper airway resistance and mild obstructive sleep apnea syndromes. Sleep Medicine, 2005. 6(5): p. 451-457.**

**44.** **Narkiewicz, K., et al., Altered Cardiovascular Variability in Obstructive Sleep Apnea. Circulation, 1998. 98(11): p. 1071-1077.**

**45.** **Khoo, M.C.K., T.S. Kim, and R.B. Berry, Spectral Indices of Cardiac Autonomic Function in Obstructive Sleep Apnea. Sleep, 1999. 22(4): p. 443-451.**

**46.** **Tasali, E., et al., Slow-wave sleep and the risk of type 2 diabetes in humans. Proceedings of the National Academy of Sciences, 2008. 105(3): p. 1044-1049.**

**47.** **Burgess, H.J., J. Kleiman, and J. Trinder, Cardiac activity during sleep onset. Psychophysiology, 1999. 36: p. 1-9.**

**48.** **Narkiewicz, K., et al., Sympathetic Activity in Obese Subjects With and Without Obstructive Sleep Apnea. Circulation, 1998. 98(8): p. 772-776.**