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Alan Michael Jones	Date

The influence of gender, weight, and age on OSA progression in REM and NREM sleep

By

Alan Michael Jones

Doctor of Philosophy

Nursing

_____

Ann E. Rogers, Ph.D.

Advisor

_____

Donald L. Bliwise, Ph.D.

Committee Member

_____

Vicki S. Hertzberg, Ph.D.

Committee Member

_____

Tene T. Lewis, Ph.D.

Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.

Dean of the James T. Laney School of Graduate Studies

_____

Date

The influence of gender, weight, and age on OSA progression in REM and NREM sleep

By

Alan Michael Jones

BSN, Georgia State University, 2012

MSN, University of West Georgia, 2016

Advisor: Ann E. Rogers, PhD, RN, FAAN, FAASM

An abstract of

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Abstract

The influence of gender, weight, and age on OSA progression in REM and NREM sleep
By Alan Michael Jones

Background

In the United States, it is estimated that obstructive sleep apnea (OSA), which greatly increases the risk of many adverse health conditions, affects over 25 million adults with prevalence rates that have drastically increased over the last two decades, likely due to the obesity epidemic. Research has identified several explanatory variables of OSA, but studies have indicated that OSA incidence and severity is mainly determined by three variables: gender, weight and age.

Purpose

The purpose of this study is to investigate the effects and importance of gender, weight, and age on OSA levels (as measured by respiratory disturbance index (RDI) values) by using a large, community-based sample with equal amounts of men and women. This study will also investigate these variables' impact on OSA in the whole-night, rapid eye movement (REM), and non-rapid eye movement (NREM) stages of sleep.

Sample/Design

A retrospective, secondary analysis of the Sleep Heart Health Study (SHHS) will be used. SHHS is a multi-center cohort study that was implemented in an effort to determine what the cardiovascular and other consequences were of sleep-disordered breathing. Participants were recruited from 9 existing epidemiological studies ("parent cohorts"), and two time points of data collection were performed (labeled SHHS1 and SHHS2). The total number of subjects at SHHS1 is 5804, and 4080 subjects were present at SHHS2.

Results

Overall, RDI values were significantly higher (indicating higher OSA levels) for men in all sleep stages. Gender, body mass index (BMI), and age were the main explanatory variables for RDI values. Overall, BMI was the main variable influencing RDI values in all sleep stages. For men and women, BMI is significantly lower in those who, over time, stay at either a normal or mild OSA level versus those who, over time, progress to moderate or severe levels.

Conclusion

Gender age, and BMI were influential on OSA and its progression or improvement. BMI was the main variable of influence in all sleep stages, and BMI median value was significantly lower in those who maintained a normal or mild OSA level over time compared to those with a more severe OSA level.

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CHAPTER 1: Dissertation Introduction

Section 1: Significance of the Problem

It is estimated that approximately 26% of adults between the ages of 30 and 70 living in the United States have obstructive sleep apnea (OSA), a condition characterized by the presence of repetitive episodes of either partial or complete cessation of breathing during sleep[29]. Patients with OSA often have nocturnal oxygen desaturations and loud snoring at night that are associated with excessive daytime sleepiness, insomnia, and non-restorative sleep[36]. The cycles of intermittent hypoxia, exaggerated negative intrathoracic pressure, and arousals that OSA can produce also have the potential to exacerbate many pre-existing health conditions in patients[28]. Although the condition is easily treated with nasal continuous positive airway pressure (CPAP), it is estimated that over 80% of the patients with moderate-to-severe OSA remain undiagnosed[13].

Health Consequences of OSA

Numerous studies have identified OSA as a significant risk factor for mortality, with moderate-to-severe OSA being independently associated with a rate of 33% all-cause mortality[23]. OSA is also an independent risk factor for developing a myriad of cardiovascular co-morbidities, including hypertension, stroke, diabetes, and heart disease[25]. Sixty percent of patients with metabolic syndrome have OSA, and the rate is even higher in those who have both obesity and diabetes[29]. In a study following 4.5 million individuals in Denmark's Danish National Patient Registry who were followed for up to 11 years, a diagnosis of OSA was independently associated with an increased risk of myocardial infarction (MI), hypertension, and stroke[18, 21]. This study also found that not only is moderate to severe OSA associated with a threefold higher stroke risk than mild or no OSA, but both stroke and atrial fibrillation are also more common in individuals with moderate-to-severe OSA compared to individuals with no OSA (30% vs. 7.2% and 4.8% vs. 0.9%, respectively)[18]. In the unadjusted arm of the Sleep Heart Health Study, prevalence rates of hypertension increased according to the severity of OSA (none: 43%, mild: 53%, moderate: 59%, severe: 62%)[27]. Findings from the Wisconsin Sleep Cohort Study also reported significantly higher average blood pressures in patients with OSA during wakefulness (131/80 $\pm$ 1.8/1.1

compared to 104/62 ± 2.0/1.3, $p<0.05$), even when controlling for obesity, gender and age (OR 2.0 to 5.0 for those with an apnea-hypopnea index (AHI) of 5-25)[1]. Results from the Cleveland Family Study suggest that the incidence and severity of OSA is independently determined by three main variables: gender, weight and age[10, 29].

Gender and OSA

Until the early 2000s, OSA was viewed as a "male" disease that occurred mainly in middle-aged, obese males. As a result, most research on OSA included only male participants and clinical data suggested that the ratio of men to women diagnosed with OSA was approximately 8:1[20]. Recent studies, however, have suggested that the ratio is closer to 2:1 in younger women, with an even smaller difference as women age[20]. At least 2% of middle-aged women in the general population are estimated to have OSA, with an estimated 93% of affected women remaining undiagnosed; approximately 82% of affected men are also undiagnosed[40]. Differences in the number of men and women diagnosed may be partially due to differences in OSA symptom presentation between men and women. For example, men are likely to report the classical OSA symptoms of excessive daytime sleepiness (EDS) and snoring[10]. While women also exhibit EDS and snoring, they are much more likely than men to report other symptoms such as insomnia, restless legs, depression, fragmented sleep, hallucinations, palpitations, nocturia, and dry mouth[14]. Research has also suggested that women may have more severe symptomatology at lower apnea-hypopnea indexes [AHI, an index notating OSA severity by counting apnea (a pause in breathing) and hypopnea (shallow, or slowed, breathing)] when compared to men[38].

Women also tend to develop obstructive sleep apnea later in life than men[14]. While the maximum prevalence for OSA peaks between ages 50–59 in men, this peak is not seen in women until after age 65[10]. OSA prevalence increases markedly after menopause, with post-menopausal women having a doubled rate of apnea compared to pre-menopausal women, even when controlling for neck circumference and body mass index (BMI) [15]. Prevalence rates for OSA range from as low as 47% to as high as 67% in post-menopausal women[10, 14, 15]. Not only is OSA less common among premenopausal women (19% vs 53%), but they have significantly lower AHI than post-menopausal women (10.3 ±

0.7/hour vs 14.3 ± 0.8/hour, respectively) [15]. In the same study, premenopausal women had lower average BMIs than post-menopausal women (mean 30.2 ± SD 0.4 vs mean 32.2 ± SD 0.4)[14]. This finding from 1,315 female sleep clinic patients supports the findings of several other studies which indicate that few slender or normal-weight women develop OSA prior to menopause, and that post-menopausal women have more severe OSA than premenopausal women with comparable BMIs[14, 15, 38].

Weight and OSA

OSA prevalence rates have significantly increased over the last two decades, likely due, in large part, to the obesity epidemic[33]. For example, the prevalence of OSA among obese and/or severely obese patients is nearly twice that of normal-weight adults[33]. One study estimated that 58% of moderate to severe OSA cases are found in those with a BMI of ≥25, with another study finding that only 0.1% of normal weighted women have severe OSA[33]. Research has shown that weight gain, for both men and women, is associated with increased OSA severity, while a weight loss is associated with a reduction in severity for both men and women[3, 4]. Those with mild OSA who gain 10% of their baseline weight are at a 6-fold increased risk of OSA progression; likewise, an equivalent weight loss can result in a ≥20% improvement in the severity of OSA[33]. Data collected by the Wisconsin Sleep Cohort Study over a 4-year period showed that, compared to participants with a stable weight, those with a 10% weight increase had a median 32% increase in their AHI and a 6-fold risk of developing moderate-to-severe OSA[41]. In addition, data collected during the Sleep Heart Health Study showed that, compared to those with a stable weight over the follow-up interval, men with ≥10 kilograms of weight gain had 5.2 times the odds of increasing their AHI by >15 events per hour[37]. In contrast, women who gained ≥10 kilograms had only 2.5 times the odds of a similar increase in apnea severity[37]. Additionally, in women, a 10% weight decrease was associated with a 26% decrease in apnea severity. In men, a similar decrease in weight has been associated with a 22% decrease in apnea severity[37].

Age and OSA

OSA prevalence is also higher in elderly and obese populations when compared to younger and leaner populations[9]. Older adults (>65 years) report more fatigue, sleepiness, insomnia and snoring than

younger adults[19]. They also have more co-morbid conditions than younger adults, and older adults with OSA were more likely than older adults without OSA to also have asthma, chronic obstructive pulmonary disorder (COPD), and obesity[7, 8]. While research has shown that OSA often progresses over time, research on the effect of age on OSA severity is quite limited. One recent study found that an obstruction event's severity is more dependent on the age than it is dependent on the AHI[19]. No study, however, has explored the progression of OSA severity when controlling for confounding factors such as gender, snoring, weight, smoking, and heart failure. Data concerning OSA progression, particularly data stratified by men and women, also remains limited in the literature.

REM-related and NREM-related OSA

There is existing data demonstrating that obstructive apneas and hypopneas are longer and associated with more severe oxygen desaturations, greater surges in heart rate, and increases in blood pressure during rapid eye movement (REM) sleep compared with during non-rapid eye movement (NREM) sleep[24]. Research has also shown that, compared with NREM sleep, REM sleep is associated with greater sympathetic activity and cardiovascular instability in healthy human subjects and in patients with OSA[26]. Despite this, there has been little research looking at certain aspects of how OSA impacts both REM and NREM sleep. Although the Wisconsin Sleep Cohort study showed that individuals with higher REM-AHI values are older, this study did not adjust for fixed variables such as gender and race/ethnicity in the models involving within-subject comparisons (i.e. changes of OSA status associated with changes in hypertension status)[41].

While apneic events are more frequent during REM sleep compared to NREM sleep in both men and women, women have been found, in previous studies, to have a greater cluster of apneic events in REM sleep when compared to men[20, 38]. Previous research has found that REM-related OSA is more common in women, particularly those presenting with depressive symptoms[37]. In addition, REM-only apnea has been associated with an increase in prevalent and incident hypertension and impaired glucose metabolism[1]. Despite the existing knowledge, research concerning the severity of OSA in the specific

stages of REM and NREM sleep, particularly concerning the long-term effects of REM sleep disruption stratified by men and women, remains largely underexplored.

Section 2: Purpose of the Dissertation

Despite the numerous studies documenting the prevalence of OSA, information about its incidence and progression is quite limited[6]. Many earlier studies have shown that gender, body weight, and age were all associated with OSA, but few studies have derived accurate correlative models between such risk factors and OSA progression, as measured by increases over time in a biological marker (i.e. AHI)[2,5]. Research examining the importance of weight and age differences, between men and women, in the clinical manifestations, treatment responses, and progression rates of OSA, particularly among community-based samples, remains limited. The research becomes even more limited when examining the aforementioned variables in terms of their relative and/or hierarchal contribution to OSA, as well as their effects on not only sleep as a whole, but also sleep as measured in separate stages (such as REM and NREM sleep).

The purpose, therefore, of this study is to investigate the effects and importance of gender, body weight, and age on OSA levels using a large community sample. As a measure of weight, this study will use BMI; we hypothesize that other weight measures, such as neck-height ratio and hip circumference, will be highly correlated to gender and will not act as a suitable independent variable alongside gender. This study will also investigate the impact of those variables on OSA in whole-night sleep, as well as in the separate REM and NREM stages of sleep. While the apnea-hypopnea index (AHI) remains a popular outcome variable of interest in sleep research, the respiratory disturbance index (RDI) is an equally valid and more precise measure of apnea severity because RDI values include not only episodes of apneas and hypopneas, but also more subtle breathing irregularities including respiratory effort related arousals (RERAs). The OSA measurement of interest in this project to be used as a measure of OSA progression and OSA improvement will be RDI values with >4% oxygen desaturation at two time points (labeled SHHS1 and SHHS2). This study will leverage the data collected during the longitudinal Sleep Heart

Health Study, which contains an ethnically diverse, community-based sample of 5,804 participants (3,039 women; 2,765 men) to address the following research questions that are accompanied by our hypotheses:

Research Question 1:

Is there a significant difference in RDI values during whole-night, REM, and NREM sleep between men and women?

Hypothesis 1:

Women will have higher RDI values (indicating higher OSA levels) during REM sleep than men; in whole-night and NREM sleep, it will be men who have higher RDI values.

Research Question 2:

During whole-night sleep, is BMI significantly different in those with varying clinically-defined OSA levels, and do the results vary between men and women?

Hypothesis 2:

BMI will be significantly different in those with normal or mild OSA versus those with moderate or severe OSA, and gender difference will not vary the results.

Research Question 3:

What are the most significant factors contributing to RDI values in whole-night, REM, and NREM sleep? Are these factors of similar importance and contribution, or is there a hierarchy of factor importance and contribution in the various sleep stages?

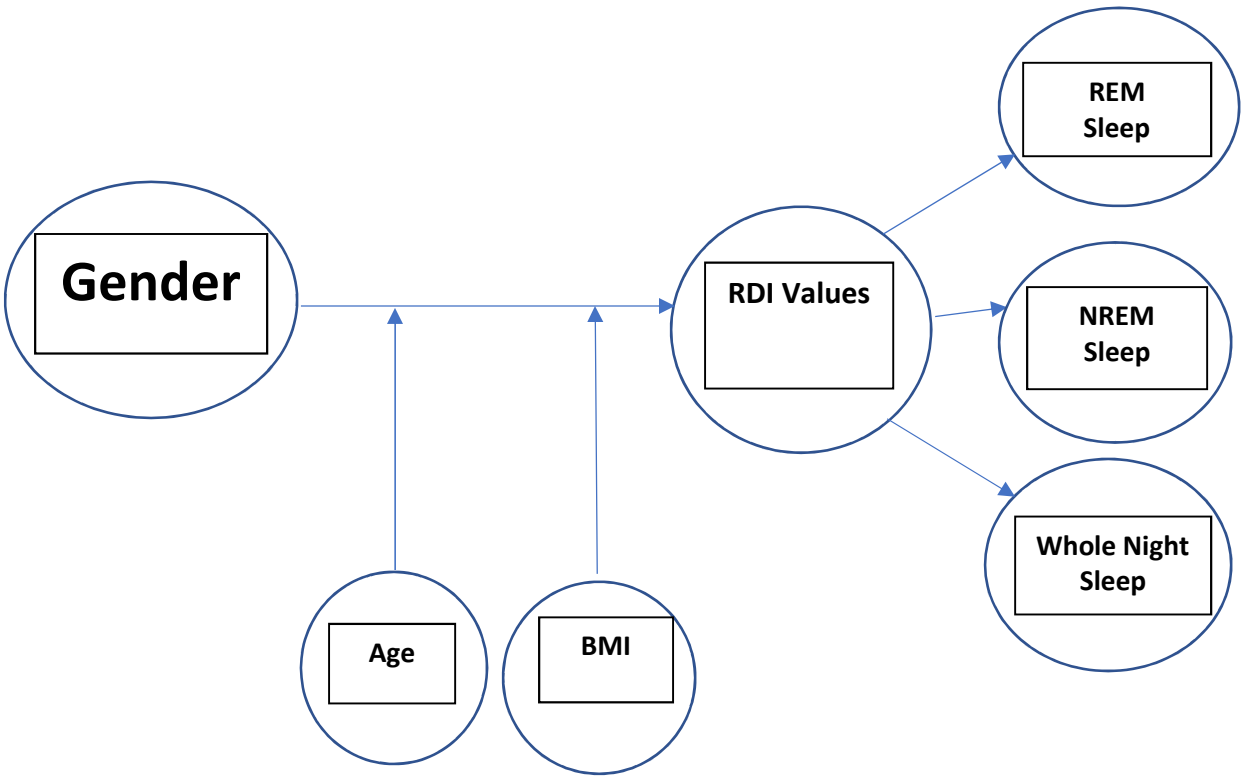
Hypothesis 3:

The most significant factors contributing to RDI values in all sleep stages will be gender, age, and BMI. Gender will be the most important contributor in REM sleep. BMI will be the most important contributor in whole-night sleep. Gender and age will be of equal value as the most important contributor in NREM sleep.

Section 3: Conceptual Model of the Dissertation

Figure 1 presents a conceptual model outlining a visual hypothesis to this study. We posit that gender has an influence on RDI values, which will be at different levels in whole-night, REM, and NREM sleep. RDI values, in this study, act as a measurement of OSA progression (when RDI values increase over time) and OSA improvement (when RDI values decrease over time). We hypothesize that women will experience higher RDI values in REM sleep, and that men will experience higher RDI values during whole-night and NREM sleep; this is based on findings in previous research studies with clinic-based samples. We also hypothesize that age and BMI are variables that help to explain the underlying mechanism of the relationship between gender and RDI values; this is based on previous research studies indicating that age and weight are important factors in OSA severity rates. We believe that age is a potential confounder in the relationship between gender and RDI, and we believe that BMI is a partial mediator in the same relationship.

Figure 1 Conceptual Model for the Dissertation



Section 4: Study Design and Methods of the Dissertation

A retrospective, secondary analysis of the Sleep Heart Health Study (SHHS) was used to address the research questions. SHHS is a multi-center cohort study that was implemented by the National Heart, Lung, and Blood Institute (NHLBI) in an effort to determine what the cardiovascular and other consequences were of sleep-disordered breathing[30]. Participants were recruited from 9 existing epidemiological studies ("parent cohorts") where data on cardiovascular risk factors had been previously collected, such as the Framingham Offspring Cohort[30, 31]. Several of these parent cohorts over-sampled snorers so that study-wide prevalence of sleep-disordered breathing could be increased[31]. From these parent cohorts, a participant sample was formed from those who met the inclusion criteria (age>40, no OSA history or treatment, no tracheostomy, no current home oxygen therapy); SHHS specifically tested whether sleep-related breathing is associated with an increased risk of several conditions including stroke, all-cause mortality, and heart disease[11]. 6,441 men and women enrolled between November 1995 and January 1998 for a baseline examination, which included an initial polysomnogram. The dataset from the first time point of measure (labeled SHHS1) represents data from the baseline and first follow-up visits performed between 1995 and 1998 with a total n of 5804[11,31]. The total n of 5,804 in SHHS1 is decreased from the originally enrolled 6,441 due to data accuracy issues from some of the participants recruited from various Strong Heart Study sites[30].

Participants in SHHS1 included 2,765 men and 3,039 women aged 40 to 90 years (men: Mean = 63.1 years, Standard Deviation = 11.0 years; women: Mean = 63.2 years, Standard Deviation = 11.4 years) [11]. In a follow-up clinic visit, 4,080 surviving men and women were studied for the second time point (labeled SHHS2), and a second polysomnogram was obtained in 3,295 participants between January 2001 and June 2003; not all cohorts and subjects took part[11]. Participants in SHHS2 were 1,861 men and 2,219 women aged 44 to 90 years (men: Mean = 68.4 years, Standard Deviation = 10.1 years; women: Mean = 68.3 years, Standard Deviation = 10.8 years) [11]. The timespan from SHHS1 to SHHS2 was an average of 5-6 years for participants; an interim clinic visit or phone call occurred 2-3 years after the SHHS1 baseline visit[11]. Adjudicated heart health outcomes (i.e. heart attack) were tracked between

baseline and through at least 2008, with some parent cohorts tracking until 2011; event-level details for the tracking of heart health outcomes (i.e. myocardial infarction requiring hospitalization) also occurred[11, 30, 31].

Section 5: Data Analysis of the Dissertation

The primary objective of this study was to explore the effects and importance of gender, body weight, and age on OSA levels and progression rates in sleep as a whole, as well as the separate REM and NREM stages of sleep. We analyzed data with the specific intent of establishing population parameters (i.e. effect sizes) for the variables under investigation. Data was analyzed using both descriptive and inferential test statistics. To identify distributions, outliers, and missing data, analysis first underwent the examination of measures of central tendency and dispersion to examine and compare the means, medians, frequencies, and variability measures for the selected variables. Standard statistics software (R version 3.5.2, Eggshell Igloo – Vienna, Austria) was used to summarize and visualize the characteristics of the data. SHHS archived descriptive statistics of all variables collected throughout the study period with visualization and numerical results provided for the 2 time points of measure (referred to as SHHS1 and SHHS2). Concerning missing data for this study, cases where data for whole-night, REM or NREM sleep were missing were excluded from the analyses since the goal was to include the same cases in the whole-night, REM, and NREM analyses. Cases with missing RDI data at either time point were also excluded from the analyses as RDI was the outcome variable of interest in the study. For the analyses in Chapters 3 and 4 concerning BMI, cases with missing BMI values were excluded from the analysis. A REM or NREM value of 0.0 did not warrant exclusion from any of the analyses since a recorded value of 0.0 is a valid result of no sleep apnea being recorded (i.e. 0.0 means no REM apnea, NREM apnea, etc.).

The initial data exploration involved determining the distributions of outcome measures to assess whether data transformation was needed. The continuous independent variable of age was normally distributed. The continuous independent variable of BMI was slightly left-skewed, but was included with no transformation, as recommended by previous SHHS manuscripts. The categorical independent

variable of gender is not privy to distribution rules and was left as is in all of the analyses. The outcome variable of RDI (in whole-night, REM, and NREM sleep) required a log-transformation since many values were at, or were very close to, 0.0. An RDI value of 0.0 for several study participants would greatly skew any results, so this resulted in SHHS providing a recommendation for variable transformation. SHHS provides a recommended formula that log-transforms the RDI values, and this transformation has been used in several other previously published analyses of the SHHS dataset. This recommended formula was used to transform the RDI values in all of this study's analyses into a new outcome variable of log-transformed RDI. We insured that underlying assumptions of statistical analyses were satisfied, identified any co-linearity issues, and identified potential outliers that would require further investigation. In building models, we checked linearity assumptions for the continuous predictors. In general, a sample size of 100 achieves 80% power to detect an R-squared of 0.05 attributed to 3 independent variable(s) using an F-test with a significance level (Alpha) of 0.05. SHHS contains >5800 subjects, and the subset of subjects used in each of the analyses exceeds the requirement for suitable statistical power. Each research question used the following statistical analyses to aid in the project:

Research Question 1

Welch's 2-independent-sample t-tests were performed to detect whether or not a statistically significant difference between men and women existed for log-transformed RDI levels in whole-night, REM, and NREM sleep. We used the unpooled t-tests for these analyses due to Bartlett's test results indicating unequal variances in the sample during whole-night, REM, and NREM sleep.

Research Question 2

Nonparametric analysis was used to examine the BMI differences between RDI level groups that were categorized as "Normal", "Mild", "Moderate", and "Severe" in accordance to similarly labeled and clinically recognized OSA groups. Parametric analysis could not be performed (e.g. Analysis of Variance (ANOVA), 1-Way Welch test) because parametric test assumptions were not met for this sample. A

Kruskall-Wallis test was conducted to examine if there was a difference between the BMI medians in any of the 10 groups that were formed; the 10 groups represent the intersection of the OSA level one was at during SHHS1 and what OSA level they ended up at during SHHS2. Since the test results suggested positive relationships between some of the groups, we then conducted post-hoc tests to further examine the results. The post-hoc tests produced positive comparisons between certain groups in the sample, and the results obtained from using the most conservative method of p-value adjustment are discussed.

Research Question 3

Multivariable linear regression analysis was used to aid in characterizing the relationship between the selected independent variables of significance (gender, BMI, and age) and the dependent variable (log-transformed RDI with >4% oxygen desaturation), controlling for selected covariates. 3 models were produced as the product of examining results from whole-night, REM, and NREM sleep. Linear regression assumptions were examined and met in the models through the following tests: 1) verifying linear parameters; 2) verifying that mean residuals were near zero; 3) evaluating the homoscedasticity of residuals/equal variance by producing a residuals-fitted plot; 4) using the Durbin-Watson test verifying no autocorrelation of residuals; 5) using Pearson's product-moment correlation verifying no correlation between the X variables and residuals; 6) verifying that the number of observation exceeded the number of X's; 7) verifying positive variability in the X values; 8) verifying that the regression models were correctly specified; 9) verifying no multicollinearity among the continuous independent variables through the diagnostic tests of condition index and variance inflation factors; and 10) verifying the normality of residuals by producing a Normal Q-Q plot. The regression models in all 3 sleep stages contained the same 3 variables of significance (gender, BMI, and age); due to this, causal mediation analysis was performed based on the initial study hypothesis that BMI and age were mediator variables acting on the relationship between gender and RDI values. Finally, calculations of both relative importance and bootstrapping significance were performed to identify the influence and hierarchy of gender, BMI, and age). Calculated relative importance is listed in the analysis results in a hierarchal percentage format; bootstrapping

importance is listed as a visualization where variables are listed in importance from left to right with applicable arrows identifying any existing variable hierarchy.

Section 6: Organization of the Dissertation

This dissertation document is comprised of five chapters: this introduction chapter (Chapter 1), three manuscripts (Chapters 2-4), and a synthesis chapter (Chapter 5). A document containing signature forms, an abstract, and a table of contents precedes the introduction chapter. The three manuscripts contained in Chapters 2-4 are briefly described below:

The first manuscript (Chapter 2), entitled *RDI value difference in men and women during whole-night, REM, and NREM sleep*, used data from SHHS to explore whether being a man or woman has a significant difference in RDI values for whole night, REM, and NREM sleep in subjects whose RDI values increased, as well as decreased, over time.

The second manuscript (Chapter 3), entitled *The significance of BMI in varying OSA levels for men and women*, used data from SHHS to explore whether BMI medians were significantly different in those with differing OSA levels (e.g. normal, mild, moderate, and severe), and also examined whether the results varied between men and women.

The third manuscript (Chapter 4), entitled *Examining the significant factors contributing to RDI in whole-night, REM, and NREM sleep*, used data from SHHS to explore what variables significantly contributed to changes in RDI values over time (results: gender, age, and BMI). We then explored the mediation effects of age and BMI on gender, and examined the contribution and importance of these 3 variables to RDI values.

Section 7: Innovation of the Dissertation

This study was innovative in that it: (1) explored RDI values as an OSA outcome variable of interest as opposed to AHI values; (2) explored RDI values in whole-night, REM and NREM sleep, as opposed to a sole focus on whole-night sleep; (3) used a dataset (SHHS) that contains an ethnically diverse, community-based sample with a higher percentage of women in its sample than men; and, (4) explored the effects of gender, BMI, and age on RDI as both a numerical value and as a categorical value of clinical interest.

Section 8: Brief Dissertation Document Summary

This study was conducted to explore the effects and importance of gender, weight, and age on OSA values and progression and improvement rates (measured as RDI increases and decreases in this study) in whole-night sleep, as well as in the separate REM and NREM stages of sleep. This study summary concludes Chapter 1 of this dissertation document. Each research question has been analyzed and is presented in Chapters 2-4 of this dissertation document. Each of those chapters is individually prepared for submission to a peer-reviewed journal as selected by the authors. An integrative summary including overall conclusions and implications for future research is outlined in Chapter 5 of this dissertation document.

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CHAPTER 2: Dissertation Manuscript 1

RDI value difference in men and women during whole-night, REM, and NREM sleep

Introduction

The prevalence rates of obstructive sleep apnea (OSA, the involuntary cessation of breathing while sleeping) have significantly increased over the last two decades[21]. Current estimates suggest that at least 26% of adults in the United States, or over 25 million adults between the ages of 30 and 70, have OSA[12, 20, 21]. Sixty percent of patients with metabolic syndrome have OSA, and OSA prevalence increases among those who are both obese and diabetic[3]. In a Danish study following over 4 million individuals that were followed for up to 11 years, a diagnosis of OSA was independently associated with an increased risk of myocardial infarction (MI), hypertension, and stroke[14]. In addition, the Sleep Heart Health Study (SHHS), a longitudinal study of 6,441 participants, reported that moderate-to-severe OSA is associated with a threefold higher risk of stroke; stroke and atrial fibrillation are also more common in individuals with OSA compared to those without it (30% vs. 7.2% and 4.8% vs. 0.9%, respectively)[15,24]. Finally, numerous other studies have identified OSA as an independent risk factor for both increased morbidity and mortality. Moderate-to-severe OSA is also independently associated with a rate of 33% all-cause mortality, compared to 7.7% and 6.5% in those with mild or no OSA, respectively[17].

OSA research was, until recently, almost exclusively conducted with men; until the early 2000s, OSA was considered a disease of middle-aged males, with 8 men being diagnosed for every 1 woman diagnosed with OSA[13]. More recent studies, however, have shown that the ratio of affected men to women is closer to 2:1, with this ratio shrinking even more as women advance in age[11]. Although under-diagnosis and under-treatment is a significant issue for both men and women, it is estimated that over 90% of affected women remain undiagnosed[21]. Men are more likely to exhibit the "classic" OSA symptoms of excessive daytime sleepiness (EDS) and snoring[19]. While women may also exhibit these symptoms, they are also much more likely to report other symptoms such as insomnia, restless legs, depression, heart palpitations, nocturia, and dry mouth[19]. Not only are there differences in symptoms reported by men and women, but men appear to have more severe illness [using, for example, apnea-

hypopnea index (AHI) as a measure of severity] than women when matched for body mass index (BMI) [21, 30]. Finally, OSA typically develops later in women. While the maximum prevalence for OSA appears to peak between ages 50–59 in men, a similar peak is not seen in women until after age 65, 10 years later than men[5].

Studies have shown that OSA risk increases when both age and BMI both increase[9]. Numerous studies have demonstrated that the menopausal transition, with its associated hormonal changes and changes in the distribution of adipose tissue, dramatically increases the risk of developing OSA[2]. In the Study of Women's Health across the Nation (SWAN), OSA prevalence ranges from 16% to 42% in premenopausal women, 39% to 47% in perimenopausal women, and 35% to 60% in postmenopausal women[9]. In fact, studies have demonstrated that even when controlling for neck circumference and BMI, the prevalence of OSA in older women is double that of younger women[5, 8]. One study of 1,315 female sleep clinic subjects found that the OSA prevalence in the premenopausal group was significantly lower than in the postmenopausal group (21% vs 47%) and that premenopausal women had significantly lower AHI levels than postmenopausal women (8.7 $\pm$ 0.6/hour vs 17.0 $\pm$ 0.9/hour, respectively)[4]. In the same study, average BMI in the premenopausal group was lower than in the postmenopausal group (mean 30.2 $\pm$ SD 0.4 vs mean 32.2 $\pm$ SD 0.4)[4]. This corroborates several other studies indicating that not only do few slender or normal-weight women develop OSA prior to menopause, but even when a comparable BMI is present, postmenopausal women have a more severe level of OSA than premenopausal women do[4].

When both sexes are matched for BMI, men appear to have higher AHI levels than women[5, 13]. In patients with OSA, events may occur not only during whole-night sleep, but events may also differ throughout rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. There are noted gender differences in OSA symptomology, with severity and distribution differing across the sleep cycle in men and women[30]. In those with OSA, women have a significantly lower overall AHI compared with men (20.2/h versus 31.8/h; $p < 0.001$); AHI during NREM sleep was also significantly lower in women than men (14.6/h versus 29.6/h; $p < 0.001$), however there was no difference between men and women with respect to AHI during REM sleep (39.9/h versus 42.7/h), suggesting that a greater clustering of

apneic events occurs during REM sleep for women[30]. In NREM sleep, it appears that men are more susceptible to pharyngeal collapse, hypocapnic dysfunction, as well as higher ventilatory response to apneas than women with a greater hypoventilation when they go back to sleep[30]. These OSA subtypes have recently sparked much research; however, despite the findings of many studies concerning both REM-related OSA and NREM-related OSA, research is underexplored in community samples with equal amounts of men and women. In this study, our aim is to evaluate the differences in apnea severity, as measured by respiratory disturbance index (RDI) values that either progress or improve over time, between men and women during whole-night, REM, and NREM sleep using data collected from the community-based sample contained in the Sleep Heart Health Study. An increase of RDI over time indicates higher levels of OSA, or OSA progression; likewise, decreases of RDI over time indicate lower levels of OSA, or OSA improvement. We hypothesize that RDI values in this sample will be higher for women during REM sleep than men, and that men will have more severe OSA during whole-night and NREM sleep as determined by comparing RDI indices.

Methods

Sleep Heart Health Study (SHHS) Overview

The Sleep Heart Health Study (SHHS) is a prospective, longitudinal multi-center cohort study designed to assess how sleep disordered breathing contributes to the development of cardiovascular disease[22]. Previous publications have detailed the SHHS design, as well as its quality control procedures[22]. To briefly summarize SHHS baseline data is based on recruitment from ongoing parent studies (Atherosclerosis Risk in Communities Study, Cardiovascular Health Study, Framingham Heart Study, Strong Heart Study, New York Hypertension Cohorts, Tucson Epidemiologic Study of Airway Obstructive Disease, and the Health and Environment Study) that began in 1995[22, 27]. Participants were required to be at least 40 years of age; they were excluded only if they being treated for OSA with continuous positive airway pressure (CPAP), an oral appliance, home oxygen therapy or had a tracheostomy for treatment of OSA[1]. Participants with existing cardiovascular disease and hypertension were included so that investigators could determine whether those with prevalent disease were at a

different risk for subsequent cardiovascular disease than those without disease[22, 27]. Finally, oversampling was done to insure adequate numbers of minority and female participants[22]. Referral bias and spurious associations were minimized by recruiting from the community rather than sleep disorders clinics. As has been described in earlier publications[22, 24, 27], baseline data for the SHHS was collected between 1995 and 1998. Study team members obtained information about participant health, medications, and obtained participant blood pressure and anthropometric measurements using standardized protocols[27]. Sleep studies were obtained at participants' homes using the Compumedics P-series portable monitor at baseline and approximately 4 years later[22].

Study Population

Of the 10,737 parent study participants that were invited to participate in the SHHS, 6,441 enrolled and completed an acceptable overnight sleep study[27]. Data issues associated with several Strong Heart Study sites (n=458) eliminated that portion of the invited participants from SHHS1, leaving a sample of 5,804 participants in SHHS1. The sample size for this aim was reduced approximately 55% due to missing RDI data in either whole-night, REM, or NREM sleep. The final sample for this analysis includes 1173 men and 1361 women with a mean age of 62. The majority of the participants were Caucasian, overweight or obese, and had never been diagnosed with obstructive sleep apnea (See Table 1).

Table 1 Participant Age, BMI, Race/Ethnicity and Prior Diagnosis of OSA (n=2534)

	Men (n=1173)	Women (n=1361)
Mean age	62.4 ±10.1 years	62.1 ± 10.7 years
Race/ethnicity		
White	1038 (88.5%)	1178 (86.6%)
Black	66 (5.6%)	94 (6.9%)

Hispanic/Latino	48 (4.1%)	72 (5.3%)
Other	21 (1.8%)	17 (1.2%)
Mean BMI	28.5 ±4.1	28.0 ±5.5
Previous diagnosis of OSA		
No	1117 (95.2%)	1320 (97.0%)
Yes	23 (2.0%)	3 (0.2%)
Did not know	16 (1.4%)	23 (1.7%)
Unknown	17 (1.4%)	15 (1.1%)

Statistical Analysis

Although various methods were used in the SHHS to calculate the RDI, we have chosen to use the more conservative method for calculating an RDI, requiring a 4% decrease in oxygen saturation (i.e. a 4% desaturation) in combination with >5 episodes of apnea, hypopneas, and/or arousals per hour. Sleep apnea severity was classified as follows: mild OSA RDI >5, but < 15 events/hour; moderate OSA ≥ 15 but < 30 events/hour; and severe OSA ≥ 30 events/hour. For this study, we used R 3.5.2 Eggshell Igloo (Vienna, Austria) in the statistical analysis. From the SHHS total number of participants, a total of 2,534 participants were included in this study. Cases with incomplete RDI value information were removed from analysis. Of the 2534 participants included in this study, 1173 are men and 1361 are women; data was analyzed for men and women separately. Due to the skewed results of the RDI values as an outcome variable (with several valued at 0.0, representing no measured RDI), use of this as an outcome variable comes with a recommendation for variable transformation provided by SHHS. Log-transformation of the RDI variable was performed using the recommended RDI log-transformation formula provided by SHHS that has been used in previous manuscripts, which is: [New Variable = log (Old Variable + 0.1)].

We used mean (+/- standard deviation) to describe the continuous variables of RDI values in all sleep stages by the categorical variable of gender. Welch independent sample t-tests were performed to compare the mean differences for RDI values between genders in whole-night, REM, and NREM sleep

stages at both the SHHS1 and SHHS2 time points. The results in mean differences with a 95% confidence interval (95% CI) are provided along with t-test statistics, p-values with 0.05 used as the threshold of statistical significance, degrees of freedom, and Cohen's D values. Satterthwaite approximations were used due to the sample containing unequal group variances during all sleep stages.

Results

Our hypothesis that women will have more severe OSA during REM sleep was not supported. As shown in Tables 2, 3, and 4, male participants had significantly higher RDI values during whole-night, REM and NREM sleep than women when comparing mean RDI for men versus mean RDI for women, $p<0.001$. Results indicate that RDI mean values were significantly higher in whole-night sleep for men (Mean RDI = 1.64, Standard Deviation = 1.33) than for women (Mean RDI = 0.86, Standard Deviation = 1.48), exceeding the alpha=0.05 significance level with $p<0.001$. Results indicate that RDI mean values were significantly higher in REM sleep for men (Mean RDI = 1.86, Standard Deviation = 1.68) than for women (Mean RDI = 1.36, Standard Deviation = 1.88), exceeding the alpha=0.05 significance level with $p<0.001$. Results indicate that RDI mean values were also significantly higher in NREM sleep for men (Mean RDI = 1.32, Standard Deviation = 1.52) than for women (Mean RDI = 0.27, Standard Deviation = 1.61), exceeding the alpha=0.05 significance level with $p<0.001$.

Table 2 T-test results and descriptive statistics for RDI means

Sleep Stage	Group						95% CI for Mean Difference				
	Male			Female							
	M	SD	n	M	SD	n		t	p	df	d
Whole-Night	1.64	1.33	1173	0.86	1.48	1361	[0.67, 0.89]	13.97	<.001	2527	0.55
REM	1.86	1.68	1173	1.36	1.88	1361	[0.36, 0.64]	7.12	<.001	2526	0.28
NREM	1.32	1.52	1173	0.27	1.61	1361	[0.94, 1.23]	16.81	<.001	2511	0.67

*Note: Satterthwaite approximation employed due to unequal group variances

Table 3 Group statistics and p-values for RDI means

Sleep Stage	Group						Prob (P-Value)
	Male			Female			
	Mean	SD	n	Mean	SD	n	
Whole-Night	1.64	1.33	1173	0.86	1.48	1361	<.001
REM	1.86	1.68	1173	1.36	1.88	1361	<.001
NREM	1.32	1.52	1173	0.27	1.61	1361	<.001

*Note: Satterthwaite approximations employed due to unequal group variances

Table 4 Differences between group means for RDI means

Sleep Stage	Group Mean ± SD		P-Value
	Male	Female	
Whole-Night	1.64 ± 1.33	0.86 ± 1.48	<.001
REM	1.86 ± 1.68	1.36 ± 1.88	<.001
NREM	1.32 ± 1.52	0.27 ± 1.61	<.001

*Note: Satterthwaite approximation employed due to unequal group variances

Discussion

In this study, we showed RDI values were significantly higher in men for SHHS participants in whole-night, REM, and NREM sleep stages. In contrast to our study, previous studies have found RDI values in REM sleep to be higher in women than men. No finding in this study indicated that women had higher RDI values at any sleep stage, whether or not the participant's RDI values increased or decreased over time (e.g. whether or not the participant's OSA severity progressed or improved over time). Most OSA research is performed on clinically-based populations with higher percentages of men in its samples; this study used SHHS which is community-based, contains a higher percentage of women than men in its adequate sample, and is longitudinal in nature. Future research may benefit from examining multiple measurements for OSA outcome variables, such as RDI with differing levels of desaturation. The

differing findings in this study, however, compared to previous findings that women have exhibited significantly worse OSA symptoms (e.g. increased RDI values) in REM sleep are of interest. The population in this study, which consists of community-based participants, may help to explain the results since sleep-clinic based participants were not used in this study's analyses. The reason for this is that clinic-based study participants tend to have exacerbated symptomatology, which often explains why they have sought clinic-based treatment. This study is using community-based participants with no history of OSA and no extreme OSA symptomatology.

This study has limitations that should be considered in future work and research in this area. Missing data lowered the applicable sample size from >5000 to approximately 2500. When comparing outcomes of participants that may have REM-related OSA versus NREM-related OSA, common comorbidities they may share (such as cardiovascular disease and hypertension) should be taken into consideration. In this study, we found significant differences between genders for RDI values in whole-night, REM, and NREM sleep using RDI >4% desaturation where men had significantly increased RDI values in whole-night, REM, and NREM sleep. Although research is beginning to be produced combining the ideas of studying both gender differences and varying sleep stages in OSA along with its disease progression, the results of this study indicate that, overall, being male does have a greater influence on higher RDI values throughout an entire night of sleep, even accounting for separate REM and NREM sleep stages.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

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CHAPTER 3: Dissertation Manuscript 2

The significance of BMI in varying OSA levels for men and women

Introduction

It is estimated that approximately 26% of adults between the ages of 30 and 70 living in the United States have obstructive sleep apnea (OSA), a condition characterized by the presence of repetitive episodes of either partial or complete cessation of breathing during sleep[3]. OSA prevalence rates have significantly increased over the last two decades, likely due, in large part, to the obesity epidemic[6]. For example, the prevalence of OSA among obese and/or severely obese patients is nearly twice that of normal-weight adults[6]. One study estimated that 58% of moderate to severe OSA cases are found in those with a body mass index (BMI) of ≥25, with another study finding that only 0.1% of normal weighted women have severe OSA[6].

Research has shown that weight gain, for both men and women, is associated with increased OSA severity, while a weight loss is associated with a reduction in severity for both sexes[1, 2]. Those with mild OSA who gain 10% of their baseline weight are at a 6-fold increased risk of OSA progression; likewise, an equivalent weight loss can result in a ≥20% improvement in the severity of OSA[6]. Data collected by the Wisconsin Sleep Cohort Study over a 4-year period showed that, compared to participants with a stable weight, those with a 10% weight increase had a median 32% increase in their apnea-hypopnea index (AHI) and a 6-fold risk of developing moderate-to-severe OSA[9]. In addition, data collected during the Sleep Heart Health Study (SHHS) showed that, compared to those with a stable weight over the follow-up interval, men with ≥10 kilograms of weight gain had 5.2 times the odds of increasing their AHI by >15 events per hour[8]. In contrast, women who gained ≥10 kilograms had only 2.5 times the odds of a similar increase in apnea severity[8]. Additionally, in women, a 10% weight decrease was associated with a 26% decrease in apnea severity. In men, a similar decrease in weight has been associated with a 22% decrease in apnea severity[8]. Despite the existing research on OSA and weight, research is underdeveloped on examining community-based samples, with equal amounts of men and women at varying clinical OSA

levels, to determine if weight has a significant influence on progression or improvement of OSA levels over time.

This study will compare participants at varying OSA levels with SHHS, which contains an adequate sample size that includes more women than men and has a longitudinal design. SHHS subjects had OSA levels documented by respiratory disturbance index (RDI) and AHI values, but they were not placed into clinical OSA categories. This study will manually place participants into groups that indicate the OSA level they were at when beginning the study and the OSA level they were at when concluding their time in the study. These OSA levels are categorized as normal, mild, moderate, and severe based on the number of negative sleep events per hour. Once we place participants into OSA level categories, this study will examine the baseline BMI information of each group to determine if significant differences exist between groups. We hypothesize that, for both men and women, there will be significant BMI differences between those who do not exceed normal or mild OSA levels over time versus those who, over time, end up at moderate or severe OSA levels.

Methods

Sleep Heart Health Study Overview

The Sleep Heart Health Study (SHHS) is a prospective, multi-center cohort study designed to assess how OSA contributes to the development of cardiovascular disease[4]. Previous publications have detailed the study's design and quality control procedures[4, 5, 7]. To briefly summarize SHHS, baseline recruitment from ongoing parent cohorts of geographic distinction in the United States initially assembled between 1976 and 1995, began in 1995[4]. SHHS was approved by the institutional review boards at all participating sites, and the study enrolled over 6400 men and women who were at least 40 years of age[4, 7]. Participants were not enrolled if they were using OSA treatment like nasal continuous positive airway pressure (CPAP) or oral appliances, receiving home oxygen therapy, or had a tracheostomy at the study's beginning period of 1995-1998[4, 5, 7]. Participants with any existing cardiovascular disease or hypertension were not excluded so that investigators could determine whether those with prevalent disease were at a different risk for subsequent cardiovascular disease than those without prevalent disease[4, 5]. Oversampling

was purposefully done in order to insure adequate numbers of minority participants[4, 5]. Additionally, recruiting participants from community settings rather than clinics provided less of a chance of referral bias or spurious associations. Subjects were weighed by SHHS research team members[7]. Height, BMI, and weight (in kilograms) were all obtained by SHHS technicians according to standardized protocols[4, 5, 7]. Polysomnograms (PSG, or sleep study) were obtained at participants' homes using the Compumedics P-series portable monitor[7]. Two time points of data were collected by the SHHS team with several years occurring between these points of collection; the time points are referred to in existing SHHS literature, and this study, as SHHS1 and SHHS2.

Study Population

Of the 10,737 parent study participants that were invited to participate in the SHHS, 6,441 enrolled and completed an acceptable overnight sleep study[7]. Data integrity issues associated with several Strong Heart Study sites (n=458) eliminated that portion of the invited participants from SHHS1; this left a sample of 5,804 participants in SHHS1[4]. The sample size for the current study was reduced approximately 55% due to missing RDI data in either whole-night, REM, or NREM sleep. The final sample for this analysis includes 1173 men and 1361 women with a mean age of 62. The majority of the participants were Caucasian, overweight or obese, and had never been diagnosed with obstructive sleep apnea (See Table 1). In this study, OSA severity will be defined by the respiratory disturbance index (RDI: the number of apneas, hypopneas, and/or arousals per hour of sleep). The severity of OSA in the RDI is classified as follows:

None/Minimal:	RDI < 5 events per hour
Mild:	RDI > 5, but <15 per hour
Moderate:	RDI $\geq$ 15, but <30 per hour
Severe:	RDI $\geq$ 30 per hour

Table 1 Participant Age, BMI, Race/Ethnicity and Prior Diagnosis of OSA (n=2513)

	Men	Women

	(n=1163)	(n=1350)
Mean age	62.3 ±10.2 years	62.2 ± 10.7 years
Race/ethnicity		
White	1029 (88.5%)	1170 (86.7%)
Black	65 (5.6%)	93 (6.9%)
Hispanic/Latino	48 (4.1%)	71 (5.2%)
Other	21 (1.8%)	16 (1.2%)
Mean BMI	28.5 ±4.1	28.0 ± 5.5
Previous diagnosis of OSA		
No	1110 (95.4%)	1310 (97.1%)
Yes	21 (1.8%)	3 (0.2%)
Did not know	15 (1.3%)	22 (1.6%)
Unknown	17 (1.5%)	15 (1.1%)

Statistical Analysis

For this study, we used R 3.5.2 Eggshell Igloo (Vienna, Austria) in our statistical analysis. From the SHHS total number of participants, a total of 2,513 participants were included in this study. Cases with incomplete respiratory disturbance index (RDI) value information were removed from analysis. Cases with incomplete body mass index (BMI) information were also removed from analysis. Of the 2,513 participants included in this study, there were 1,163 men and 1,350 women. The 2,513 participants were divided into 2 subsamples based on gender. In those subsamples, we manually separated the participants into 10 groups (see Table 2) that contain information about the participants' OSA levels at the SHHS1 and SHHS2 time points, as measured by their RDI values at both timepoints. The group each participant is assigned to indicates the result of 1) their initial clinical OSA level at the SHHS1 time point and 2) their ending clinical level OSA level at the SHHS2 time point (e.g. Group 2 contains participants whose OSA was at a normal level at the SHHS1 time point and increased to mild, moderate or severe at

the SHHS2 time point). An increase in OSA levels over time indicates worsening symptoms and/or outcomes; likewise, an OSA level decrease over time indicates an improvement in symptoms and/or outcomes. To determine variance homogeneity of each group, parametric tests were performed to see if analysis of variance (ANOVA) or 1-way Welch tests could be applied. We could not perform an ANOVA test because Levene's test for homogeneity of variance was performed which did not satisfy ANOVA assumptions. We then performed a Welch 1-way test with a Shapiro-Wilk normality test as an ANOVA alternative; the results, along with a produced Q-Q plot, showed that there were not normal distributions among the variances, which also violated ANOVA assumptions. For these reasons, we moved to a non-parametric method of analysis. A Kruskall-Wallis test was conducted to examine the difference between the BMI of subjects in the 10 groups we formed based on clinical OSA levels. When the Kruskall-Wallis test suggested positive relationships between some of the groups, we then conducted post-hoc tests to further examine the Kruskall-Wallis test results. The post-hoc tests produced positive comparisons between certain groups in the sample, and these tests were conducted with a more conservative p-value adjustment method to obtain a strict set of results.

Results

Table 2 Men - Group number reference

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		Group 1	Group 2
Mild	Group 3	Group 4	Group 5
Moderate	Group 6	Group 7	Group 8
Severe	Group 9	Group 10	

Table 3 Men - Sample Distribution (n=1163)

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		318	194
Mild	70	200	132
Moderate	67	57	42
Severe	34	49	
	Total: 171	Total: 624	Total: 368

Table 4 Men – Mean Baseline BMI

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		26.5	28.4
Mild	28.1	29.3	29.6
Moderate	28.9	30.8	30.1
Severe	30.3	31.7	

Table 5 Men - Median Baseline BMI

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		26.3	28.1
Mild	27.9	28.9	29.7
Moderate	28.6	30.8	29.0
Severe	30.0	32.7	

Figure 1 Men – Pairwise Comparison results

	1	2	3	4	5	6	7	8	9
2	**2.6e-06**	-	-	-	-	-	-	-	-
3	**0.03628**	1.00000	-	-	-	-	-	-	-
4	**1.5e-13**	0.46496	0.52351	-	-	-	-	-	-
5	**3.5e-12**	0.08393	0.14476	1.00000	-	-	-	-	-
6	**3.2e-05**	1.00000	1.00000	1.00000	1.00000	-	-	-	-
7	**7.7e-12**	**0.00086**	**0.00230**	0.21879	1.00000	0.21588	-	-	-
8	**0.00086**	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	-	-
9	**6.9e-05**	0.39648	0.33093	1.00000	1.00000	1.00000	1.00000	1.00000	-
10	**2.0e-09**	**0.00113**	**0.00434**	**0.04229**	0.19632	0.06922	1.00000	1.00000	1.00000

Table 6 Women - Group number reference

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		Group 1	Group 2
Mild	Group 3	Group 4	Group 5
Moderate	Group 6	Group 7	Group 8
Severe	Group 9	Group 10	

Table 7 Women - Sample Distribution (n=1350)

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		632	266
Mild	86	164	78
Moderate	42	28	24
Severe	13	17	
	Total: 141	Total: 841	Total: 368

Table 8 Women – Mean Baseline BMI

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		26.4	28.9
Mild	28.2	29.6	30.7
Moderate	31.6	31.3	33.0
Severe	31.0	30.7	

Table 9 Women - Median Baseline BMI

SHHS1 OSA Level	Subjects with SHHS2 OSA Level Decrease	Subjects with the Same SHHS2 OSA Level	Subjects with SHHS2 OSA Level Increase
Normal		25.8	28.1
Mild	28.4	28.7	29.5
Moderate	31.8	31.4	32.5
Severe	28.0	31.4	

Figure 2 Women – Pairwise Comparison results

	1	2	3	4	5	6	7	8	9
2	**1.1e-09**	-	-	-	-	-	-	-	-
3	**0.00957**	1.00000	-	-	-	-	-	-	-
4	**3.0e-09**	1.00000	1.00000	-	-	-	-	-	-
5	**8.9e-08**	0.91553	1.00000	1.00000	-	-	-	-	-
6	**1.1e-05**	0.24564	0.18979	1.00000	1.00000	-	-	-	-
7	**0.00723**	1.00000	1.00000	1.00000	1.00000	1.00000	-	-	-
8	**0.00051**	0.27922	0.21208	1.00000	1.00000	1.00000	1.00000	-	-
9	**0.02581**	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	-
10	**0.00375**	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000

Our hypothesis that, in both men and women, there will be significant BMI differences between those who do not exceed normal or mild OSA levels over time versus those who, over time, end up at moderate or severe OSA levels, was not supported. We can conclude, based on the Kruskall-Wallis test results which are less than the alpha=0.05 significance level (Men: Chi-Square = 146.87, $p<.001$, df=9; Women: Chi-Square = 128.59, $p<.001$, df=9), that there are significant differences between the groups. Even though we know there is a difference between groups, we had to further examine which pairs of groups in the subsamples of men and women were different. We performed a Wilcoxon rank sum test to calculate pairwise comparisons between group levels with corrections for multiple testing. Any pairs with $p<.05$ show a significant difference, and these pairs are bolded in Figures 2 and 3. R statistical software has built-in methods to adjust a series of p-values to control the family-wise error rate. This adjustment method (Holm p-value adjustment method) was used as it attempts to limit the probability of even 1 false

discovery, which would incorrectly reject the null hypothesis when no real effect is present. This adjustment method is also strong and conservative, so it was used in the statistical analysis to produce the strongest results.

For men, when we examine the pairwise comparison results with the Holm p-value adjustment method, there is a significant difference from Groups 1 to 2-10. There is also a significant difference from Group 2 to 7 & 10, from Group 3 to 7 & 10, and from Group 4 to 10. Figure 3 below provides a narrative explanation of the groups, in the subsample of men, with significant differences in BMI medians:

Figure 3 Men – Pairwise Comparison Results narrative

Group 1 (Normal-Same)	to	Groups 2-10 (All other groups)
Group 2 (Normal-Higher)	to	Groups 7 & 10 (Moderate-Moderate; Severe-Severe)
Group 3 (Mild-Lower)	to	Groups 7 & 10 (Moderate-Moderate; Severe-Severe)
Group 4 (Mild-Same)	to	Group 10 (Severe-Severe)

For women, when we examine the pairwise comparison results with the Holm p-value adjustment method, there is only a significant difference from Group 1 to Groups 2-10. Figure 4 below provides a narrative explanation of the groups, in the subsample of women, with significant differences in BMI medians:

Figure 4 Women – Pairwise Comparison Results narrative

Group 1 (Normal-Same)	to	Groups 2-10 (All other groups)

Discussion

In this study, for both men and women, we found that there were significant differences in BMI medians between some of the groups that represent OSA progression and/or improvement in the study participants. This finding indicates that, for some of the groups within both subsamples, BMI is influential in placing participants in a particular OSA level that is identified by an RDI value increase, or decrease, over time. In examining the pairwise comparison results using the conservative Holm p-value adjustment method, several observations are noted. For both men and women, those who stayed at a

normal OSA level during both the SHHS1 and SHHS2 time points have a statistically different BMI from all 9 other groups that represent an increase in RDI value and subsequent OSA level. This suggests that those who stayed at a normal OSA level at both SHHS1 and SHHS2 have a significant difference in BMI than those at any other OSA group which falls into mild, moderate, and/or severe OSA levels at SHHS1 and SHHS2. This means that BMI is a significant variable, or factor, in keeping those who started out at normal OSA levels at a normal OSA level over time. For women, there are no other significant group differences. The conclusion of this study for women, then, is that BMI in women is only significantly different in those who remained at normal OSA levels during SHHS1 and SHHS2; therefore, in all of the other groups with an ending OSA level higher than normal, BMI difference is not statistically significant.

For men, there are some additional group differences to note. In men, BMI is statistically different for those who have normal or mild OSA levels at SHHS2 versus those who, at the SHHS2 time point, have moderate or severe OSA levels. In men, there is also a significant difference in those who stayed at mild OSA levels during the SHHS1 and SHHS2 time points than in those who stayed at severe OSA levels during the SHHS1 and SHHS2 time points. The indication is that those who stayed at a normal OSA level at both SHHS1 and SHHS2 have a significant difference in BMI than those at any other OSA group that fall into mild, moderate, and/or severe OSA levels at the SHHS1 and/or SHHS2 time points. This finding indicates that BMI is an important variable in keeping such participants at a normal OSA level over time. These results also indicate that those who advance over time from a normal to increased OSA level (most likely mild) by SHHS2 have a significant BMI difference than those who reach moderate or severe OSA levels by SHHS2. This finding suggests that BMI, in men, is important in differentiating participants whose OSA increases over time to a mild level versus those whose OSA increases over time to a more advanced level (moderate or severe). In men, this study indicates that BMI is significantly different in those who end up at a normal or mild OSA level at SHHS2 versus those who end up at a moderate or severe OSA level. For those men whose OSA stayed at a mild level at both SHHS1 and SHHS2, there was a significant difference from those whose OSA levels stayed at a severe level at both SHHS1 and SHHS2; this suggests that BMI is significantly different in those men who

maintained a mild OSA level over time than in those who maintained a moderate or severe OSA level over time.

This study has limitations that should be considered in future work and research in this area. Missing data lowered the applicable sample size from >5000 to approximately 2500. More liberal p-value adjustment methods were not performed in this analysis which could have produced more significant findings, albeit with the understanding that the use of such methods is, by nature, more liberal in statistical application and interpretation than the Holm method used in our study will be. This study found that, in both men and women, BMI is significantly different in the study participants who maintained a normal OSA level throughout SHHS versus those in any of the other groups where a higher OSA level was achieved at either SHHS1 or SHHS2. Men have additional findings of interest. For men, there are significant BMI differences in those who started SHHS at a normal OSA level and ended at a mild OSA level versus those who end SHHS at a moderate or severe level that was maintained over time from SHHS1 to SHHS2. Also, in men, those who started SHHS at a mild OSA level and ended at a normal OSA level also had significant BMI differences than those who ended SHHS at a moderate or severe level that was maintained over time from SHHS1 to SHHS2. Additionally, in men, those who maintained mild OSA levels throughout SHHS had significant BMI differences from those who maintained severe OSA levels throughout SHHS.

Our findings suggest that, for both men and women, BMI is an important variable for those who do not progress, over time, from normal OSA levels (e.g. no OSA) to mild, moderate or severe OSA levels. This implies that, in both genders, BMI is statistically different in those who never advance to an OSA level of clinical significance compared to those who do, whether the ending OSA level is mild, moderate, or severe. For men, BMI also appears to be an important variable for who those who either maintain normal OSA levels over time or do not progress beyond mild OSA levels over time when compared to those who maintain moderate or severe OSA levels over time. Clinically, this has implications for future research and practice. Although weight (as represented in our study by BMI) has been known to be a factor of influence in OSA and its disease progression, these findings suggest that, in

men and women, those who stay in a normal OSA level over time have a significant difference in their BMI compared to those who reach a mild, moderate, or severe OSA level over time. These findings also suggest that, in men, those who stay in either a normal or mild OSA level over time have a significant difference in their BMI versus those who maintain moderate or severe OSA levels over time. This may coincide with research findings that have suggested that cardiovascular disease risk may not significantly increase if OSA goes from moderate to severe levels, implying that one needs to only progress to moderate OSA levels to reach the tipping point of significantly increasing several health risks. Future research and clinical practice may benefit from these findings based on results from a large community sample that not only highlights the importance that weight has on keeping OSA levels at either mild or normal over time, but also demonstrates the importance of BMI as a largely patient-controlled variable that can have a significant influence on OSA's progression or improvement over time.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

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CHAPTER 4: Dissertation Manuscript 3

Examining the significant factors contributing to RDI in whole-night, REM, and NREM sleep

Introduction

Obstructive sleep apnea (OSA, a condition involving involuntary breathing pauses while sleeping) affects millions of Americans with its prevalence and incidence amounts increasing on an annual basis. Results from the Cleveland Family Study suggest that the incidence and severity of OSA is independently determined by three main variables: gender, weight and age[10, 29]. Earlier studies have shown that gender, body weight, and age were all associated with OSA, but few studies have derived accurate correlative models between such risk factors and OSA progression, as measured by increases over time in a biomedical marker (i.e. apnea-hypopnea index, or AHI)[1]. Research examining the importance of weight and age differences, between genders, in the clinical manifestations, treatment responses, and progression rates of OSA, particularly among community-based samples, remains limited. The research becomes even more limited when examining the aforementioned variables in terms of their relative and/or hierarchal contribution to OSA, as well as their effects on not only sleep as a whole, but also sleep as measured in separate stages (such as rapid eye movement (REM) and non-rapid eye movement (NREM) sleep).

Patients with REM-related OSA frequently lack excessive daytime sleepiness (EDS), which may lead to delays in both diagnosis and therapy[5]. This is a clinically relevant statement because OSA treatment is often limited to the first half of the sleep period; because REM sleep is dominant during the latter part of the sleep period, REM-related OSA may often remain untreated even with 3–4 hours of nightly continuous positive airway pressure (CPAP) use, leaving REM-related OSA symptoms untreated. In some patients, respiratory events occur predominantly during REM sleep[15]. The typical finding is that respiratory events during REM sleep are longer and are associated with a greater degree of hypoxemia than events that occur during NREM sleep[6]. The differential impact of sleep-disordered breathing during NREM and REM sleep on clinical sequelae, such as daytime sleepiness, remains to be determined; therefore, sleep-state dependent indices, such as NREM-RDI and REM-RDI, may provide a better

characterization of disease severity than the typical use of an overall OSA summary measure of whole-night sleep[11].

The prevalence and impact of REM-predominant OSA in individuals older than 65 years is not well known. The average age of the subjects in most studies has been between 49-65 years of age, and individuals older than 65 have been excluded in most studies[3]. In some studies, REM related OSA was more common in the younger population, while in other studies such as the Sleep Heart Health Study (SHHS), subjects with more severe REM-predominant OSA were older[3]. While many OSA patients demonstrate increased respiratory distress during REM sleep, this phenomenon occurs more commonly in women; the entire clinical significance of REM-related sleep disordered breathing (SDB), however, is still unknown[4]. The long-term effects of REM sleep disruption in women may support reports of a more severe symptomatology at lower apnea-hypopnea index (AHI) levels when compared to men[2]. Some research has found that women with OSA have a greater proportion of adverse respiratory events during REM sleep than men; women also, in some findings, have had a higher prevalence of OSA occurring almost exclusively during REM sleep than men[8]. In one study, during REM sleep, apnea severity was similar in women and men, but during NREM sleep, apnea was less severe in women than in men[8]. In another study, women's AHI was higher in REM than NREM, with AHI higher in REMs than NREMs in both tested sleeping positions[9]. One study found that not only was the prevalence of REM-related OSA higher among women, but that the female patients with REM-related OSA were younger and less obese than those with NREM-related OSA[15, 16]. In a study conducted by O'Connor et al., OSA was found to be milder in women than in men, but the significant respiratory events recorded in women were associated with REM sleep; therefore, it was concluded that REM-related OSA is more common in women[8].

With OSA treatment being focused on certain periods of sleep, key differences in symptoms, presentation, and outcomes of OSA in both REM and NREM sleep is an area that demands further exploration in order to improve the efficacy of treatment modalities. Although gender, weight, and age have often been cited as important variables that contribute to OSA, identifying what variables are of the greatest contribution to OSA in various sleep stages is a question to consider and investigate.

Most studies that have examined OSA during both REM and NREM have had relatively small sample sizes with women under-represented in the samples. This study will compare OSA, by gender, with SHHS data that has an adequate sample size, includes more women than men, and has a longitudinal design to determine the most significant factors contributing towards respiratory disturbance index (RDI) values in whole-night, REM, and NREM sleep. This study will also examine the importance and contribution of the factors determined to be significant in all sleep stages; this will allow us to examine if a hierarchy of importance and contribution of variables exists in various sleep stages, and we will examine if any existing hierarchy changes depending on whether we are viewing participants in whole-night, REM, or NREM sleep. We hypothesize that gender, weight (with body mass index, or BMI, acting as the main variable of interest followed by various body circumferences) and age will be significant variables in explaining RDI values for this sample. We also hypothesize that both BMI and age will act as partial mediators on gender in explaining RDI values for whole-night, REM, and NREM sleep. We further hypothesize that the calculated relative, and statistically significant, importance across all sleep stages of interest will have BMI as the most significant factor followed by gender and then age.

Methods

Sleep Heart Health Study Overview

The Sleep Heart Health Study (SHHS) is a prospective cohort study designed to assess the contribution of OSA in the development of cardiovascular disease. Previous publications have detailed the SHHS design, as well as its quality control procedures. 6,441 men and women enrolled between November 1995 and January 1998 for a baseline examination, which included an initial polysomnogram. The dataset from the first time point of measure (labeled SHHS1) represents data from the baseline and first follow-up visits performed between 1995 and 1998 with a total n of 5804[12, 13, 14]. The total n of 5,804 in SHHS1 is decreased from the originally enrolled 6,441 due to data integrity issues from some of the participants recruited from various Strong Heart Study sites[12, 13]. Participants in SHHS1 included 2,765 men and 3,039 women aged 40 to 90 years (men: Mean = 63.1 years, Standard Deviation = 11.0 years; women: Mean = 63.2 years, Standard Deviation = 11.4 years) [12, 13, 14]. In a follow-up clinic visit, 4,080

surviving men and women were studied for the SHHS2 time point (labeled SHHS2), and a second polysomnogram was obtained in 3,295 participants between January 2001 and June 2003[14]. The timespan from SHHS1 to SHHS2 was an average of 5-6 years for participants; an interim clinic visit or phone call occurred 2-3 years after the SHHS1 baseline visit[14]. Adjudicated heart health outcomes (i.e. heart attack) were tracked between baseline and through at least 2008, with some parent cohorts tracking until 2011; event-level details for the tracking of heart health outcomes (i.e. myocardial infarction requiring hospitalization) also occurred[14].

Study Population

At the SHHS1 time point, 5,804 subjects enrolled in SHHS and completed an overnight sleep study[12, 13]. The sample size for the current study was reduced approximately 55% due to missing RDI data in either whole-night, REM, or NREM sleep as well as missing BMI data throughout the study. The final sample for this analysis includes 1163 men and 1337 women with a mean age of 62. The majority of the participants were Caucasian, overweight or obese, and had never been diagnosed with OSA (see Table 1). Some calculations in SHHS used a 3% decrease in oxygen saturation, while others used a 4% decrease in oxygen saturation to define an episode of hypopnea and/or apnea[12, 13]. Definitions also varied in terms of whether they included arousals when calculating RDI. For this research, we have chosen to use a 4% decrease in oxygen saturation (i.e. a 4% desaturation) in combination with >5 episodes of apnea, hypopnea, or arousals. A positive correlation has been identified between RDI and cardiovascular comorbidities; research shows this correlation to be significant using a $\geq$4% oxygen desaturation criterion for RDI scoring, with a correlation being nonsignificant when using the $\geq$3% desaturation criterion with or without an arousal.

Table 1 SHHS Participant Age, BMI, Race/Ethnicity and Prior Diagnosis of OSA (n=2500)

	Men (n=1163)	Women (n=1337)
Mean age	62.4 ±10.1 years	62.2 ± 10.7 years

Race/ethnicity		
White	1028 (88.4%)	1160 (86.8%)
Black	66 (5.7%)	90 (6.7%)
Hispanic/Latino	48 (4.1%)	70 (5.2%)
Other	21 (1.8%)	17 (1.3%)
Mean BMI	28.4 ±4.0	28.0 ±5.5
Previous diagnosis of OSA		
No	1107 (95.2%)	1296 (96.9%)
Yes	23 (2.0%)	3 (0.2%)
Did not know	16 (1.4%)	23 (1.7%)
Unknown	15 (1.4%)	17 (1.2%)

Statistical Analysis

For this study, we used R 3.5.2 Eggshell Igloo (Vienna, Austria) in our statistical analysis. From the SHHS total number of participants. Cases with incomplete RDI value information were removed from analysis. Once BMI was found to be a variable of significance, cases with missing BMI information were also removed from analysis. This left a remaining sample of 2,500 participants to be used in this study. Due to the skewed results of the respiratory disturbance index (RDI) as an outcome variable (with several valued at 0.0, representing no measured RDI), use of this as an outcome variable comes with a recommendation for variable transformation provided by SHHS. Log-transformation of the RDI variable was performed using the recommended RDI log-transformation formula provided by SHHS that has been used in previous manuscripts, which is: [New Variable = log (Old Variable + 0.1)].

Multivariable linear regression analysis was used to test whether a variety of variables significantly predicted participants' RDI values. 3 models were produced as the product of examining results from whole-night, REM, and NREM sleep. The 3 models were validated with training and test data; all 3 produced similar test data results for Adjusted R-squared, F-test statistic, residual sum of

squares (RSS), mean squared error (MSE), and root mean squared error (RMSE) as the original models produced. 10 linear regression assumptions, including Durbin-Watson tests and analyzing multicollinearity, were then tested and met in the 3 models. The results in Tables 2-5 provide the independent variable being tested along with its corresponding unstandardized Beta/slope (B), standard error for the slope (SE B), standardized Beta, t-test statistic (t), and probability level (p) set at a 0.05 level of significance. The final model selected is provided in each table that includes the constant/intercept, R-squared, Adjusted R-squared, F-test statistic with degrees of freedom, standard error, sample size, and probability value set at a significance level of alpha=0.05. Causal mediation analysis was then performed on BMI towards the relationship between gender and RDI as BMI was a significant variable of interest in our models and this relationship was postulated in our hypothesis. Age is not a mediator variable for gender, and is considered instead to act as a potential confounder on the relationship between gender and RDI. Calculated relative importance percentages were produced and bootstrapping was performed to create an importance hierarchy visualization consisting of the significant variables of interest produced from the linear regression models.

Results

The results of multiple linear regression analysis of RDI on a variety of independent variables are shown in Table 2. Results for regression analysis of RDI on the same independent variables in REM sleep are shown in Table 3, and the results in NREM sleep are shown in Table 4. Variable lines that are bolded indicate variables of statistical significance ($p<.05$) in each of the tables.

Table 2 Multiple linear regression results for Whole-Night sleep

Independent Variable	B	SE B	β	t	p
Age	**0.03**	**<0.01**	**0.22**	**11.44**	**<0.001**
BMI	**0.11**	**<0.01**	**0.38**	**21.12**	**<0.001**
Neck-Height Ratio	**27.58**	**1.29**	**0.39**	**21.43**	**<0.001**

Gender	**-0.70**	**0.05**	**-0.24**	**-14.03**	**<0.001**
Change in Weight	<0.01	<0.01	0.05	1.72	0.09
Hip Circumference	<0.01	<0.01	-0.03	-0.95	0.34
Waist Circumference	<0.01	<0.01	0.05	1.67	0.09
Neck Circumference	**0.05**	**0.01**	**0.15**	**4.59**	**<0.001**
Total Cholesterol	**<0.01**	**<0.01**	**0.05**	**3.00**	**<0.01**
HDL	<0.01	<0.01	-0.01	-0.52	0.61
Race	-0.07	0.05	-0.03	-1.51	0.13
Ethnicity	<0.01	0.12	<0.01	0.05	0.96
Smoking Status	-0.03	0.03	-0.02	-1.26	0.21
Surgery for OSA Treatment	**0.73**	**0.33**	**0.05**	**2.19**	**0.03**
Surgery for Snoring	1.13	1.34	0.15	0.85	0.41
Height in cm	<0.01	<0.01	-0.32	-1.19	0.23
Sleep Efficiency	-0.01	<0.01	-0.06	-2.39	0.17
Sleep Latency	<0.01	<0.01	-0.02	0.89	0.37
Stroke	0.06	0.05	0.02	1.36	0.18
Stroke History	0.38	0.37	0.04	1.03	0.30
MI History	0.18	0.21	0.03	0.84	0.40
Antidepressant Use	-0.03	0.13	<0.01	-0.20	0.84
Beta Blocker Use	0.10	0.08	0.02	1.25	0.21
Beta Blocker with Diuretic Use	**-1.04**	**0.47**	**-0.04**	**-2.21**	**0.03**
Insulin Use	0.25	0.23	0.02	1.12	0.26
Benzodiazepine Use	-0.16	0.12	-0.02	-1.34	0.18
Hypertension Medication Use	0.05	0.06	0.02	0.92	0.36

Final Model:

Constant = -2.87

R^2 = 25.27%

Adj. R^2 = 25.18%

F(3, 2496) = 281.4

SEE =	1.25
n =	2500
p =	<0.001

Our hypothesis that gender, BMI, and age will be significant variables in explaining RDI values was supported for this sample during whole-night sleep. At a significance level of alpha=0.05, 8 predictors explained 25.18% of the variance (R^2=0.2527, Adj. R^2=0.2518, F (3, 2496) =281.4, p<.001). With p-values <.05, it was found that age significantly predicted RDI values as did BMI, neck-height ratio, gender, neck circumference, total cholesterol, surgery for OSA, and the use of beta blockers with diuretics. Neck-height ratio and neck circumference were highly correlated to BMI and gender and do not improve the final model F-test and Adjusted R squared; these variables were removed from the final model. Cholesterol, surgery for OSA, and beta blockers with diuretics did not improve the final model F-test and Adjusted R squared; these variables were removed from the final model.

Table 3 Multiple linear regression results for REM sleep

Independent Variable	B	SE B	β	t	p
Age	**0.02**	**<0.01**	**0.13**	**6.76**	**<0.001**
BMI	**0.14**	**<0.01**	**0.38**	**20.79**	**<0.001**
Neck-Height Ratio	**28.41**	**1.68**	**0.32**	**16.96**	**<0.001**
Gender	**-0.41**	**0.07**	**-0.11**	**-6.25**	**<0.001**
Change in Weight	<0.01	<0.01	0.01	1.23	0.22
Hip Circumference	-<0.01	<0.01	-0.01	-0.35	0.73
Waist Circumference	<0.01	<0.01	0.05	1.40	0.16
Neck Circumference	**0.04**	**0.01**	**0.09**	**2.77**	**<0.01**
Total Cholesterol	<0.01	<0.01	0.03	1.34	0.18

HDL	-<0.01	<0.01	-0.01	-0.39	0.70
Race	-0.09	0.06	-0.03	-1.37	0.17
Ethnicity	-0.06	0.16	0.01	0.40	0.69
Smoking Status	-0.01	0.04	-<0.01	-0.17	0.87
Surgery for OSA Treatment	0.73	0.42	0.04	1.73	0.08
Surgery for Snoring	-1.22	1.68	-0.13	-0.72	0.48
Height in cm	-<0.01	<0.01	-0.01	-0.40	0.69
Sleep Efficiency	-<0.01	<0.01	-0.03	-1.39	0.17
Sleep Latency	-<0.01	<0.01	-0.02	-0.80	0.42
Stroke	0.03	0.06	0.01	0.57	0.57
Stroke History	0.09	0.47	<0.01	0.20	0.84
MI History	0.37	0.26	0.06	1.40	0.16
Antidepressant Use	**-0.59**	**0.17**	**-0.06**	**-3.54**	**<0.001**
Beta Blocker Use	-<0.01	0.11	-<0.01	-0.04	0.97
Beta Blocker with Diuretic Use	-1.11	0.62	-0.03	-1.80	0.07
Insulin Use	-0.02	0.29	-<.01	-0.08	0.94
Benzodiazepine Use	-0.19	0.16	-0.02	-1.19	0.23
Hypertension Medication Use	0.03	0.07	<0.01	0.40	0.69

Final Model:

Constant =	-3.32
R^2 =	17.58%
Adj. R^2 =	17.48%
F(3, 2496) =	177.30
SEE =	1.63
n =	2500
p =	<0.001

Our hypothesis that gender, BMI, and age will be significant variables in explaining RDI values was supported for this sample during REM sleep. At a significance level of alpha=0.05, 6 predictors

explained 17.48% of the variance (R^2=0.1758, Adj. R^2=0.1748, F (3, 2496) =177.3, p<.001). With a p-value of <.05, it was found that age significantly predicted RDI values as did BMI, neck-height ratio, gender, neck circumference, and antidepressant use. As with whole-night sleep, both neck-height ratio and neck circumference were highly correlated to BMI and gender and both did not improve the final model F-test and Adjusted R squared; these variables were removed from the final model. Antidepressant use does not improve the final model F-test and Adjusted R squared; this variable was also removed from the final model.

Table 4 Multiple linear regression results for NREM sleep

Independent Variable	B	SE B	β	t	p
Age	**0.03**	**<0.01**	**0.22**	**11.38**	**<0.001**
BMI	**0.10**	**<0.01**	**0.31**	**16.61**	**<0.001**
Neck-Height Ratio	**29.48**	**1.48**	**0.36**	**19.89**	**<0.001**
Gender	**-0.98**	**0.06**	**-0.30**	**-16.92**	**<0.001**
Change in weight	<0.01	<0.01	0.02	1.32	0.19
Hip Circumference	-0.01	<0.01	-0.06	-1.87	0.06
Waist Circumference	<0.01	<0.01	0.01	0.44	0.66
Neck Circumference	**0.06**	**0.01**	**0.16**	**4.86**	**<0.001**
Total Cholesterol	**<0.01**	**<0.01**	**0.06**	**3.52**	**<0.001**
HDL	<0.01	<0.01	<0.01	0.06	0.95
Race	-0.05	0.06	-0.02	-0.94	0.35
Ethnicity	-0.05	0.14	-0.01	-0.35	0.73
Smoking Status	-0.06	0.03	-0.04	-1.81	0.07
Surgery for OSA Treatment	**0.79**	**0.39**	**0.05**	**2.04**	**0.04**
Surgery for Snoring	1.75	1.64	0.19	1.07	0.29
Height in cm	-0.01	<0.01	-0.04	-1.41	0.16
Sleep Efficiency	-0.01	<0.01	-0.04	-1.59	0.11

Sleep Latency	-0.01	<0.01	-0.03	-1.27	0.20
Stroke	0.09	0.05	0.03	1.71	0.09
Stroke History	0.25	0.41	0.02	0.62	0.54
MI History	0.09	0.23	0.02	0.40	0.69
Antidepressant Use	0.21	0.15	0.03	1.46	0.14
Beta Blocker Use	0.15	0.09	0.04	1.57	0.12
Beta Blocker with Diuretic Use	-0.96	0.54	-0.03	-1.77	0.08
Insulin Use	0.49	0.26	0.03	1.90	0.06
Benzodiazepine Use	-0.14	0.14	-0.02	-1.00	0.32
Hypertension Medication Use	0.06	0.06	0.02	0.89	0.38

Final Model:

Constant =	-2.87
R^2 =	23.20%
Adj. R^2 =	23.11%
F(3, 2496) =	251.3
SEE =	1.44
n =	2500
p =	<0.001

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Our hypothesis that gender, BMI, and age will be significant variables in explaining RDI values was supported for this sample during NREM sleep. 7 predictors explained 23.11% of the variance (R^2=0.2320, Adj. R^2=0.2311, F (3, 2496) =251.3, p<.001). With a p-value <.05, it was found that age significantly predicted RDI values as did BMI, neck-height ratio, gender, neck circumference, total cholesterol, and surgery for OSA treatment. Neck-height ratio and neck circumference are both highly correlated to BMI and gender, and both variables do not improve the final model F-test and Adjusted R squared; these variables were removed from the final model. Total cholesterol and surgery for OSA treatment also did not improve the final model F-test and Adjusted R squared; these variables were removed from the final model.

Table 5 Multiple linear regression results for REM sleep (modified with REM > 15 minutes)

Independent Variable	B	SE B	β	t	p
Age	0.02	<0.01	0.13	6.78	<0.001
BMI	0.14	<0.01	0.39	21.03	<0.001
Neck-Height Ratio	28.31	1.67	0.32	16.93	<0.001
Gender	-0.40	0.07	-0.11	-6.15	<0.001
Change in Weight	<0.01	<0.01	0.01	1.22	0.22
Hip Circumference	-<0.01	<0.01	-0.02	-0.55	0.58
Waist Circumference	<0.01	<0.01	0.06	1.72	0.09
Neck Circumference	0.05	0.01	0.10	3.02	<0.01
Total Cholesterol	<0.01	<0.01	0.02	1.21	0.23
HDL	-<0.01	<0.01	-0.01	-0.57	0.57
Race	-0.09	0.06	-0.03	-1.44	0.15
Ethnicity	-0.06	0.16	0.01	0.35	0.72
Smoking Status	<0.01	0.03	<0.01	0.02	0.98
Surgery for OSA Treatment	0.74	0.42	0.04	1.75	0.08
Surgery for Snoring	-1.22	1.68	-0.13	-0.72	0.48
Height in cm	-<0.01	<0.01	<-0.01	-0.15	0.88
Sleep Efficiency	-<0.01	<0.01	-0.04	-1.68	0.09
Sleep Latency	-<0.01	<0.01	-0.02	-0.86	0.39
Stroke	0.03	0.06	0.01	0.57	0.57
Stroke History	0.09	0.47	<0.01	0.20	0.84
MI History	0.37	0.26	0.06	1.40	0.16
Antidepressant Use	-0.62	0.17	-0.07	-3.75	<0.001
Beta Blocker Use	-<0.01	0.11	-<0.01	-0.04	0.97
Beta Blocker with Diuretic Use	-1.09	0.66	-0.03	-1.65	0.10
Insulin Use	0.01	0.30	<0.01	0.04	0.97
Benzodiazepine Use	-0.20	0.16	-0.02	-1.23	0.22

Hypertension Medication Use	0.02	0.07	<0.01	0.30	0.77

Final Model:

Constant =	-3.37
R^2 =	17.94%
Adj. R^2 =	17.84%
F(3, 2479) =	180.6
SEE =	1.61
n =	2483
p =	<0.001

An additional model was run that analyzed subjects who had at least 15 minutes of REM sleep recorded in SHHS. One point of concern we had was that any results in REM sleep may be skewed if a fair number of subjects had very low amounts of REM sleep in minutes. The concern is that RDI is calculated as a ratio with the number of negative episodes as a numerator and the number of sleep minutes as a denominator. If REM sleep had an extremely small number of sleep minutes (thus, producing an extremely small denominator), any resulting ratios in REM sleep could provide very large results that are not necessarily accurate in any standardized comparison. Table 5 shows the regression results from a model run using only subjects with over 15 minutes of REM sleep. The results were, however, the same as Table 3 with REM sleep for all subjects because there were only 17 out of 2,500 subjects that had <15 minutes REM sleep. The vast majority of subjects, in fact, had well over 20 minutes of documented REM sleep. Table 5 has a slight uptick in Adjusted R-squared and F-test statistic than Table 3, but the difference was not significant for us to use only those with REM > 15 minutes in any further analyses.

Table 6 Causal mediation analysis results [Treatment: Gender; Mediator: BMI]

Mediator Variable	ACME Estimate	95% CI Lower	95% CI Upper	P-value [Prob]
Whole-Night BMI	-0.04	-0.08	0.00	0.05

REM BMI	-0.04	-0.08	0.00	0.05
NREM BMI	-0.04	-0.07	0.00	0.04

Our hypothesis that BMI will act as a partial mediator on gender's relationship to RDI was not supported for whole-night, REM, or NREM sleep. Gender, age, and BMI were the three consistent variables of independent significance in each of the preceding linear regression models. Before examining the relative and statistical importance of these variables towards RDI values, we performed a causal mediation analysis based on our hypothesis that both BMI and age were variables that helped to explain the underlying mechanism of the relationship between gender and RDI values. Age is not a mediator variable on gender, so only BMI was used in this analysis; age acts as a potential confounder on the relationship between gender and RDI. In whole-night sleep, at a significance level of alpha=0.05, BMI is at the threshold of being a partial mediator to partially explain the underlying mechanism of the relationship between gender and RDI (p=0.05). The same result is seen in REM sleep (p=0.05) with a slight increase of explanation in NREM sleep (p=0.04).

Table 7 Relative Variable Importance in 3 sleep stages

Whole-night sleep

Calculated Relative Importance:

BMI	53.64%
Gender	24.95%
Age	21.41%

REM sleep

Calculated Relative Importance:

BMI	79.76%
Age	11.82%
Gender	8.42%

NREM sleep

Calculated Relative Importance:

Gender	39.77%
BMI	37.61%

Age	22.62%

Our hypothesis that the calculated relative, and statistically significant, importance of gender, BMI, and age will list BMI as the variable of greatest significance followed by gender and then age in whole-night, REM, and NREM sleep was not supported. Relative importance was calculated for age, BMI, and gender in the models for whole-night, REM, and NREM sleep; bootstrapping was also performed to establish statistically significant differences in importance between age, BMI, and gender (see Table 7). In whole-night sleep, looking at how these variables contribute to RDI, BMI is significantly more important than both gender and age. In REM sleep, the results are the same as whole-night with BMI being significantly more important than both gender and age. In NREM sleep, both gender and BMI are significantly more important than age; however, there is no statistically significant difference between gender and BMI in importance from both a calculated relative importance standpoint and from a bootstrapping standpoint.

Discussion

Age, gender, and BMI were explanatory variables for RDI values in whole-night, REM, and NREM sleep. Concerning weight, BMI and a neck circumference measure (either neck circumference in cm or neck circumference represented as a neck-height ratio) were significant in all analyses; we eliminated all neck circumference measures from the final models as these variables had a significant correlation to other significant independent variables (e.g. gender and BMI) which could confound regression results. Once significant explanatory variables were discovered in the analyses, we were interested in their relative importance to the outcome variable of RDI values. By analyzing this, we could determine if a similar order in relative and significant variable importance existed among sleep as a whole, as well as sleep in separate REM and NREM states. BMI was the explanatory variable of the highest relative and statistically significant importance for whole-night and REM sleep. Gender and age were of similar relative importance, and were of the same statistical significance when bootstrapping analysis was performed. In NREM sleep, gender was the explanatory variable of the highest relative

importance followed very closely by BMI; gender was equal to BMI in terms of the variable(s) of the most statistically significant importance.

Although other variables of significance were dismissed in our study's models due to adding no benefit to the final model's fit, it is worth mentioning that a couple of variables may be of interest in future studies. One finding of particular interest is that antidepressant use was a significant variable in REM sleep for decreasing RDI values with a negative slope value in this community-based study with higher numbers of women than men. Further studies may benefit from examining this potentially interesting drug effect on REM sleep. This study has limitations that should be considered for any future work and research in this area. Missing data lowered the applicable sample size from >5000 to approximately 2500. When comparing outcomes of participants that may have REM-related OSA versus NREM-related OSA, common comorbidities they may share (such as cardiovascular disease and hypertension) should be taken into consideration. Various anthropomorphic measures have been previously cited as more accurate weight measures for research considerations than BMI, however, in our study, the correlations of any anthropomorphic measure shared between the SHHS1 and SHHS2 time points (e.g. neck circumference) had significant correlation between other significant variables that prohibited use. Whether participants received treatment or not at SHHS2 or in the interim between SHHS1 and SHHS2 is largely unknown.

In this study, we found that age, gender, and BMI are the most significant variables to explain RDI values. This coincides with previous research that indicated those same variables are significant explanatory variables to OSA. This study also examined this finding among different sleep stages that included not only whole-night sleep, but also separated sleep into REM and NREM stages for analysis. Similar findings were found across all sleep stages with the same three explanatory variables. This study also examined the relative importance of any explanatory variables that were found to be significant across multiple sleep stages to discover patterns of importance. BMI was the variable of most importance in whole-night and REM sleep; in NREM sleep, BMI was equal to gender as the variable of most

importance. For whole-night and REM sleep, gender and age had no statistically significant difference between each other, and both were significantly behind BMI in terms of importance.

These findings suggest that, overall, BMI (a measure of weight) is the driving variable behind the RDI value results in almost all sleep stages. The findings highlight the importance of this one variable, and it is further noteworthy that this variable is the one of most significance that is largely managed by patient behavior. BMI was the driving variable in the majority of sleep stages for RDI values; thus, BMI is the variable of most importance in those who improve upon, as well as increase, their OSA levels. Previous research has recognized the effects of age, gender, and weight on OSA levels as well as its disease progression. This study examines the effects of those variables across multiple sleep stages and our findings show that BMI appears to be the most significant variable in terms of RDI values. The results of this study indicate that age, gender, and BMI are still significant explanatory variables towards OSA levels in this large, community-based sample that contains adequate numbers of women. Our findings also suggest that, overall, BMI is the most influential variable on RDI either increasing over time when OSA progresses or decreasing over time when OSA improves. Future research and clinical practice may benefit from these findings that not only highlight the importance of age, gender, and BMI on OSA levels in a large community sample, but demonstrate the importance of BMI as a largely modifiable variable that has a significant influence on OSA's progression or improvement over time.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

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CHAPTER 5: Dissertation Summary

Section 1: Dissertation Overview

The purpose of this dissertation was to investigate the effects and importance of gender, body weight, and age on obstructive sleep apnea (OSA) levels in whole-night, REM, and NREM sleep. The aims of the study were to: (1) Examine if there are significant differences in respiratory disturbance index (RDI) values between men and women in whole-night, REM, and NREM sleep; (2) Compare BMI levels among varying OSA groups for both men and women; and (3) Identify the most significant factors contributing to RDI values in whole-night, REM, and NREM sleep. An abundance of research has identified OSA as a significant risk factor for mortality. OSA, particularly at moderate and severe levels, is not only associated with a rate of 33% all-cause mortality, but is also an independent risk factor of many other co-morbidities ranging from stroke to diabetes to cardiovascular disease[2, 3]. Results of the Cleveland Family Study indicated that OSA incidence and severity was primarily determined by gender, weight, and age; these results have also been indicated in other research findings, and this conclusion spearheaded the direction of this study[1, 4]. Limitations of previous studies in this area include a lack of community-based samples, a lack of equal amounts of men and women in samples, and a lack of examining many aspects of OSA in certain sleep stages, such as REM and NREM sleep, versus examining OSA over the whole night. This study sought to explore what contribution one's gender, weight, and age had on RDI levels. This study also sought to explore this topic not only in whole-night sleep, but in the more specific REM and NREM stages of sleep, as well.

Section 2: Summary of Research Findings

Each of the three manuscripts (Chapters 2, 3, and 4) included in this dissertation adds a unique contribution to existing literature. Chapter 2 presents results from an exploration of whether gender differences contributed to significant differences in RDI values for whole-night, REM, and NREM sleep. Chapter 3 presents results from a comparison of BMI medians in varying OSA levels for both men and

women during whole-night sleep. Chapter 4 presents results from an examination of the contribution and importance of gender, age, and weight (specifically body mass index (BMI)) to RDI values in whole-night, REM, and NREM sleep. Chapters 2, 3, and 4 (Manuscripts 1, 2, and 3) are discussed in more detail below.

Chapter 2 (Manuscript 1)

OSA subtypes, such as REM and NREM OSA, have recently sparked much research; however, despite the findings of many studies, research is underexplored in community samples with equal amounts of men and women. The first manuscript (Chapter 2) presents findings from the analysis of Research Question 1, which sought to examine whether significant differences existed in RDI values between men and women during whole-night, REM, and NREM sleep. In Chapter 2, the Sleep Heart Health Study (SHHS) was used as the sample for data analysis. SHHS contains adequate numbers of women in its sample, is community-based, and collected longitudinal data[5].

In this study, we showed RDI was significantly higher in men for SHHS participants in whole-night, REM, and NREM sleep. Independent sample t-test analysis was used for Chapter 2. The results indicated that RDI values, which were log-transformed for analysis, were significantly higher in whole-night sleep for men (M=1.64, SD=1.33) than for women (M=0.86, SD=1.48), exceeding the alpha=0.05 significance level: t (2527) =13.97, $p<0.001$, d=0.55, 95% CI for Mean Differences [0.67, 0.89]. Results also showed that RDI values, again log-transformed, were also significantly higher in REM sleep for men (M=1.86, SD=1.68) than for women (M=1.36, SD=1.88), exceeding the alpha=0.05 significance level: t (2526) =7.12, $p<0.001$, d=0.28, 95% CI for Mean Differences [0.36, 0.64]. Results further showed that log-transformed RDI values were also significantly higher in NREM sleep for men (M=1.32, SD=1.52) than for women (M=0.27, SD=1.61), exceeding the alpha=0.05 significance level: t (2511) =16.81, $p<0.001$, d=0.67, 95% CI for Mean Differences [0.94, 1.23]. In contrast to our study, previous studies have found RDI values in REM sleep to be higher in women than men. No finding in this study indicated that women had higher RDI values at any sleep stage, whether or not the participant's RDI values

increased or decreased over time (e.g. whether or not the participant's OSA severity progressed or improved over time). The results of this study indicate that, overall, being male has a greater influence than being female regarding higher RDI values throughout an entire night of sleep; this finding remains consistent even when accounting for, and analyzing, sleep in the separate REM and NREM stages.

Chapter 3 (Manuscript 2)

Despite the existing research on OSA and weight, research is underdeveloped on examining community-based samples, with equal amounts of men and women at varying clinical OSA levels, to determine if weight has a significant influence on the progression or improvement of OSA levels over time. The second manuscript (Chapter 3) presents findings from the analysis of Research Question 2, which sought to discover whether BMI, for both men and women, had significant differences between varying OSA levels. This study compared participants at varying OSA levels with the Sleep Heart Health Study (SHHS) that has an adequate sample size, includes more women than men, and has a longitudinal design[5]. Nonparametric data analysis was used for Chapter 3, due to unequal group variances in whole-night, REM, and NREM sleep. A Kruskall-Wallis test examined differences between groups in BMI medians; post-hoc tests were then performed to further highlight positive comparisons between groups, and results were filtered with the Holm p-value adjustment method.

In this study, for both men and women, the results found significant differences in BMI medians between some of the groups that represented OSA progression and/or improvement in the study participants. This finding indicates that, for some of the groups within both subsamples, BMI is influential in placing participants in a particular OSA level that is identified by an RDI value increase, or decrease, over time. When examining pairwise comparison post-hoc analyses, in both men and women, those who stayed at a normal OSA level during both the SHHS1 and SHHS2 time points have a statistically different BMI from all 9 other groups that represent an increase in RDI value and subsequent OSA level. This finding suggests that those who stayed at a normal OSA level at both SHHS1 and SHHS2 have a significant difference in BMI than those at any other OSA group which falls into mild,

moderate, and/or severe OSA levels at SHHS1 and SHHS2. This means that BMI is a significant variable, or factor, in keeping those who started out at normal OSA levels at a normal OSA level over time. While no additional positive pairwise comparisons existed for women, there were some additional findings for men. In men, this study found that BMI is significantly different in those who ended at a normal or mild OSA level during SHHS2 versus those who ended at a moderate or severe OSA level during SHHS2. For men whose OSA stayed at a mild level during both SHHS1 and SHHS2, there was a significant difference from those whose OSA levels stayed at a severe level during both SHHS1 and SHHS2; this suggests that BMI is significantly different in those men who maintained a mild OSA level over time than in those who maintained a moderate or severe OSA level over time. The results of this study indicate that, for both men and women, BMI is significantly different in those who do not progress, over time, from normal OSA levels (e.g. no OSA) to mild, moderate or severe OSA levels compared to those who do progress to either a mild, moderate, or severe OSA level. This finding implies that, in both men and women, BMI is statistically different in those who never advance to an OSA level of mild, moderate, or severe compared to those who do advance over time to mild, moderate, or severe OSA. For men, BMI also appears to be significantly different for who those who either maintain normal OSA levels over time or do not progress beyond mild OSA levels over time when compared to those who began SHHS at moderate or severe OSA levels and maintained those same levels over time.

Chapter 4 (Manuscript 3)

Although gender, weight, and age have often been cited as important variables that contribute to OSA, identifying what variables are of greatest contribution to OSA in various sleep stages is an important question to consider and investigate. Most studies that have examined OSA during both REM and NREM have had relatively small sample sizes with women under-represented in the samples. The third manuscript (Chapter 4) presents findings from the analysis of Research Question 3, which sought to discover the most significant factors contributing to RDI in whole-night, REM, and NREM sleep; additionally, we were interested in the hierarchy of importance and statistical significance of contribution

for any significant factors that were found. In Chapter 4, OSA was compared by gender with the Sleep Heart Health Study (SHHS) that has an adequate sample size, includes more women than men, and has a longitudinal design to determine the most significant factors contributing towards RDI values in whole-night, REM, and NREM sleep[5]. Multiple linear regression was used to produce models of prediction for whole-night, REM, and NREM sleep with RDI as the outcome variable of interest; several independent variables, used due to positive correlations with OSA in previous research, were analyzed for significance. The results indicated that age, gender, and BMI were the significant explanatory variables for RDI in whole-night, REM, and NREM sleep. Causal mediation analysis was then performed on the significant factor of BMI that was found to address the hypothesis posited by the conceptual model of the dissertation. The results indicated that BMI was at the $p=0.05$ threshold of being a partial mediator to partially explain the underlying mechanism of the relationship between gender and RDI in whole-night, REM, and NREM sleep. Age is not considered to be a mediator between gender and RDI for either whole-night, REM, or NREM sleep; it is instead identified as a potential confounder. This study also used SHHS as the sample for data analysis to examine the calculated relative, and bootstrapped, importance and contributions of the factors determined from the final linear regression models to be significant variables in whole-night, REM, and NREM sleep contributing towards RDI.

The results indicated that, for whole-night sleep, BMI is significantly more important than both gender and age in their contribution to RDI; REM sleep resulted in similar findings. For NREM sleep, both gender and BMI were of equal importance to RDI, and both variables were significantly more important than age. We showed that, with a significance level of 0.05, the final regression model for whole-night sleep RDI had the significant predictor variables of age ($p<.001$), BMI ($p<.001$), and gender ($p<.001$). Additionally, the final regression model for REM RDI had the same predictor variables of age ($p<.001$), BMI ($p<.001$), and gender ($p<.001$), as did the final regression model for NREM RDI with age ($p<.001$), BMI ($p<.001$), and gender ($p<.001$). In these three sleep stages, additional variables were significant (e.g. neck-height ratio, total cholesterol) but were not included due to either multicollinearity

or a lack of contribution to model F-statistic and Adjusted R-squared. An additional model was constructed for REM sleep that only included participants with >15 minutes of REM sleep recorded. The reasoning for this was that, for REM RDI, there could be spurious results if the RDI being calculated consists of a large numerator (indicating number of adverse events in sleep) divided into a very small numerator (indicating REM sleep in minutes). This additional REM model, however, produced the same results as the previous REM sleep model; only 17 out of 2500 cases had REM sleep <15 minutes, producing an n of 2483 in this model compared to the n of 2500 used in the previous REM RDI model.

In this study, we showed that, in whole-night sleep at a significance level of alpha=0.05, BMI is a partial mediator to partially explain the underlying mechanism of the relationship between gender and RDI (ACME: -0.04, p=0.05). The same result, at a significance level of alpha=0.05, is seen in REM sleep (ACME: -0.04, p=0.05); additionally, similar results appear for NREM sleep (ACME: -0.04, p=0.04). In whole-night sleep, when we examined how the variables of age, gender, and BMI contributed to RDI using both calculated relative importance and bootstrapping methods, we showed that BMI is significantly more important than both gender and age when viewing their contributions as a percentage [BMI: 53.6%, Gender: 25.0%, Age: 21.4%]. In REM sleep, the results are the same as whole-night with BMI being significantly more important than both gender and age [BMI: 79.8%, Age: 11.8%, Gender: 8.4%]. In NREM sleep, both gender and BMI are significantly more important than age; however, there is no significant difference between the contribution of gender and BMI [Gender: 39.8%, BMI: 37.6%, Age: 22.6%]. The results of this study indicate that age, gender, and BMI are significant factors contributing to RDI. While this has been reported in previous research, this study also confirmed this finding in REM and NREM sleep. Additionally, the mediation effects of BMI were measured, and the statistical importance of age, gender, and BMI were calculated for whole-night, REM, and NREM sleep. We found that BMI was, at least, a partial mediator on how gender contributes to RDI values. Additionally, this study indicates that when examining the significant importance of age, BMI, and gender on RDI, BMI is the most important variable in whole-night and REM sleep over both gender and age. In NREM sleep, gender and BMI are

more important variables than age, but gender and BMI have no significant difference between them in terms of importance to RDI in NREM sleep.

Section 3: Discussion

Collectively, these dissertation study findings suggest that weight, as measured in this study as BMI, is a variable of particular significance on RDI levels. In these findings, BMI acted as a significant mediator on how gender contributes to RDI. BMI was a significant predictor for RDI in whole-night, REM, and NREM sleep. BMI was the most important predictor variable for RDI in whole-night and REM sleep, and it tied with gender as the most important predictor variable for RDI in NREM sleep. In men and women, BMI is significantly different in those who do not progress, over time, from normal/no OSA to mild, moderate, or severe OSA levels when compared to those who do progress to a mild, moderate, or severe OSA level over time. Additionally, in men, BMI is significantly different for those who maintained normal OSA levels or only progressed to mild OSA levels over time when compared to those who maintained moderate or severe OSA levels over time. Being male also appears to have a greater influence on RDI in whole-night, REM, and NREM sleep; this finding differs, in terms from RDI, from some findings in the literature suggesting that women have worse outcomes during REM sleep.

The majority of previous studies in OSA research have been conducted in primarily clinical populations with subjects that are already seeking treatment at sleep clinics for conditions such as OSA or narcolepsy. It is possible that the use of more community-based samples, such as SHHS that was used in this study, in OSA research can expand the applicability and relevance of research findings. To better understand the process, future studies may benefit from exploring such samples, in addition to obtaining samples for analysis that contains equal amounts of both men and women. The finding that gender and age appear to trail behind BMI in predicting RDI does not mean that these variables are of no importance; rather, this study's findings reveal that the most important variable is also the most modifiable one. There are many factors that influence RDI, and other measures of OSA severity, which may warrant further

exploration in future research to determine the entirety of how this complex system functions and can be better understood for clinical solutions.

Section 4: Strengths and Limitations

Strengths

This study examined RDI in whole-night, REM, and NREM which has been understudied in the context of community-based populations with samples containing equal amounts of men and women. RDI was also used as the OSA outcomes variable as opposed to AHI, which is the most frequently used; using a relatively unique outcome variable expanded findings beyond what is exclusively applicable to AHI as RDI includes the AHI measures of apneas and hypopneas, as well as respiratory event related arousals (RERAs). This study used SHHS which contained an ethnically diverse, community-based sample with a higher percentage of women in its sample than men. Additionally, this study explored the effects and importance of age, BMI, and gender on RDI as not only a numerical value of interest, but also as a categorical value of clinical interest by analyzing RDI in groups corresponding to OSA levels over time. The findings from this study provide contributions to the literature in the following areas:

Chapter 2 (Manuscript 1)

Identifying how gender differences contribute to RDI in whole-night, REM, and NREM sleep

Chapter 3 (Manuscript 2)

Identifying whether, for both men and women, BMI significantly differs depending on OSA level progression or improvement over time

Chapter 4 (Manuscript 3)

Identifying the significant variables that predict RDI in whole-night, REM, and NREM, as well as identifying the statistical importance of each variable in hierarchy format for each sleep stage

Limitations

There were limitations to note in this dissertation study. First, in using a secondary dataset for analysis, there are inherent issues with missing data and acknowledging that analyses can only cover what is measured in the dataset. As an example of this limitation, the effects of menopause were a topic that the team was interested in exploring. SHHS, however, did not have menopause as a variable of measurement, so exploring this option was not an option. While SHHS started with >5800 subjects at SHHS1, to perform analysis on RDI required completed RDI data in both SHHS1 and SHHS2 to look at change in RDI over time (a measure of OSA progression). Missing RDI data at time points reduced the dataset to less than half of the SHHS1 subject number. While approximately 2500 subjects is a large sample size for analysis with adequate power on the almost 2000 measured variables, results from a larger portion of SHHS would have been, perhaps, even more enlightening. OSA is also a highly complicated disease process with many influential factors that can vary from person to person. In this study, RDI was used as the outcome variable of interest with a selected set of independent variables tested for predictive significance. It could have been of further benefit to examine additional outcome variables along with more independent variables of interest to understand a broader scope of the disease process in this sample. Additionally, while treatment was suggested to many of the study participants, whether or not those participants partook of any treatment options (such as losing weight or using CPAP) is largely answered in SHHS as "unknown", with over 95% of respondents having this as an answer for most treatment options.

Section 5: Future Steps

Implications for Future Research

This study found significant associations between BMI and RDI. This study also found that men, in this sample, experienced increased RDI in whole-night, as well as in REM and NREM, sleep. This work suggests that more research into other aspects of weight's effects on RDI may be important to investigate in future studies. Weight was primarily measured in this project as BMI; this is a primarily modifiable variable, and it can be a focus in future research and clinical practice for patients to monitor

and control while at normal/no OSA levels before advancing to more serious OSA levels where BMI median differences, in this study sample, were not found. More studies are needed to confirm how BMI appears to override many other variables in influencing RDI, as the activities of this system and the disease process of OSA are complex. Measuring only a handful of variables may be insufficient to rule out (or in) other variables analyzed in this study's regression models as contributors to adverse outcomes. The next steps to move research forward in this topic are outlined in Figure 1 below:

Figure 1 Next Steps

1: Consider a more comprehensive evaluation of the variables that can impact RDI values throughout the sleep cycle

2: Explore the relationships between age, gender, and BMI with different outcome variables of OSA progression

3: Investigate the role of various weight measures on RDI for those <40, particularly in children/young adults as this is a population where OSA incidence is on the rise

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