INFLAMMATION, EXCESSIVE DAYTIME SLEEPINESS, AND FATIGUE
IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

A Dissertation in
Nursing
by
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ABSTRACT

Background/Significance: OSA in adults contributes to poor health outcomes such as cardiovascular diseases and increased risk of morbidity and mortality. Evidence suggests that inflammation is the causal mechanism of the adverse cardiovascular events in OSA as well as a biological factor that may cause everyday symptoms in adults with OSA. There is a paucity of research that identifies the relationship between inflammatory biomarkers and OSA symptoms; there is also relatively known about the relationship between diurnal variation of inflammatory biomarkers and OSA symptoms such as sleepiness and fatigue. Even though OSA symptoms are prevalent in adults with OSA, not all patients with OSA express these symptoms. Therefore, studies exploring molecular signatures related to OSA symptom expression are needed.

Study Purpose: The primary aim of the study was to identify the relationship between inflammatory biomarkers, i.e., cytokines, and everyday symptoms in adults with OSA. Two secondary aims were also addressed; (1) to identify if there is a relationship between diurnal variation of inflammatory biomarkers and everyday symptoms in OSA, and (2) to explore symptom phenotype in OSA.

Theoretical Framework: An adapted conceptual model that combines a physiological model of inflammation in OSA and the Theory of Unpleasant Symptoms was developed to support this study. The theory addresses the diverse factors affecting multiple symptoms and the association between the factors and multiple symptoms in adults with OSA.

Methods: A cross-sectional cohort study of adults with OSA (n=22) employed convenience sampling with strict inclusion/exclusion criteria for adults undergoing diagnostic polysomnography was conducted. Symptom questionnaires and blood samples were measured pre-sleep bout (8pm; Time 1) and post-sleep bout (6am; Time 2); objective sleep characteristics
were measured by polysomnography. Cytokine plasma concentrations including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10(IL-10), and tumor necrosis factor-α (TNF-α) were measured by using a Luminex Human MMP 5-Plex Panel; C-reactive protein (CRP) was measured by using ELISA. Symptom measures included Stanford Sleepiness Scale (higher mean score=sleepier), Lee Fatigue and Energy Scale (higher fatigue score=more fatigue; lower energy score=less energy). Diurnal variation was calculated as (Time 2 – Time 1). Descriptive statistics, Mann-Whitney test, and Spearman's partial rank-order correlation adjusting for the confounding effects of age, body mass index (BMI), cardiovascular disease, and type 2 diabetes mellitus were used. Cluster analysis for symptom phenotype exploration, used cosine similarity to measure the distances between symptom vectors and clusters were visualized with network graphs by using yED Graph Editor.

**Results:** The sample (n=22) was middle aged (49 ± 17.12), overweight or obese, men and women. Most of the sample was non-Hispanic white (86.4%) and had mild OSA (68.2%). There was a significant difference between evening TNF-α and morning TNF-α, with a higher level at post-sleep bout (p=0.0012). There were no significant relationships between each inflammatory biomarker and AHI or between diurnal variation of each inflammatory biomarker and AHI; however, there were significant relationships between diurnal variation of IL-6 and oxygen desaturation indices and between morning IL-6 and oxygen desaturation indices. Evening IL-6 was negatively correlated with evening energy level (r=−0.685, p=0.001); whereas, morning fatigue was positively correlated to both morning IL-8 and morning TNF-α (r=0.582, p=0.021; r=0.453, p=0.05, respectively). Only IL-10 diurnal variation had a significant relationship with evening energy level. After removing outliers, the diurnal variation of IL-8 had a significant association with both morning fatigue and diurnal variation of energy level (r=0.548, p=0.03; r=−
0.537, \( p=0.03 \), respectively). Two symptom clusters, momentary symptom cluster and lasting symptom cluster, were identified in adults with mild OSA.

**Conclusions & Implications:** Pre-sleep bout IL-6 was significantly associated with symptom expression in OSA; post-sleep bout IL-8 and IL-8 diurnal variation were correlated with morning symptoms. These findings may provide mechanistic insights for symptom management in OSA. The study findings highlight the importance of continued research for identification of the causal factor(s) of symptom expression in OSA.

**Keywords:** Obstructive Sleep Apnea, Cytokine, Inflammatory Biomarker, Molecular Signature, Symptom, Sleepiness, Fatigue, Energy, Symptom Phenotype, Momentary Symptom, Lasting Symptom
# TABLE OF CONTENTS

List of Tables ......................................................................................................................... xi
List of Figures ........................................................................................................................... xiii
Acknowledgements .................................................................................................................. xiv
Dedication ................................................................................................................................. xv

Chapter 1 Introduction ............................................................................................................. 1
  Statement of the Problem .......................................................................................................... 3
    Diurnal Variation of Inflammatory Biomarkers as a Response to Hypoxia and Sleep
    Fragmentation ....................................................................................................................... 4
    Circadian Rhythm of Cortisol in OSA and Healthy Adults ................................................... 5
    Circadian Rhythms of Inflammatory Biomarkers in OSA ..................................................... 6
    A Molecular Signature for OSA ............................................................................................ 10
  The Relationship Between OSA and the Level of Inflammatory Biomarkers ...................... 11
  The Relationship Between Inflammatory Biomarker Diurnal Variation and Everyday
  Symptoms in OSA ................................................................................................................... 13
  Summary: Statement of the Problem ...................................................................................... 14

Purpose of the Study ................................................................................................................. 15
  Conceptual Framework .......................................................................................................... 16
  Definitions of Key Terms ....................................................................................................... 17
  Assumptions ............................................................................................................................ 21
  Significance of the Study ....................................................................................................... 22
  Chapter 1 Summary ............................................................................................................... 22

Chapter 2 Review of the Literature .......................................................................................... 24
  Obstructive Sleep Apnea in Adults .......................................................................................... 25
  Definition of Obstructive Sleep Apnea .................................................................................... 26
  The Epidemiology of OSA ....................................................................................................... 28
Pathophysiology of OSA ................................................................. 29
Sleep, OSA, and Inflammation ..................................................... 30
  The Classification of Inflammatory Cytokines .............................. 32
  C-Reactive Protein (CRP) in OSA .................................................. 32
  Tumor Necrosis Factor-α (TNF-α) in OSA .................................... 34
  Interleukin-1β (IL-1β) in OSA ..................................................... 35
  Interleukin-6 (IL-6) in OSA ......................................................... 36
  Interleukin-8 (IL-8) in OSA ......................................................... 38
  Interleukin-10 (IL-10) in OSA ...................................................... 39
Potential Confounding Factors ..................................................... 40
  Age ............................................................................................. 40
  Obesity ...................................................................................... 40
  Cardiovascular Disease ............................................................. 42
  Type 2 Diabetes ......................................................................... 43
Symptoms of OSA ....................................................................... 43
  Excessive Daytime Sleepiness and Fatigue .................................... 44
Symptom Science ....................................................................... 46
  Relevance of Symptom Management in OSA to Nursing Science .................................................................................. 48
Conceptual Framework for the Study ......................................... 49
  Major Concept of Theory of Unpleasant Symptoms ......................... 51
    Symptoms ............................................................................... 51
    Influencing Factor ................................................................... 53
    Performance Outcomes ............................................................ 54
Relationships among Concepts ................................................... 55
  Hypothesized Models for Understanding the Relationship between Inflammation, Excessive Daytime Sleepiness, and Fatigue in OSA ................................................................. 57
Chapter 2 Summary ................................................................... 59
Chapter 3 Methods ................................................................. 61
  Design of the Study ........................................................................... 61
  Sample and Setting ................................................................. 62
    Study Population ........................................................................ 62
    Sample Size Calculation .................................................... 62
    Inclusion and Exclusion Criteria ........................................ 63
    Recruitment Process .......................................................... 64
  Study Measures ........................................................................ 64
    Demographic Questionnaire ............................................ 64
    Berlin Questionnaire .......................................................... 65
    Epworth Sleepiness Scale (ESS) ........................................ 65
    Stanford Sleepiness Scale (SSS) .......................................... 66
    Lee’s Fatigue and Energy Scales ......................................... 66
    Profile of Mood States (POMS) 2nd Edition-Adult Short .... 67
    Quebec Sleep Questionnaire (QSQ) ..................................... 67
    Perceived Stress Scale (PSS) .................................................. 68
    Beck’s Depression Inventory (BDI) ...................................... 68
    Polysomnography (PSG) ....................................................... 69
    Inflammatory Biomarkers .................................................... 70
      Enzyme-Linked Immunosorbent Assay (ELISA) ............... 70
      Multiplex Assays ............................................................... 71
  Procedures ............................................................................. 73
  Data Collection and Management ........................................ 75
    Maintaining Confidentiality and Data Security .................. 75
      Storage of and Access to Data and/or Specimens .......... 76
      Transferring Data and/or Specimens ............................. 76
    Privacy ............................................................................... 77
    Protection of Human Subjects .......................................... 77
Consenting Process: Obtaining Informed Consent ........................................ 77
Consent Documentation .................................................................................. 77
Plan for Recording and Managing Any Adverse Events ................................. 78
Adverse Events ............................................................................................... 78
Data Analysis .................................................................................................. 78
Research Question 1 ....................................................................................... 79
Research Question 2 ....................................................................................... 79
Research Question 3 ....................................................................................... 80
Research Question 4 ....................................................................................... 80
Chapter 3 Summary ....................................................................................... 81

Chapter 4 Results .......................................................................................... 83
Sample Recruitment ......................................................................................... 83
Characteristics of Study Sample ..................................................................... 85
Description of Research Variables: Inflammatory Biomarkers, Symptoms, and
Polysomnography Variables .......................................................................... 87
Inflammatory Biomarkers .............................................................................. 87
Description of Inflammatory Biomarkers ...................................................... 87
Distribution of Inflammatory Biomarkers ..................................................... 88
Symptoms ....................................................................................................... 91
Description of Symptom Variables ............................................................... 91
Distribution of Evening and Morning Symptoms ......................................... 93
Sleep-Related Variables ............................................................................... 94
Description of Sleep-Related Variables ........................................................ 94
Distribution of Sleep-Related Variables ......................................................... 95
Research Question 1 ...................................................................................... 97
Research Question 1.1 ............................................................................... 97
Research Question 1.2 ............................................................................... 99
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research Question 2</td>
<td>106</td>
</tr>
<tr>
<td>Research Question 3</td>
<td>109</td>
</tr>
<tr>
<td>Research Question 4</td>
<td>111</td>
</tr>
<tr>
<td>Chapter 5 Discussion</td>
<td>115</td>
</tr>
<tr>
<td>References</td>
<td>142</td>
</tr>
<tr>
<td>Appendix A: Human Research Approval</td>
<td>186</td>
</tr>
<tr>
<td>Appendix B: Study Instrument</td>
<td>197</td>
</tr>
<tr>
<td>Appendix C: Copyright Permission Letter</td>
<td>214</td>
</tr>
</tbody>
</table>
List of Tables

Table 1.1. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: CRP ................................................................. 7
Table 1.2. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: TNF-α ................................................................. 8
Table 1.3. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-1β ................................................................. 8
Table 1.4. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-6 ................................................................. 9
Table 1.5. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-8 ................................................................. 9
Table 1.6. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-10 ................................................................. 10
Table 2.1. Abbreviation List ........................................................................ 25
Table 2.2. Severity Classification of OSA .................................................. 27
Table 3.1. Sample Size Estimation .............................................................. 63
Table 3.2. Measures: Purpose, Study Variables and Protocol Timing of Measurement ................................................................. 71
Table 4.1. Baseline Characteristics of the Study Sample ................................ 84
Table 4.2. Description of Inflammatory Biomarkers ..................................... 86
Table 4.3. Description of Symptom Variables ............................................. 89
Table 4.4. Description of Sleep-Related Variables in OSA ........................... 92
Table 4.5. Pre-Sleep and Post-Sleep Bout Inflammatory Biomarkers in OSA ................................................................................................. 97
Table 4.6. Partial Correlations Between Diurnal Variation of Inflammatory Biomarkers and AHI, AI, and HI Including Outliers ....................................................... 97
Table 4.7. Partial Correlations Between Diurnal Variation and Other Sleep Related Variables ............................ 98
Table 4.8. Partial Correlations Between Diurnal Variation and Other Sleep Related Variables After Excluding Outliers ........................................................................ 100
Table 4.9. Partial Correlations Between Morning Cytokines and Sleep-Related Variables After Excluding Outliers ........................................................................ 101
Table 4.10. Partial Correlations Between Morning Arousal and RERA Index and Inflammatory Biomarkers ................................................................. 102
Table 4.11. Partial Correlation Matrix: Sleep Fragmentation and Oxygen Desaturation ........ 103
Table 4.12. Relationship Between Evening Inflammatory Biomarkers and Momentary Symptom Including Outliers ................................................................. 105
Table 4.13. Relationship Between Morning Inflammatory Biomarkers and Momentary Symptom Including Outliers ................................................................. 105
Table 4.14. Relationship Between Evening Inflammatory Biomarkers and Momentary Symptom Excluding Outliers ................................................................. 106
Table 4.15. Relationship Between Morning Inflammatory Biomarkers and Momentary Symptom Excluding Outliers ................................................................. 107
Table 4.16. Relationship Between Diurnal Variation of Inflammatory Biomarkers and Symptoms Excluding Outliers ................................................................. 108
List of Figures

Figure 1.1. Circadian Rhythm of Cortisol in Healthy .................................................. 5
Figure 1.2. The Middle-Range Theory of Unpleasant Symptoms ................................. 17
Figure 2.1. Obstructive Apnea .................................................................................. 26
Figure 2.2. Obstructive Hypopnea ........................................................................... 27
Figure 2.3. Hypothesized Model .............................................................................. 60
Figure 3.1. Study Protocol ....................................................................................... 75
Figure 4.1. Enrollment and Evaluable Data Flowchart .............................................. 83
Figure 4.2. Distribution of the Evening and Morning Inflammatory Biomarkers in OSA .... 87
Figure 4.3. Distribution of Momentary Symptoms in OSA ........................................ 91
Figure 4.4. Distribution of Sleep-Related Variables ................................................... 93
Figure 4.5. Evening to Morning Inflammatory Biomarkers in OSA ............................ 96
Figure 4.6. Scatterplot Illustrating the Relationship Between Diurnal Variation of IL-10 and Evening Energy ............................................................... 108
Figure 4.7. Symptom Phenotype in OSA ................................................................. 111
Figure 5.1. An Adapted Model of Symptom Phenotypes in Mild OSA ....................... 133
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Dedication

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Chapter 1

Introduction

The increasing prevalence of obstructive sleep apnea (OSA), affecting an estimated 25 million adults in the U. S., is threatening public health (The Healthy Sleep Project, 2014). OSA is increasingly recognized as the most common cause of cardiovascular morbidity and mortality (Durán-Cantolla et al., 2010; Punjabi, Newman, Young, Resnick, & Sanders, 2008; Somers et al., 2008). If OSA is left untreated, it results in an increased risk of fatal cardiovascular events and sudden death during sleep (Gami, Howard, Olson, & Somers, 2005; Marin, Carrizo, Vicente, & Agusti, 2005). A recent longitudinal cohort study has also found that moderate to severe OSA defined by ≥15 of apnea-hypopnea index (AHI) is associated with an increased risk of all-cause mortality, including cancer (Marshall, Wong, Cullen, Knuiman, & Grunstein, 2014).

The major health burden associated with OSA is increased risk of cardiovascular diseases (McNicholas & Bonsignore, 2007). Although the underlying mechanisms with regard to the association between OSA and cardiovascular diseases remain unclear, the literature suggests an inflammatory etiology (Nadem et al., 2013). The development of inflammation in response to hypoxia in OSA may lead to endothelial dysfunction, and eventually, to fatal or non-fatal cardiovascular events (Davignon & Ganz, 2004; Eltzschig & Carmeliet, 2011). Patients with OSA have elevated levels of inflammatory biomarkers, including C-reactive protein (CRP) and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) (Born, Lange, Hansen, Mölle, & Fehm, 1997; Maeder et al., 2015; Redwine, Hauger, Gillin, & Irwin, 2000). These inflammatory biomarkers are also closely linked with the pathogenesis of cardiovascular disease and thus have been hypothesized as a causal link between OSA and cardiovascular disease.
Several studies have found that the proinflammatory cytokine levels, including IL-6, are subject to the diurnal variation in healthy adults (Entzian, Linnemann, Schlaak, & Zabel, 1996; Vgontzas et al., 1999). In OSA adults, there is accumulating evidence that proinflammatory cytokine levels are different from healthy adult levels; some proinflammatory biomarkers demonstrate diurnal variability as compared to healthy adult levels. Depending on the individual biomarker, an absence, heightened variance, or reversed diurnal variation pattern has been identified in OSA adults compared to healthy adults (Entzian et al., 1996). Yet, the evidence for diurnal variability of individual proinflammatory biomarkers is not consistent; the reason for such inconsistent findings is, in part, the confounding effects of obesity, age, type 2 diabetes mellitus (T2DM), and cardiovascular diseases, that influence the development of both OSA and expression of inflammatory biomarkers (Imagawa et al., 2004; Peker, Hedner, Norum, Kraiczi, & Carlson, 2002; Taheri, Austin, Lin, Nieto, Young, & Mignot, 2007; Yaggi, Concato, Kernan, Lichtman, Brass, & Mohsenin, 2005). Further research is required to fully understand whether OSA per se influences the level of and diurnal variability of inflammatory biomarkers, independent of confounding factors.

OSA is a disorder characterized by repetitive events of upper airway collapses over the sleep period. The reduction or cessation of airflow is accompanied by intermittent hypoxia and is terminated by arousals, which lead to sleep fragmentation and decreased amount of slow wave sleep, referred to as deep sleep (N3; non-rapid eye movement [NREM] stage 3) as well as rapid eye movement sleep (REM sleep) (McNicholas, 2002; Steirpoulos et al., 2010). These changes in sleep architecture (i.e., the quantity of time spent in each phase of sleep such as NREM and REM sleep) result in excessive daytime sleepiness and fatigue in OSA (Gislason & Sunnergren, 2014; Steirpoulos et al., 2010).
Although excessive daytime sleepiness and fatigue are prevalent symptoms in patients with OSA and considered important symptoms when determining the severity and clinical implications of OSA, not all patients with OSA develop these symptoms (Barbé et al., 2001; Young, Palta, Dempsey, Skatrud, Weber, & Badr, 1993). The question that should be answered is why some patients with OSA develop excessive daytime sleepiness and fatigue while others do not? The gene or protein expression related to a specific disease (i.e., its molecular signature) may explain the symptoms, or consequences, of OSA (Barratt & Topham, 2007; Kittleson & Hare, 2005; Mohr & Liew, 2007). There is no research that has examined specific protein expressions (i.e., molecular signatures) in OSA that may confer risk for specific symptom phenotypes (i.e., excessive daytime sleepiness, fatigue).

There is little known about the relationship between the level of inflammatory biomarkers and everyday symptoms such as excessive daytime sleepiness and fatigue in adults with OSA. There is also little known about the relationship between diurnal variability of inflammatory biomarkers and everyday symptoms in adults with OSA. The primary purpose of this study is to identify whether inflammatory biomarkers are associated with excessive daytime sleepiness and fatigue in adults with OSA when controlling for obesity, age, T2DM, and cardiovascular disease. A secondary goal of this study is to explore whether the diurnal fluctuation of inflammatory biomarkers influences everyday symptoms in adults with OSA, independent of confounding factors. The study was conducted using a cross-sectional cohort design and employed a convenience sample of adults with OSA.

Statement of the Problem

The key pathophysiological characteristic in OSA is that the upper airway obstruction occurs only during sleep, not wakefulness. The obstructive episodes that occur during sleep
affect daytime symptoms such as excessive daytime sleepiness and fatigue in patients with OSA. To phenotype these symptoms in patients with OSA, it is important to identify the biologic mechanism(s) of excessive daytime sleepiness and fatigue. To date, the molecular signatures of OSA have not been examined in relationship to expressed symptoms, or symptom phenotypes. Symptom phenotypes will be identified through both biologic and self-reported symptom measures. The relationship between the level of inflammatory biomarkers and everyday symptoms as well as between the diurnal variability of inflammatory biomarkers and everyday symptoms after controlling for confounding factors have not been broadly explored. Further research is needed to provide mechanistic insights for subsequent symptom management studies.

**Diurnal Variation of Inflammatory Biomarkers as a Response to Hypoxia and Sleep Fragmentation**

OSA is characterized by apneas (i.e., complete airway collapses/closures) and hypopneas (i.e., partial airway collapses/closures) during sleep that result in intermittent chronic hypoxia and sleep fragmentation (American Academy of Sleep Medicine, 2012). In response, inflammatory pathways are activated. These inflammatory responses to stress, which in the case of OSA is intermittent hypoxia and sleep architecture disruption, have been substantiated in the literature (Alzoghai & BaHammam, 2005; Arnardottir et al., 2012; Ohga et al., 2003; Steiropoulos et al., 2010). By measuring inflammatory biomarkers in healthy and OSA adults, the evidence clearly suggests a pro-inflammatory state in OSA (Entzian et al., 1996; Mills, Natarajan, von Känel, Ancoli-Israel, &Dimsdale, 2009). Furthermore, the diurnal variation of these inflammatory biomarkers is different in OSA than in healthy adults (Entzian et al., 1996; Mills et al., 2009). This difference in the variability of inflammatory biomarkers is based on the diurnal and circadian rhythm of these markers in healthy controls. In essence, the majority of
these inflammatory biomarkers have a naturally occurring circadian rhythm; yet, early evidence in OSA suggests an altered or shifted rhythm of inflammatory biomarkers. Hypoxia and sleep fragmentation occur during sleep in OSA and are therefore hypothesized to cause inflammation and potentially shift the circadian expression of inflammatory biomarkers relative to the timing/occurrence of these stressors during the sleep period.

**Circadian Rhythm of Cortisol in OSA and Healthy Adults**

A circadian rhythm is an organism’s internal body clock regulating biological processes that suggest an endogenous variation of approximately 24 hours (Vitaterna, Takahashi, & Turek, 2001). The circadian clock is controlled by a central pacemaker in a region of the brain located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The most distinct circadian variation is the sleep-wake cycle (Vitaterna et al., 2001). Certain hormones, such as cortisol and melatonin, play an important signaling role for the circadian clock in a body (Entzian et al., 1996). The peak levels of cortisol occur after waking and gradually decrease until the nadir, approximately during the first two hours of sleep (Davidson, Moldofsky, & Lue, 1991) (Figure 1.1).

![Circadian Rhythm of Cortisol](image)

**Figure 1.1. Circadian Rhythm of Cortisol in Healthy**
The circadian cortisol production in OSA is different than seen in healthy adults, where the highest levels of cortisol occur after sleep, typically in the early morning for night-time sleepers, and the lowest levels occur after extended wakefulness, typically in the evening (Ghiciuc, et al., 2015; Raff, Ettema, Eastwood, & Woodson, 2011). Adults with OSA show significantly lower cortisol concentrations in the morning, after sleep bout, compared to adults without OSA (4.6±1.0ng/ml and 7.2±0.6ng/ml, p<.05, respectively). Unlike the cortisol concentrations in the morning, in adults with OSA, the cortisol concentration in the evening was lower when compared to adults without OSA, but it was not statistically significant (2.1±1.0ng/ml and 2.5±0.4ng/ml, p>.05, respectively) (Ghiciuc, et al., 2015). Decreased levels of cortisol in OSA may result from repeated nocturnal episodes (i.e., frequent apneas and hypoxia and the resultant sleep architecture alterations), which likely alters the hypothalamic–pituitary–adrenal (HPA) axis (Nater et al., 2008). HPA axis, acting as the maintenance of homeostasis in rest and stress, is believed to be disrupted in OSA (Karaca et al., 2013). The dysregulation of HPA axis in OSA is likely to cause physiological and psychological comorbidities such as excessive daytime sleepiness and fatigue observed in patients with OSA (Ghiciuc, et al., 2015). The circadian rhythm of cortisol promulgates expression of more specific biomarkers that indicate inflammation, such as CRP, TNF-α, and IL-6.

**Circadian Rhythms of Inflammatory Biomarkers in OSA**

There is little known about the diurnal variation of inflammatory biomarkers during sleep-wake pattern (Haack, Pollmächer, & Mullington, 2004). Despite the sparse evidence, it is important to address whether circadian rhythm differences of inflammatory biomarker release exist between OSA and healthy adults. Any impairment in the circadian rhythm of inflammatory biomarkers is likely an indicator of disease state (Mullington et al., 2016).
In healthy adults, CRP concentrations throughout the 24-h day show absence of a diurnal variation (Meier-Ewert, Ridker, Rifai, Price, Dinges, & Mullington, 2001; Mills et al., 2009). On the other hand, patients with OSA express a rhythmic diurnal variation of CRP levels, where higher levels occur in the daytime and levels decline from the peak, during daytime, to a nadir during night time (Mills et al., 2009) (Table 1.1). The variability of CRP concentrations in OSA is likely related to the CRP half-life of approximately 18 hours (Ingle & Patel, 2011). CRP increases in response to hypoxia caused by sleep apnea during sleep, and increased CRP levels can be normalized, within a day, when inflammation is eliminated with treatment (Hartmann et al., 2000).

**Table 1.1. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults:**

<table>
<thead>
<tr>
<th>CRP</th>
<th>OSA</th>
<th>Healthy Humans</th>
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<tbody>
<tr>
<td></td>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Mills et al., 2009</td>
<td>Nighttime (22:00 until 06:00)</td>
<td>Daytime (08:00 until 20:00)</td>
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*Notes. OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time; h, hours*

TNF-α is known as a sleep-inducing cytokine because it is associated with the increased duration of NREM sleep. It also exhibits somnogenic potency, increasing sleepiness or promoting sleep in an experimental group of healthy adults that received injection of endotoxin compared to a healthy adult control group that received injection of endotoxin-placebo (Pollmächer et al., 1993). In healthy adults, TNF-a levels increase as sleep bout approaches, typically during the evening and peak during early sleep bout; TNF-a levels are lowest post sleep bout, typically in the morning. Whereas in OSA, there is a shift in the circadian rhythm of TNF-
a, with peak levels occurring post-sleep bout and the nadir occurs later in the sleep bout compared to healthy adults. The circadian rhythm of TNF-α is inversely related to the cortisol rhythm. The peak level of TNF-α occurred at noon and its lowest level occurred during night (Entzian et al., 1996) (Table 1.2). These findings indicate that circadian rhythm of TNF-α release in OSA is shifted, or advanced. The peak level of TNF-α at noon is likely to cause excessive daytime sleepiness in OSA.

**Table 1.2. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: TNF-α**

<table>
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<th>OSA</th>
<th>Healthy Humans</th>
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<tr>
<td></td>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Entzian et al., 1996</td>
<td>04:21</td>
<td>12:03</td>
</tr>
<tr>
<td></td>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td></td>
<td>08:20</td>
<td>02:32</td>
</tr>
</tbody>
</table>

*Notes. OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time*

When it comes to the circadian rhythm of interleukin-1β (IL-1β), the diurnal variation of IL-1β was constant throughout 24-hours in healthy adults although its lowest level occurred in the morning, whereas diurnal variation of IL-1β in OSA was distinct, wherein the peak levels occurred at 01:04 and the lowest levels were observed at 08:00 in the morning (Entzian et al., 1996) (Table 1.3).
**Table 1.3. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-1β**

<table>
<thead>
<tr>
<th>OSA</th>
<th>Healthy Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Entzian et al., 1996</td>
<td>08:00 01:04</td>
</tr>
</tbody>
</table>

**Notes.** OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time

IL-6 is expressed in a circadian manner. IL-6 has a peak level during sleep which suggests that IL-6 is likely to be a sleep factor. Increased IL-6 levels are also associated with excessive daytime sleepiness and fatigue (Vgontzas et al., 2005). Even though the peak levels of IL-6 in OSA occur earlier than in healthy adults, the circadian rhythm between OSA and healthy adults was not significantly different (Entzian et al., 1996) (Table 1.4). However, in OSA, IL-6 levels between 15:00 and 22:00 have a rising curve, whereas in healthy adults, IL-6 levels between 15:00 and 22:00 have a falling curve (Entzian et al., 1996). These findings suggest that elevated IL-6 levels may be a mediator of excessive daytime sleepiness and fatigue.

**Table 1.4. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-6**

<table>
<thead>
<tr>
<th>OSA</th>
<th>Healthy Humans</th>
</tr>
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<tbody>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Entzian et al., 1996</td>
<td>08:00 20:47</td>
</tr>
</tbody>
</table>

**Notes.** OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time
The circadian rhythm of IL-8 in healthy humans is inversely related to the cortisol rhythm (Hermann et al., 2006). There is no published evidence that addresses the circadian rhythm of IL-8 in OSA (Table 1.5).

**Table 1.5. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-8**

<table>
<thead>
<tr>
<th>OSA</th>
<th>Healthy Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Hermann et al., 2006</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

*Notes. OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time*

In healthy adults, interleukin-10 (IL-10) showed constant levels throughout the 24-hour day (Lissoni, Rovelli, Brivio, Brivio, & Fumagalli, 1998). There is no published evidence with regard to the circadian rhythm of IL-10 adults with OSA (Table 1.6).

**Table 1.6. Circadian Clock Variations in Inflammatory Biomarkers in OSA and Healthy Adults: IL-10**

<table>
<thead>
<tr>
<th>OSA</th>
<th>Healthy Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. Clock Time</td>
<td>Max. Clock Time</td>
</tr>
<tr>
<td>Hermann et al., 2006</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

*Notes. OSA, obstructive sleep apnea; Min. Clock Time, minimum clock time; Max. Clock Time, maximum clock time*

**A Molecular Signature for OSA**

A molecular signature is a marker of gene or protein expressions in particular cells or tissues related to a disease. The molecular signatures expressed in blood, urine, or saliva provide...
diagnostic information on the presence of disease (Barratt & Topham, 2007; Kittleson & Hare, 2005; Mohr & Liew, 2007). OSA is associated with an increased incidence of excessive daytime sleepiness and fatigue; however, not all, patients experience the aforementioned consequences (Johns, 1993; Lavie, Herer, & Hoffstein, 2000; Nieto et al., 2000; Peppard et al., 2000b; Young et al., 1993). The question yet to be answered is why some, not all, patients experience OSA negative consequences, such as excessive daytime sleepiness or fatigue. Gaining an understanding of the molecular signatures for OSA may provide insight into potential mechanisms that are responsible for variations in consequences of the disorder.

The unique features of OSA include obstructive events, accompanied by intermittent hypoxia, reoxygenation, and sleep fragmentation during sleep (i.e. temporal changes across the sleep period) (Zamarrón, Valdés Cuadrado, & Álvarez-Sala, 2013). Intermittent hypoxia and frequent arousals during sleep in OSA produce molecular changes in the brain as well as in the carotid body and chemoreceptors; subsequently down-stream molecular pathways are altered, which can be observed in blood or urine. These biologic alterations can be identified by measuring the temporal fluctuation in specific inflammatory biomarkers (i.e., CRP and cytokines) from pre-sleep to post-sleep (Arnardottir, Sunwoo, & Pack, 2011). The molecular signatures can be used to predict who is likely to develop OSA-related symptoms such as excessive daytime sleepiness and fatigue. Therefore, the biologic data (i.e., identified molecular signatures) will potentially lead to a better understanding of symptom phenotypes and support continued symptom management research in OSA.

**The Relationship Between OSA and the Level of Inflammatory Biomarkers**

Studies in adults with OSA have found overall elevated levels of inflammatory biomarkers, including CRP and proinflammatory cytokines, TNF-α and IL-6 (Huiguo et al.,
2000; Kokturk, Ciftci, Mollarecep, & Ciftci, 2005; Minoguchi et al., 2004; Yokoe et al., 2003). However, the exact mechanism of increased inflammatory biomarkers in OSA are yet to be determined (Minoguchi et al., 2004; Ryan, Taylor, & McNicholas, 2006; Vgontzas et al., 2004a).

Inflammation is hypothesized to be a response to hypoxia in OSA. In support of this potential mechanism, inflammatory biomarkers are positively related to the duration of hypoxia during total sleep time and the number of apneas and hypopneas in OSA (Alberti et al., 2003; Huiguo et al., 2000). The severity of OSA, measured by AHI (i.e., the number of apneic and hypopnic events per hour over the sleep period), was also associated with increased cytokine levels (American Academy of Sleep Medicine, 2012). Maeder et al. (2015) have found higher levels of IL-6 in moderate to severe OSA when compared to mild or no OSA. The repetitive hypoxia during sleep with apneas is likely to be the important causative factor in the mechanism of the inflammation in OSA (Calvin, Albuquerque, Lopez-Jimenez, & Somers, 2009).

In a meta-analysis examining the effect of treating OSA with continuous positive airway pressure (CPAP) on inflammatory biomarkers, 23 studies demonstrated that CPAP therapy improves circulating levels of inflammatory biomarkers in terms of CRP, TNF-α, and IL-6 (Baessler et al., 2013). These findings are important because CPAP is the most prevalent treatment for OSA (Malhotra, Ayas, & Epstein, 2000). CPAP works by eliminating upper airway obstruction during sleep and normalizing oxygenation and consequently leads to improved sleep quality (i.e., sleep architecture); the consequences of OSA are also decreased with CPAP, including excessive daytime sleepiness (Patel, White, Malhotra, Stanchina, & Ayas, 2003). Duration of hypoxia during total sleep time has been identified as a powerful predictor of TNF-α production; one month of CPAP treatment resulted in significantly improved sleep quality as well as decreased levels of TNF-α (Minoguchi et al., 2004). These findings suggest that
untreated OSA is likely a major contributor in promoting inflammation and that the inflammation observed in OSA is reversible with treatment of OSA.

Yet, some studies report conflicting results regarding the relationship of TNF-α with OSA. Guasti et al. (2011) reported no difference in TNF-α levels in OSA patients when compared to control groups. Similarly, Imagawa and colleagues (2004) reported no change in circulating TNF-α levels from pre- to post- CPAP treatment in an OSA group (Imagawa et al., 2004). Similar findings have been reported for IL-8 as well (Guasti et al., 2011). Inconsistent prior results, therefore, suggest the need for additional research to better define the relationship between OSA and levels of inflammatory biomarkers.

The Relationship Between Inflammatory Biomarker Diurnal Variation and Everyday Symptoms in OSA

Excessive daytime sleepiness and fatigue are considered the major clinical symptoms of OSA (Vgontzas, et al., 2004a). The prevalence of excessive daytime sleepiness in the general population is estimated at 5-15% (Young, 2004) and its prevalence in OSA is 87.2% (Seneviratne & Puvanendran, 2004). Excessive daytime sleepiness is increasingly recognized as a major threat to public safety since it is related to fatal motor vehicle accidents and impaired cognitive and social function (Aldrich, 1989; American Sleep Disorders Association Diagnostic Classification Steering Committee, 1997; Ohayon, Caulet, Philip, Guilleminault, & Priest, 1997). The Cardiovascular Health Study of 5,888 adults reported that excessive daytime sleepiness related to OSA was related to increased risk of cardiovascular morbidity and mortality (Newman, Spiekerman, Lefkowitz, Manolio, Reynolds, & Robbins, 2000). Excessive daytime sleepiness is common in OSA and has important implications for safety, quality of life and health outcomes.
Fragmented sleep due to frequent cortical arousals during sleep may, in part, explain the relationship between OSA and everyday symptoms (de la Peña Bravo, Serpero, Barceló, Barbé, Agustí, & Gozal, 2007); however, this may not fully explain the everyday symptoms of OSA. Inflammatory cytokines influence sleep regulation, including central neural regulation of sleep and wakefulness, and may thereby promote excessive daytime sleepiness and fatigue in OSA. For example, both increased TNF-α and IL-6 levels in OSA have been found to be inactivated by TNF-α antagonist, which resulted in decreased excessive daytime sleepiness in patients with OSA (Vgontzas et al., 2004a). In contrast, a recent study found no statistically significant differences in the levels of TNF-α and IL-6 between OSA patients with excessive daytime sleepiness and OSA patients without excessive daytime sleepiness (de la Peña Bravo et al., 2007).

To date, a relatively small number of studies have examined the relationship between inflammatory biomarkers and everyday symptoms in OSA and have contributed conflicting results; no studies have examined the relationship between diurnal variation of inflammatory biomarkers and everyday symptoms of OSA. The temporal conditions, or stressors, during sleep in OSA, including hypoxia and sleep fragmentation, are causal mechanisms of inflammation; the temporality of these mechanisms are likely to cause altered diurnal expression of inflammatory biomarkers and may explain symptom expression in OSA.

**Summary: Statement of the Problem**

OSA is a highly prevalent sleep disorder in the general adult population and leads to fatal and non-fatal health consequences. The repetitive hypoxia and sleep fragmentation in OSA are associated with sympathetic nerve activation, oxidative stress, and systemic inflammation. However, not all patients have the same health outcomes or symptoms.
The protein expressions in cells or tissues related to specific diseases may shed light on the consequences (i.e., symptoms) of the disorder. In OSA, inflammation occurs in response to hypoxia. Systemic inflammatory biomarkers such as CRP and pro-inflammatory cytokines such as TNF-α, IL-6, and IL-8 are up-regulated in OSA. Yet little is known about the OSA molecular signatures and how varied molecular signatures may result in differences in expression of symptoms (i.e., symptom phenotypes of OSA). Inflammatory biomarkers have a naturally occurring diurnal variation (e.g. highest expression in the evening and lowest expression in the morning). Preliminary studies in OSA suggest the diurnal variation of inflammatory biomarkers is different than in healthy adults. It is therefore plausible that altered diurnal fluctuation of inflammatory biomarkers in OSA, a stress response to repetitive hypoxia and frequent cortical arousals that alter normal sleep architecture and occur during sleep, may influence the daytime consequences of OSA, or symptoms, such as excessive daytime sleepiness and fatigue. If this is true, then symptom phenotypes are potentially discoverable based on OSA molecular signatures.

**Purpose of the Study**

The purpose of this study was to determine a molecular signature for OSA. Specifically, a cross-sectional cohort study examined; 1) the relationship between the level of inflammatory biomarkers and everyday symptoms in OSA after controlling for confounding factors and 2) the relationship between the diurnal variation of inflammatory biomarkers and everyday symptoms in OSA, independent of confounding factors. The following research questions were explored:

1. What is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA, and is there a significant relationship between diurnal variation and AHI?
2. Is there an association between inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP and everyday symptoms, including excessive daytime sleepiness and fatigue, in adults with OSA?

3. Is diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP associated with symptoms in adults with OSA?

4. What is symptom phenotype in adults with OSA?

**Conceptual Framework**

A conceptual model was employed in the study with the combination of the physiological framework and the theory of unpleasant symptoms to examine the relationships: 1) between inflammatory biomarkers and everyday symptoms including excessive daytime sleepiness and fatigue; and 2) between the diurnal fluctuation of inflammatory biomarkers and everyday symptoms in adults with OSA. It is essential to address the physiological framework to determine the underlying molecular mechanisms of everyday symptoms including excessive daytime sleepiness and fatigue associated with OSA.

Adults with OSA commonly experience impaired daily function due to excessive daytime sleepiness and fatigue (Bardwell, Moore, Ancoli-Israel, & Dimsdale, 2003). There is a need to identify physiological and/or biological markers related to those symptoms as well as psychological conditions, such as a mood disturbances, in order to phenotype excessive daytime sleepiness and fatigue in OSA. A nursing middle range theory, providing an understanding of the nature of symptoms, may establish further understanding of the interaction between symptom experience and its influencing factors and between symptom experience and its consequences. The theory of unpleasant symptoms, illustrating the relationships between symptoms experiences, outcomes, and influencing factors will provide a scientific perspective into both
biologic and other features of symptoms (Lenz, Pugh, Milligan, Gift, & Suppe, 1997) (Figure 1.2).

![Middle-Range Theory of Unpleasant Symptoms](image)


**Definitions of Key Terms**

The following definitions are used in this study;

**Obstructive Sleep Apnea (OSA):** Repetitive episodes of partial or total upper airway collapse during sleep that occur despite continuous respiratory effort. It is defined by an AHI of 5 or greater (American Academy of Sleep Medicine, 2005).

**Molecular Signature:** A subset of genes, proteins, or mRNA transcripts that are expressed in particular cell or tissue. Molecular signatures are useful to diagnose diseases or predict health-related risks (Barratt & Topham, 2007; Kittleson & Hare, 2005).
**Inflammatory biomarker**: A measurable biological indicator of the severity or presence of inflammation (Tamariz & Hare, 2010). Biomarkers included in this research were:

1. **CRP (CRP)**: A protein produced by the liver in response to inflammation. The activation of the immune system in response to inflammation has been substantiated in the prognosis of cardiovascular diseases. CRP release is prompted by IL-6. It plays a role in the binding of CRP to lysophosphatidylcholine in dead or dying cells to activate host defense (Anand et al., 2005).

2. **Proinflammatory cytokines**: Are involved in cell signaling and systemic inflammatory reactions. Activated macrophages promote the production of proinflammatory cytokines and increased levels of proinflammatory cytokines lead to worsened diseases (Dinarello, 2000).

   2.1. **Interleukin-1β (IL-1β)**: As a member of interleukin-1 family, it is produced by hematopoietic cells such as monocytes and macrophages and plays a role in the regulation of the inflammatory responses by cell proliferation, differentiation, and apoptosis (Garlanda, Dinarello, & Mantovani, 2013).

   2.2. **Interleukin-6 (IL-6)**: Interleukin-6 is released by T cells in order to stimulate immune response during infection or tissue damage (Askevold, Gullestad, Dahl, Yndestad, Ueland, & Aukrust, 2014).

   2.3. **Interleukin-8 (IL-8)**: IL-8 is a chemokine produced by macrophages and mesenchymal cells in response to inflammatory stimuli. Increased production of IL-8 is affected by hypoxia (Hirani et al., 2001).
2.4. Tumor necrosis factor-α (TNF-α): One of the inflammatory cytokines that increase in the acute phase inflammatory reaction. The primary role of TNF-α is to mediate the immune response to inflammation (Petersen & Felker, 2006).

3. Anti-inflammatory Cytokine: Known as immunomodulatory cytokine. The series of anti-inflammatory cytokines play a role in the immunosuppressive response by controlling proinflammatory reactions (Opal & DePalo, 2000).

3.1. Interleukin-10 (IL-10): A potent anti-inflammatory cytokine, it is produced by monocytes, macrophages, T-cells (T helper cell type 2; Th2), and B cells. The major role of IL-10 is to inhibit proinflammatory cytokine synthesis (Opal & DePalo, 2000).

**Diurnal variation:** The level of variability of a substance that is measured per day, before and after sleep (Maeder et al., 2015).

**Symptom:** The perceived change in normal function experienced by individuals. Symptoms are influenced by physiological, psychological, and situational factors and affect individuals’ performance outcomes (Lenz et al., 1997). Symptoms included in the present study were momentary symptoms and lasting symptoms.

1. Momentary Symptom: For the purposes of this study, momentary symptom is operationalized to be concurrent self-recognized symptom levels in daily life for adults with OSA. The present study included the levels of sleepiness, fatigue, and energy.

1.1. Excessive Daytime Sleepiness: The increase tendency to fall asleep as a physiological need. The propensity to fall asleep, or sleepiness, can be measured by the presence, frequency, endurance, and intensity to fall asleep during the daytime (Roehrs, Carskadon, Dement, & Roth, 2010).
1.2. Fatigue: Self-recognized physical and mental exhaustion. It is characterized by an overwhelming exhaustion and tiredness, which individuals experience currently (Lee, 1993).

1.3. Energy: Perceived level of vitality for an individual to perform physical and mental activity (Lee, 1993).

2. Lasting Symptom: For the purposes of this study, lasting symptom is operationalized as persistent, self-recognized symptoms in the daily lives of adults with OSA; lasting symptoms persist over time. The present study measured daytime sleepiness, mood disturbance, quality of sleep, perceived stress, and depressive symptoms.

2.1. Daytime sleepiness: Sustained way of sleepiness with dozing off or falling asleep during daytime (Johns, 1992).

2.2. Mood Disturbance: Perceptions of negative or positive states of emotions and moods from recent events (Lin, Hsiao, & Wang, 2014).

2.3. Quality of Sleep: Sustained symptoms related to adults with obstructive sleep apnea in daily living (Lacasse, Bureau, & Series, 2004). This study measured diurnal symptoms, social interactions, nocturnal symptoms, emotions, and hypersomnia, which are denoted as sub-domains in the Quebec Sleep Questionnaire.

2.3.1. Diurnal Symptoms: Persistent perceptions of the lack of concentration and difficulties with memory and performance at work (Lacasse et al., 2004).
2.3.2. Social Interactions: Persistent recognition related to the interruptions regarding social interactions due to sleep apnea (Lacasse et al., 2004).

2.3.3. Nocturnal Symptoms: Sustained awareness of the unrefreshed feeling and tiredness during daytime (Lacasse et al., 2004).

2.3.4. Emotions: Sustained mood of anxiety and fear with regards to suffocation, sleep apnea-related comorbidities, and sudden death (Lacasse et al., 2004).

2.3.5. Hypersomnolence: Perceived difficulties in staying awake during daytime and struggling with drowsiness (Lacasse et al., 2004).

2.4. Perceived Stress: Unpredictable, uncontrollable, and overloaded perception from the events of an individual’s life (Cohen, Karmarck, & Maermelstein, 1983).

2.5. Depressive Symptoms: Perceived recognition with regards to not only hopelessness, irritability, and feeling guilty, but also physical symptoms such as weight loss and lack of interest in sex (Beck & Beamesderfer, 1974).

**Phenotype**: All the observable physical and biological traits of an organism that result from interactions between genes and the environment (Griffiths, Wessler, Lewontin, Gelbart, Suzuki, & Miller, 2005).

**Symptom phenotype**: Phenotypic traits, reflecting a manifestation of a disease. Symptom phenotypes can be measured by examining the association of symptom(s) with particular genes or proteins (Wanke & Augustson, 2009).

**Assumptions**

The following assumptions are acknowledged prior to the conduct of this study:
1. Adults with OSA have increased levels of inflammatory biomarkers as compared to adults without OSA.
2. OSA has particular molecular signatures.
3. Increased levels of cytokines in adults with OSA affect symptoms including excessive daytime sleepiness and fatigue.
4. Inflammatory biomarkers in OSA have diurnal variation.
5. The symptoms, excessive daytime sleepiness and fatigue, vary in relationship to the diurnal variation of inflammatory biomarkers.
6. Obesity, age, type 2 diabetes and cardiovascular disease are confounding variables in the relationship between inflammation and symptoms in OSA.

**Significance of the Study**

This study explores the molecular signature for OSA; specifically, this study identifies if a relationship exists between basal inflammation and everyday symptoms of excessive daytime sleepiness and fatigue in adults with OSA. Diurnal variation in inflammation is also described and explored as potentially associated with different symptom phenotypes in OSA. The knowledge acquired will help guide the science of symptom management in several ways: 1) provide a critically needed foundation to move forward symptom science research in OSA; 2) provide insight to the pathophysiological significance of molecular signatures; and 3) enable an understanding of the relationship between diurnal variation of inflammatory biomarkers and symptom phenotypes in OSA to potentially guide symptom management approaches in OSA.

**Chapter 1 Summary**

OSA is a common chronic disease in the general population. The most prevalent symptom of OSA is excessive daytime sleepiness and fatigue, contributing to poor quality of life,
impaired daily function, and increased risk of motor vehicle accidents. Multiple factors including physiological factors (i.e., inflammation, hypoxia and arousal induced by OSA), psychological factors (i.e., mood disturbance), and situational factors (i.e., short sleep duration) likely contribute to excessive daytime sleepiness and fatigue in OSA.

Inconsistent findings of inflammatory biomarkers in OSA prevent further understanding of the disease and the development of symptom management science that addresses OSA. Intermittent hypoxia and arousal are induced only during sleep in OSA and these distinct characteristics are likely important causative factors. The approaches to identify the molecular signatures for OSA include the assessment of changes in specific protein expression (i.e., CRP, IL-6, IL-8, and TNF-α) and the evaluation of the response of inflammatory biomarkers along with diurnal variation.

This study is guided by a combination of a physiological framework and the Theory of Unpleasant Symptoms. This research framework supports the hypothesis that inflammatory biomarkers are associated with excessive daytime sleepiness and fatigue in adults with OSA. There is little known about inflammatory biomarkers in OSA and, in particular, the diurnal variation of inflammatory biomarkers, and the relationship of inflammatory biomarkers with excessive daytime sleepiness and fatigue in OSA. This study, therefore, addresses this gap to identify the expression of symptoms or symptom phenotypes in OSA.
Chapter 2

Review of the Literature

Large population-based studies have identified the relationship between OSA and cardiovascular disease, independent of obesity, but the mechanism(s) underlying this association remain unclear (Nieto et al., 2000; Young, Evans, Finn, & Palta, 1997). It is believed that a multifactorial causal process, including inflammatory pathways, oxidative stress, and endothelial dysfunction, influences the pathogenesis of cardiovascular disease in OSA (McNicholas, 2009). Recent evidence suggests inflammatory biomarkers, including CRP, TNF-α, IL-6, and IL-8, are elevated in OSA as compared to healthy adults (Liu et al., 2011; Sahlman et al., 2010). Little is known about diurnal variation of these biomarkers; this is specifically relative to OSA as the disease is characterized by hypoxia during sleep that stimulates or causes inflammation. With the periodicity of sleep bouts and thereby hypoxia, inflammatory biomarkers may exhibit a diurnal pattern of expression relative to sleep bouts. The net effect of this chronic inflammation is heightened risk for cardiovascular disease. Less is known about the relationship of symptoms and inflammation in OSA and the symptom expressions, or symptom phenotypes, relative to the periodicity of the biological processes of inflammation in OSA.

OSA is a chronic disease with common symptoms such as excessive daytime sleepiness and fatigue. Hypoxia and arousals induced by OSA are thought to contribute to excessive daytime sleepiness and fatigue. The mechanisms of OSA symptoms are not explicitly understood; examining inflammation in OSA and the relationship of inflammation with symptoms may contribute to the development of symptom management approaches in OSA. The following literature review, therefore, evaluated the scientific evidence regarding: 1) OSA in adults; 2) OSA and inflammation; and 3) symptom science.
The most common abbreviations included in this review of the literature are listed below (Table 2.1).

**Table 2.1. Abbreviation List**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>OSA</td>
<td>Obstructive Sleep Apnea</td>
</tr>
<tr>
<td>AHI</td>
<td>Apnea-Hypopnea Index</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-α</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>NREM sleep</td>
<td>Non-Rapid Eye Movement Sleep</td>
</tr>
<tr>
<td>REM sleep</td>
<td>Rapid Eye Movement Sleep</td>
</tr>
<tr>
<td>CPAP</td>
<td>Continuous Positive Airway Pressure</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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**Obstructive Sleep Apnea in Adults**

OSA is the most common type of sleep related breathing disorder (Garvey, Pengo, Drakatos, & Kent, 2015). OSA is characterized by recurrent events of partial (hypopneas) or total (apneas) upper airway obstruction with continuous respiratory effort (American Academy of Sleep Medicine, 2012). The following review included a definition of OSA, diagnostic recommendations for OSA, and the epidemiology and pathophysiology of OSA.
**Definition of Obstructive Sleep Apnea**

The American Academy of Sleep Medicine ([AASM]; 2012) defines obstructive apnea as complete or nearly complete cessation of airflow (≥90% of baseline amplitude, lasting at least 10 seconds) with persistent respiratory muscle effort to breathe (Figure 2.1).

![Figure 2.1. Obstructive Apnea. Complete cessation of airflow ≥ 10 seconds in duration, accompanied by persisting respiratory effort. From “Sleep Breathing Disorders” by Lee-Chiong, T. L., & Polnitsky, C. A., 2007, Review of Sleep Medicine (2nd ed.), p.44. Reprinted with permission from Elsevier.](image)

An obstructive hypopnea is related to partial collapse of the upper airway (i.e., > 30% decrease in airflow with respect to baseline, lasting at least 10 seconds) with persistent respiratory muscle effort (American Academy of Sleep Medicine, 2012). In addition, the event is accompanied by at least 3% oxygen desaturation from pre-event baseline (Figure 2.2) (American Academy of Sleep Medicine, 2012).
Figure 2.2. Obstructive Hypopnea. Partial cessation of airflow > 50% from baseline, accompanied by persisting respiratory effort. From “Sleep Breathing Disorders” by Lee-Chiong, T. L., & Polnitsky, C. A., 2007, Review of Sleep Medicine (2nd ed.), p.45. Reprinted with permission from Elsevier.

Obstructive apneas and obstructive hypopneas measured by polysomnography (i.e., sleep study) are combined as a single metric of OSA severity, the apnea hypopnea index, or AHI (Ruehland, Rochford, O'Donoghue, Pierce, Singh, P., & Thornton, 2009). The AHI is defined as the total number of apneic and hypopneic events per hour of sleep. The AHI is a diagnostic criterion of OSA and classifies the severity of OSA in adults (American Academy of Sleep Medicine, 1999; Table 2.2).

Table 2.2. Severity Classification of OSA

<table>
<thead>
<tr>
<th>Sleep-Related Obstructive Events, Apnea Hypopnea Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Severe</td>
</tr>
</tbody>
</table>
The Epidemiology of OSA

According to the World Health Organization (WHO) (2010), the prevalence of OSA is estimated at greater than 100 million around the world. A rigorous epidemiologic study, the Wisconsin Sleep Cohort Study, reported that 4% of men and 2% of women have OSA (Young, et al., 1993). Likewise, another study with a large random sample (1,741 individuals) in Pennsylvania investigated the prevalence of OSA (Bixler, et al., 2001). This study defined OSA by AHI ≥ 10 events/hour, plus excessive daytime sleepiness; an estimated 3.9% of men and 1.2% of women had OSA (Bixler, et al., 2001). Despite the high prevalence of OSA in the general population, greater than 80% of moderate and severe OSA has not been clinically diagnosed (Young et al., 1997).

Beyond the U.S., a number of studies in other countries have investigated the prevalence of OSA with similar estimates (Bearpark, et al., 1995; Durán, Esnaola, Rubio, & Iztueta, 2001; Ip, et al., 2001; Ip, et al., 2004). The prevalence of OSA with AHI ≥5 events/hour and excessive daytime sleepiness in Australia is 3.1% (Bearpark, et al., 1995). The Spanish cohort study showed prevalence estimates of 3.4% in men and 3% in women (Durán et al., 2001). Interestingly, the prevalence of OSA in Asian countries, where the mean body mass index (BMI) is lower than those of Western countries, is similar to Western counterparts (WHO, 2004). The prevalence of OSA in China is 4.1% in men and 2.1% in women (Ip, et al., 2001; Ip, et al., 2004), and likewise, cohort studies of OSA in South Korea identified prevalence estimates of 4.5% in men and 3.2% of women (Kim, et al., 2004). OSA is a worldwide public health problem that results in significant adverse health and functional outcomes.
Pathophysiology of OSA

The predisposing factors and physiological causes of OSA considerably differ among individuals, but most evidence suggests that OSA is associated with structural abnormalities (i.e., narrowed upper airway by muscles and tissues in the throat and mouth) (Dempsey, Veasey, Morgan, & O'Donnell, 2010). The airway includes muscles and soft tissues; however, the inherent lack of bone support contributes to collapsibility of the airway during sleep. The collapsible airway includes the area from the hard palate to the larynx. Anatomically, a narrow upper airway is at higher risk to collapse than a large airway (Fogel et al., 2004). Patients with OSA have decreased area of the upper airway compared with those without OSA (Haponik, Smith, Bohlman, Allen, Goldman, & Bleecker, 1983; Schwab, Gupta, Gefter, Metzger, Hoffman, & Pack, 1995) which likely predisposes the upper airway to collapse during sleep.

An important pathophysiologic consideration in OSA is the relationship between pharyngeal anatomy and the upper airway dilating muscles, which ensure the upper airway patency during sleep (Dempsey, 2010). Studies that measured muscle tone via electromyography have found the reduction of the upper airway dilator muscle tone at sleep onset in both healthy groups and OSA groups. OSA developed when individuals rely on the muscle tone to maintain a patent airway in the presence of abnormal anatomy of upper airway (Mezzanotte, Tangel, & White, 1996; Worsnop, Kay, Pierce, Kim, & Trinder, 1998). Thus, the reduction of the activity in upper airway dilator muscles combined with a narrowed upper airway anatomy play an important role in OSA. Collapse and obstruction affect air flow and subsequently results in decreased ventilation. Individuals with OSA have increased ventilatory effort, hypoxia, frequent arousal, and sleep fragmentation (Fogel et al., 2004).
Sleep, OSA, and Inflammation

Sleep is a fundamental biological activity occurring in all humans and plays a role in maintaining health and cognitive function throughout the lifespan (Krueger, Rector, Roy, Van Dongen, Belenky, & Panksepp, 2008). During sleep, two main stages of sleep, rapid eye movement (REM) and non-rapid eye movement (NREM), occur in periods of approximately 90 minutes (Carskadon & Dement, 2011). A sleep episode begins with NREM stage 1 and progresses through NREM sleep stages 2 and 3 (Altevogt & Colten, 2006). During NREM sleep, the brain is less active but the body repairs and restores tissues and strengthens the immune system. REM sleep, occupying 20% to 25% of total sleep time, involves the restoration of brain function and the amount of REM sleep is associated with intellectual function (Carskadon & Dement, 2011; Prinz, 1977). Previous studies show that sleep duration and quality contributes to daily function and physical and mental health; and impaired sleep duration and quality increase morbidity and mortality (Durmer & Dinges, 2005; Hublin, Partinen, Koskenvuo, & Kaprio, 2007).

Sleep also influences the homeostatic regulation of immune functions. The level of inflammatory biomarkers, indicators of immune function and activity, exhibit fluctuations over the 24-hour day along with during the sleep-wake cycle (Simpson & Dinges, 2007). Inflammatory biomarkers such as, TNF-α, IL-6, and IL-8 in the blood have the highest expression in the early evening and the lowest expression in the morning in healthy adults (Redwine, Dang, & Irwin, 2004). Several cytokines, including TNF-α, IL-1, and IL-6, have been found to be associated with sleep. These cytokines, termed and measured as inflammatory biomarkers, induce or progenerate sleep and reach peak levels at night, or during sleep, demarcated by
increased amplitude of slow wave delta activities measured by electroencephalography (EEG) (Motivala & Irwin, 2007).

OSA impairs sleep quality, defined as short sleep duration, sleep disturbances, and prolonged sleep latency due to sleep fragmentation and frequent arousals (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). Poor sleep quality is associated with inflammation. For example, previous studies have found that the morning level of IL-6, an inflammatory biomarker, was positively associated with sleep fragmentation and wakefulness during the preceding sleep bout and inversely correlated to slow wave sleep (NREM sleep stage 3) and sleep efficiency (Carpagnano, Kharitonov, Resta, Foschino-Barbaro, Gramiccioni, & Barnes, 2002; Huiguo et al., 2000; Vgontzas et al., 2000). In patients with OSA it is therefore likely that frequent arousals and sleep fragmentation during sleep disrupt not only the normal sleep-wake cycle but also contribute to systemic inflammation.

Frequent apneas during sleep lead to oxygen deprivation; SaO$_2$ is rapidly normalized when ventilation resumes (i.e., re-oxygenation). Each episode of re-oxygenation leads to repetitive ischemic reperfusion and production of free radicals. The resultant release of proinflammatory biomarkers, including CRP, TNF-α, IL-6, and IL-8, affect impaired endothelial function (Arnardottir, Mackiewicz, Gislason, Teff, & Pack, 2009; Lavie, 2003; McNicholas & Bonsigore, 2007). Increased IL-6 and CRP levels were significantly correlated with minimum SaO$_2$ in OSA (Arnardottir et al., 2012). With CPAP treatment, increased inflammatory biomarkers including CRP, TNF-α, and IL-6 were decreased (Oyama et al., 2012; Yokoe et al., 2003). The poor sleep quality and repetitive nocturnal hypoxia in OSA are hypothesized causal mechanisms of inflammation in OSA.
The Classification of Inflammatory Cytokines

Inflammatory biomarkers, or cytokines, are increasingly included in the cardiovascular risk assessment of patients with and without OSA. Cytokines, a biological marker of the inflammatory system, are low-molecular weight proteins released by cells of the immune system that act as chemical messengers. Cytokines play a role in mediating and regulating the pathophysiological functions in terms of innate immunity, acquired immunity and inflammatory responses (Feliciani, Gupta, & Saucier, 1996). They can be classified into chemokines, inducing chemotactic activities (chemotaxis) and interleukins, produced mainly by leukocytes and send signals out to other leukocytes. Interleukins communicate between leukocytes and play a role in the growth, maturation and activation of immune cells (Zhang & An, 2007).

As there is no standard classification of cytokines, they can be variously classified: 1) by primary cell of origin such as lymphokine and monokine; 2) by the numeric order that was discovered to be expressed by leukocytes such as interleukin-1 through interleukin-35; 3) by functional activities such as tumor necrosis factor (TNF); and/or 4) by functional role in infection and/or inflammation such as proinflammatory, promoting inflammation, or anti-inflammatory, suppressing the activity of proinflammatory cytokines (McInnes, 2013). In patients with OSA, many studies have demonstrated increased proinflammatory biomarkers in terms of CRP, TNF-α, IL-6, and IL-8. However, study findings regarding whether or not the relationship between OSA and proinflammation biomarkers is independent of BMI have not been consistent (Drager et al., 2010; Huiguo et al., 2000; Imagawa et al., 2004; Taheri et al., 2007; Yokoe et al., 2003).

C-Reactive Protein (CRP) in OSA

The acute phase response protein, CRP, is an important serum marker of inflammation. It is synthesized in the liver in response to proinflammatory cytokine IL-6 released by monocytes
or macrophages as a result of tissue damage (Castell, Gómez-lechón, David, Fabra, Trullenque, & Heinrich, 1990; Pasulka, Bristian, & Blackburn, 1985; Roytblat, Rachinsky, Fisher, Greemberg, Shapira, Douvdevani, & Gelman, 2000). The circulating levels of CRP suggest presence and severity of inflammation. Repetitive hypoxic episodes are likely to play a causative role in the elevated CRP levels in patients with OSA. Steiropoulos et al. (2010) found a positive correlation between CRP levels and nocturnal hypoxia in patients with OSA. In another study of normal humans, the hypoxia of high altitude led to increased levels of CRP (Hartmann et al., 2000; Imoberdorf et al., 2001). Unlike other inflammatory biomarkers, CRP levels are not subject to diurnal variation (i.e., individuals have quite stable CRP across 24 hours) (Meier-Ewert et al., 2001).

Previous studies examining the relationship between CRP and OSA have identified higher levels of CRP in patients with OSA compared to age- and BMI-matched controls without OSA (Bhushan et al., 2009; Chien, Lee, Tsai, Yang, & Wu, 2012; Shamsuzzaman et al., 2002; Yokoe et al., 2003). A meta-analysis of studies examining a total of 4,283 participants identified that those with OSA had a statistically significant higher level of CRP compared to controls (Nadeem et al., 2013). Several studies have found an association between OSA and increased levels of CRP independent of BMI (Bhushan et al., 2009; Drager et al., 2010; Guven, Turkkani, Ciftci, Ciftci, & Erdogan, 2012; Shamsuzzaman et al., 2002; Yokoe et al., 2003). Also, increased levels of CRP have been identified as a strong predictor of cardiovascular risk (Danesh et al., 2000; Rider, 2001). However, there are conflicting results regarding the relationship between OSA and increased levels of CRP as well as whether the relationship is independent of obesity.

A longitudinal study of 907 subjects from the Wisconsin Sleep Cohort study found that the relationship between OSA and CRP was not significant after controlling for BMI, age, and sex
(Taheri et al., 2007). Several studies have found that obesity (i.e., high BMI), rather than OSA, is the key risk factor of CRP levels (Arnardottir et al., 2012; Barceló et al., 2004; Guilleminault, Kirisoglu, & Ohayon, 2004). Even though Shamsuzzaman et al. (2002) reported increased CRP levels in a study of patients with OSA, wherein BMI was controlled, the study included only severe OSA with mean AHI of 60±5 events/hour. Therefore, studies are needed to determine if the severity of OSA may be associated with circulating CRP levels when BMI is controlled.

**TNF Alpha (TNF-α) in OSA**

TNF-α is a proinflammatory cytokine involved in the development of systemic inflammation and is mainly secreted by monocytes/macrophages (Idriss & Naismith, 2000). It is a cell signaling cytokine that leads to apoptotic cell death, cellular differentiation/proliferation, and inflammation. TNF-α primarily plays a role in the regulation of immune cells (Aderka, 1996; Idriss & Naismith, 2000). It is also involved in sleep regulation by promoting NREM sleep (Krueger, 2008).

Inflammatory cytokines are known to demonstrate a circadian rhythm with evidence that the peak levels of inflammatory cytokines occur between 1:00 to 2:00 a.m., when slow wave sleep (i.e., NREM sleep stage 3) usually occurs (Bauer et al., 1994; Gudewill, Pollmächer, Vedder, Schreiber, Fassbender, & Holsboer, 1992). Vgontzas et al. (1997) found that elevated TNF-α levels in patients with OSA occurred between 6:00 to 7:00 a.m., suggesting a physiological role of cytokine on the sleep architecture. The study also identified that nocturnal sleep disturbance was the primary driver of TNF-α levels. Similarly, several studies have found a significant relationship between elevated levels of TNF-α and OSA (Huiguo et al., 2000; Kanba, Kokturk, Ciftci, Tavil, & Bukan, 2008).
Several studies have found the elevation of TNF-α in patients with OSA and the increase of TNF-α was independent of BMI (Ciftci et al., 2004; Steiropoulos et al., 2010). Steiropoulos and colleagues (2010) also identified that TNF-α levels were positively correlated with AHI and oxygen desaturation index (ODI). In another study, elevated TNF-α levels were independently related to the duration of hypoxia. In addition, the circulating levels of cytokines significantly decreased after one month of treatment with CPAP (Minoguchi et al., 2004). These results may suggest a pathophysiological role of hypoxia on the circulating levels of cytokines.

However, there are discrepancies with regard to the relationship between OSA and circulating TNF-α levels. Imagawa et al. (2004) examined the increase of TNF-α in patients with severe OSA compared to those of non-OSA controls. Although they found a relationship between oxygen desaturation index and the AHI, TNF-α levels did not differ between groups. In addition, BMI was significantly correlated with the severity of the AHI. In contrast, Guasti and colleagues (2011) found no differences of TNF-α levels in OSA patients as compared to controls matched for cardiovascular risk factors. The provision of 12 weeks of CPAP treatment in patients with OSA did not change TNF-α levels. These inconsistent findings suggest the need for further studies on the pathophysiological role of cytokines, independent of BMI, in patients with OSA.

**Interleukin-1β (IL-1β) in OSA**

IL-1β is a member of the interleukin-1 family, which consists of a group of 11 cytokines including IL-1α, IL-1β, IL-1Ra, IL-18, IL-36Ra, IL-36α, IL-37, IL-36β, IL-36γ, IL-38, and IL-33. It is produced by monocytes and macrophages and involved in cellular activities (Dinarello, 1996). IL-1β as a proinflammatory cytokine plays a crucial role for host-defense responses to acute infection and trauma (Dinarello, 1996). It also contributes to the regulation of sleep by
promoting sleep in the brain (i.e., the activity of sleep-active neurons in the hypothalamus and hippocampus) and the activity of the thermoregulatory center (Kang, Park, Chung, & Kim, 2013).

In spite of the identified role of IL-1β in sleep regulation by activating sleep-active neurons and inhibiting wake-active neurons, there are few studies that examined the level of IL-1β in adults with OSA and the relationship between IL-1β and OSA-related symptoms. Vgontzas et al. (1997) examined the differences of morning IL-1β levels between patients with sleep apnea and healthy controls; there was no significant difference between the two groups. When the study explored the association between IL-1β and excessive daytime sleepiness, there were no significant findings (Vgontzas et al., 1997). In keeping with the findings, the study with 176 adults with OSA (AHI ≥5) explored the association between morning IL-1β and indices of OSA severity (i.e., AHI and Index 90; the percentage of sleep time spent below a saturation of 90%). There were negative correlations between morning IL-1β and AHI and between morning IL-1β and Index 90, but the correlations did not reach statistical significance (r=-0.094, p=-.22; r=-0.08, p=0.27, respectively) (Al Lawati et al., 2009). In contrast to human studies, in animal studies, IL-1β clearly enhanced non-rapid eye movement sleep and prolonged sleep (Obal et al., 1990; Opp, Obal, & Krueger, 1991). These inconsistent findings, that is, the level of IL-1β and its relationship with sleep between human studies and animal studies, suggest further studies are needed to identify the role of IL-1β in OSA.

**Interleukin-6 (IL-6) in OSA**

IL-6 plays a role as both a pro-inflammatory cytokine and an anti-inflammatory cytokine. IL-6 is a circulating cytokine that is produced by T cells and macrophages. It stimulates the hepatic production of CRP and is an important mediator of the acute phase response (Yudkin,
Kumari, Humphries, & Mohamed-Ali, 2000). Circulating IL-6 is also related to the pathogenesis of atherosclerosis as well as increased mortality rate in patients with unstable coronary artery disease (Lindark, Diderholm, Wallentin, & Siegbahn, 2001; Yudkin et al., 2000). A significant proportion of circulating IL-6 originates from adipose tissue; increased levels of IL-6 in patients with OSA may be attributable to high BMI as many OSA patients are obese (Fain, Madan, Hiler, Cheema, & Bahouth, 2004). In accordance with the evidence, several studies have reported that obesity rather than OSA was positively correlated with IL-6 levels (Ciftci, Kokturk, Bukan, & Bilgihan, 2004; Imagawa et al., 2004; Yokoe et al., 2003).

On the other hand, several studies have found that the levels of IL-6 in patients with OSA were significantly higher than those of controls, and a significant correlation between serum IL-6 levels and AHI has been identified (Liu, Xu, Hua, Wang, Liu, & Yang, 2011; Sahlman et al., 2010). In a recent study, Maeder et al. (2015) examined serum IL-6 levels, measured before (between 8:00 p.m. and 10:00 p.m.) and after sleep (between 6:00 a.m. and 7:00 a.m.) in patients with moderate or severe OSA compared with those with no or mild OSA. They found higher IL-6 levels after sleep in patients with moderate or severe OSA as compared to those with no or mild OSA. The IL-6 levels before sleep did not differ between the groups. The study suggested diurnal variability of IL-6 is present in OSA, with higher levels in the morning after sleep and that IL-6 measured after sleep was as an independent predictor of severe OSA.

The association between intermittent hypoxia in patients with OSA and IL-6 levels has been well documented by several studies. Liu et al. (2011) identified a positive correlation between serum IL-6 levels and the duration of oxygen desaturation. Similarly, in a cross-sectional cohort study, IL-6 levels were significantly correlated with oxygen desaturation index, the number of minutes with oxygen saturation (SaO2) < 90%, and minimum oxygen saturation
(SaO$_2$) (Arnardottir et al., 2012). Repetitive hypoxia during the night is likely to cause oxidative stress as well as the production of reactive oxygen species (ROS) (Prabhakar, 2002; Suzuk, Jain, Park, & Day, 2006). As a consequence of oxidative stress, IL-6 is activated as an inflammatory response in patients with OSA (Lavie, 2005). With CPAP treatment, the IL-6 levels were significantly decreased in several studies (Oyama et al., 2012; Yokoe et al., 2003). A decrease in AHI with treatment may inhibit the oxidative stress and consequently decrease the inflammation response.

**Interleukin-8 (IL-8) in OSA**

OSA is increasingly recognized as one of the most important risk factors for cardiovascular disease (Ohga et al., 2003). Hypoxic stress, induced by OSA, activates inflammatory mediators such as adhesion molecules and cytokines in order for leukocyte migration to inflamed tissue. IL-8, a chemokine produced by macrophages, epithelial cells, and endothelial cells, plays a role in leukocyte attachment to endothelium and proliferation of endothelial cells (Gerszten et al., 1999; Haught et al., 1996; Simonini et al., 2000). Simonini and colleagues found increased IL-8 levels in atherosclerotic lesions; that is, the results suggest that increased expression of IL-8 is involved in the development of cardiovascular disease (Simonini et al., 2000).

Studies showed increased circulating IL-8 levels in the OSA group when compared to control group matched for age and BMI (Alzoghaibi & BaHammam, 2005; Ohga et al., 2003). Ohga and colleagues (2003) found a significant correlation between increased IL-8 levels and desaturation, defined as hypoxia with SaO$_2$ <90%. Increased IL-8 levels are also positively related to apnea index. In addition, studies have found that CPAP treatment for at least three months resulted in decreased IL-8 levels (Alzoghaibi & BaHammam, 2005; Oyama et al., 2012).
These findings suggest that hypoxia during sleep activates IL-8 levels and the improvement of OSA with CPAP treatment leads to decreased chemokine levels. However, not all study findings are consistent. Guasti and colleagues (2011) found no significant differences in IL-8 levels between OSA group and non-OSA group. In addition, CPAP treatment for 12 weeks did not result in decreased IL-8 levels.

**Interleukin-10 (IL-10)**

IL-10, which is referred to as a cytokine synthesis inhibitory factor, impedes cytokine production and enhances both T-cell production and B-cell growth. It plays a role in not only the activation of the immune system, but also the production of corticotropin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) (Hughes, Cadet, Rady, Tyring, Chin, & Smith, 1994). IL-10 may be an important regulator in glucocorticosteroid production in response to stress (Coutinho & Chapman, 2011). Considering sleep regulation, IL-10 inhibits the production of IL-1β and TNF-α known as prosomnogenic cytokines and other somnogenic substances related to sleep regulation including nerve growth factor (NGF), and consequently increases the production of sleep-inhibitory substances such as CRF (Krueger, 2008). Previous studies demonstrated that administration of exogenous IL-1β (Krueger, Walter, Dinarello, Wolff, & Chedid, 1984; Opp et al., 1991) and TNF-α (Kapas et al., 1992; Nistico, DeSarro, & Rotiroti, 1992) enhances NREM sleep, whereas administration of exogenous IL-10 inhibits spontaneous NREM sleep (Opp, Smith, & Hughes, 1995).

In spite of the important role of IL-10 on the sleep regulation, there are few studies that have examined the systemic levels of IL-10 in adults with OSA and the association between IL-10 and the severity of OSA. There is growing evidence with regards to the relationship between anti-inflammatory response and OSA (Leon-Cabrera et al., 2015) and between promoter
polymorphisms of IL-10 gene and OSA (Özdaş et al., 2016). Leon-Cabrera et al. (2015) have found a negative relationship between IL-10 and AHI and between IL-10 and total arousal index ($r=-0.64$, $p<0.0001$; $r=-0.6$, $p=0.0065$). Additionally, systemic levels of IL-10 were significantly lower in obese adults with OSA compared to obese adults without OSA ($p=<0.0001$); however, the levels of IL-10 were significantly decreased in obese with severe OSA, which suggests further studies are needed to identify the stimulatory mechanisms of IL-10 in obese adults with OSA.

**Potential Confounding Factors**

**Age.** The prevalence of OSA tends to increase with age (Punjabi, 2008). Between the ages of 65 to 100 years, OSA was present in 18.1%, compared to that of 3.2% in the 20 to 44 year old group. The odds ratio of AHI indicating OSA in 65 to 100-year-old adults compared with 20 to 44-year-old adults was 6.6 (95% CI 2.6-16.7) (Bixler, Vgontzas, Have, Tyson, & Kales, 1998).

In spite of the absence of disease, older adults have a chronic inflammatory state with increased levels of IL-1, IL-6 and IL-18 (Dinarello, 2006; Giuliani et al., 2001). With advancing age, oxidative stress is increasingly activated and, as a result, cellular structure and function are damaged (Yu & Chung, 2006). A meta-analysis identified a positive correlation between age and inflammatory biomarkers including CRP, IL-6, and IL-8 (Nadeem et al., 2013). Another study found that older adults with OSA had increased CRP levels when compared to controls (Hall et al., 2014). Increased inflammatory levels in OSA are associated with advancing age; therefore, studies of inflammatory responses to stress must control for age as a confounder as in this study.

**Obesity.** The most important risk factor of OSA is obesity and the incidence of obesity is rapidly increasing (Stevens, et al., 2012). The estimated prevalence of obesity in U.S. adults is
35.7%, classified by a BMI $\geq 30 \text{ kg/m}^2$. In addition, approximately one-third of the population is overweight with a BMI of 25-29.9 kg/m$^2$ (Fryar, Carroll, & Ogden, 2012). The epidemic of OSA may be significantly associated with the obesity epidemic since approximately 70% of adults with OSA are obese (Daltro, Fontes, Santos-Jesus, Gregorio, & Araújo, 2006; Malhotra, & White, 2002). Several studies have demonstrated the strong relationship between OSA and elevated BMI (Daltro et al., 2007; Wu et al., 2015). The odds of 5-year incidence of OSA is 1.14 (95% CI 1.10-1.19) in obese compared to non-obese adults (Tishler, Larkin, Schluchter, & Redline, 2003). Previous evidence suggests that a 10% weight gain may lead to a 6-fold increased incidence of OSA and a 30% increase in the severity of OSA (Peppard, Young, Palta, Dempsey, & Skatrud, 2000a).

Both OSA and obesity lead to similar pathogenic pathways. For instance, both are considered a major risk factor for cardiovascular disease and result in oxidative stress and an inflammatory state (Van Gaal, Mertens, & Christophe, 2006; Vincent, Innes, & Vincent, 2007; Vincent & Taylor, 2006; Young et al., 1993). The alteration in upper airway structure, patency and the reduction of the functional residual capacity often associated with obesity can be predisposing factors for the occurrence of OSA (Makki, Froguel, & Wolowczuk, 2013). Frequent cortical arousals during sleep in OSA activate the sympathetic nerve system. Increased sympathetic nerve activation contributes to release of free fatty acids (FFAs), and increased FFAs activate inflammatory pathways and oxidative stress (McNicholas & Bonsignore, 2007; Ryan, Taylor, & McNicholas, 2009). Adipose tissue also promotes inflammatory responses such as CRP, TNF-α, IL-6, and IL-8. Increased levels of circulating inflammatory biomarkers contribute to arterial plaque formation and plaque rupture, and consequently increase the possibility of myocardial infarction (Makki, Froguel, & Wolowczuk, 2013). However, the
relationship between OSA and elevated inflammatory biomarkers is not yet clearly discerned since systemic inflammation in OSA may merely indicate the confounding effects of obesity (Akashiba, Akahoshi, Kawahara, Majima, & Horie, 2005; Barceló et al., 2004; Can et al., 2006; Guilleminault, Kirisoglu, & Ohayon, 2004; Saletu et al., 2006).

**Cardiovascular Disease.** Increasing evidence suggests that OSA is an independent risk factor for cardiovascular morbidity and mortality (American Academy of Sleep Medicine, 2005; McNicholas & Bonsignore, 2007). Although the mechanisms underlying the relationship remain unclear, one possible mechanism is that increased sympathetic activity due to hypoxia and/or sleep fragmentation may result in increased risks of developing cardiovascular disease. Such episodic hypoxia in OSA is likely a cause of oxidative stress and the generation of reactive oxygen species (ROS), which may contribute to the activation of the inflammatory response (Lavie, 2005; Prabhakar, 2002; Schulz et al., 2000; Suzuki, Jain, Park, & Day, 2006) and consequently lead to endothelial dysfunction. Normal endothelium plays a role in mediating coagulation, homeostasis, and immune function and regulating vasomotor tone (Aird, 2007). Impaired endothelial function substantially contributes to the development of cardiovascular manifestations of OSA (Atkeson & Jelic, 2008).

A large epidemiological study identified that inflammatory biomarkers CRP, TNF-α, IL-6, and IL-8 are independent predictors of cardiovascular events and mortality (Koenig et al., 1999). In patients with OSA, medical comorbidities such as cardiovascular disease, and hypertension, all of which are independently associated with elevated inflammatory biomarkers, are likely also confounding factors in delineating the relationship between OSA and inflammation (Toraldo, De Nuccio, De Benedetto, & Scoditti, 2015). Therefore, any study aimed at understanding
inflammatory biomarkers in OSA must carefully address the confounding effect of cardiovascular disease.

**Type 2 Diabetes.** Type 2 diabetes is highly prevalent among adults with OSA, with a 70% prevalence in moderate to severe OSA (Brooks et al., 1994). In OSA, increased fasting plasma glucose levels and insulin levels (i.e., impaired glucose–insulin metabolism) are independent of obesity (Brooks et al., 1994; Ip et al., 2004; Punjabi, Sorkin, Katzel, Goldberg, Schwartz, & Smith, 2002; Vgontzas et al., 2000). The progress of type 2 diabetes show features of chronic, low-level systemic inflammation (Dandona et al., 2005). In OSA, the activated sympathetic response induced by hypoxia and frequent arousal leads to decreased glucose tolerance and impaired insulin resistance. This causes increased risk of type 2 diabetes in OSA (Ip & Mokhlesi, 2007). Increased circulating monocytes into adipose tissue are related to type 2 diabetes. In OSA, the production of TNF-α, secreted by monocytes, is increased (Minoguchi et al., 2004; Yamauchi et al., 2006). Proinflammatory cytokine levels such as TNF-α, IL-6, and IL-8 are increased in patients with type 2 diabetes. Therefore, type 2 diabetes is an important confounding variable that must be accounted for when evaluating the net effect of increased inflammatory biomarkers in OSA (Mohamed-Ali et al., 1997; Weisberg, McCann, Desai, Rosenbaum, Leibel, & Ferrante, 2003).

**Symptoms of OSA**

Beyond excessive daytime sleepiness and fatigue, patients with OSA experience loud snoring or choking at night, witnessed apneas, and awaking with dryness of mouth (American Academy of Sleep Medicine, 2009). Loud snoring is the most common symptom in OSA. Snoring is caused by a narrowing upper airway during sleep due to large tonsils, a long uvula, and/or nasal congestion from deformities of the respiratory cartilage or allergies (Eckert &
Loud snoring is associated with poor sleep quality and quality of life in patients with OSA as well as their partners (Beninati, Harris, Herold, & Shepard, 1999; Goncalves, Paiva, Ramos, & Guilleminault, 2004). Though snoring is a common symptom of OSA, it is not necessarily specific to OSA and thereby not a good candidate symptom for phenotyping OSA.

Another OSA symptom is morning headache, likely caused by repeated airway obstruction with recurrent hypoxia and hypercarbia and cortical arousals (American Academy of Sleep Medicine, 2009). However, morning headaches in patients with OSA may not be a specific OSA symptom since frequent morning headaches are common in patients with other causes of sleep-disordered breathing, such as obesity hypoventilation syndrome and central sleep apnea, and/or comorbidities (Aldrich & Chauncey, 1990). Rather, morning headaches are a non-specific symptom. Aldrich et al. (1990) found that 18% of patients with OSA had morning headaches compared with 21% in other sleep disorder group and 6% of controls (matched for sex and age). Furthermore, the frequency of morning headaches was not related to the severity of OSA (Aldrich et al., 1990). Morning headaches, a non-specific symptom of OSA, are less likely contributive to symptom phenotypes of OSA.

**Excessive Daytime Sleepiness and Fatigue**

Patients with OSA have worse excessive daytime sleepiness than in the general population. The prevalence of excessive daytime sleepiness in OSA is 72% to 87.2% as compared with 5% to 15% in the general population (Seneviratne & Puvanendran, 2004; Puvanendran & Goh, 1998). The adverse outcomes of excessive daytime sleepiness and fatigue in OSA include not only impaired daytime cognitive function, poor quality of life, and threatened public safety, but also increased risk of hypertension and all-cause mortality (Feng, He, Zhang, & Chen, 2012; Gooneratne et al., 2011; Kapur, Resnick, & Gottlieb, 2008; Pagel, 2009).
In the U.S. in the year 2000, a reported 800,000 OSA-related motor vehicle accidents occurred, leading to the death of 1,400 drivers and costs of approximately $15.9 billion (Sassani, Findley, Kryger, Goldlust, George, & Davidson, 2004). A quarter of untreated OSA patients often experience falling asleep while driving (Findley, Levinson, & Bonnie, 1992). If OSA drivers are treated with CPAP, the estimated savings is estimated at 980 lives per year as well as $11.1 billion annually (Sassani et al., 2004). In addition to safety consequences related to OSA symptoms of excessive daytime sleepiness, a rigorous longitudinal cohort study reported a significant relationship between excessive daytime sleepiness and risk of mortality (i.e., mortality hazard ratio of 1.5). The mortality hazard ratio increased to 2.3 when considering AHI ≥ 20 events/h (Gooneratne et al., 2011). These findings suggest the importance of symptom evaluation and management in OSA.

There are several possible mechanisms with regard to the association between excessive daytime sleepiness/fatigue and OSA: 1) frequent cortical arousals and 2) hypoxia (de la Peña Bravo et al., 2007). During sleep, patients with OSA experience airway re-opening against partially or totally blocked respiratory airflow; the episodes are accompanied by arousals. These arousals result in a decrease in the duration of restorative, deep sleep (i.e., slow wave sleep; N3 sleep) and decreased percentages of REM sleep (Jacobsen, Shi, & Mokhlesi, 2013). Frequent arousals stimulate brainstem nuclei, controlling sleep-wake cycles and cardiac function; and subsequently lead to increased sympathetic nerve activation (Bonsignore et al., 2006). An increase in sympathetic drive during sleep causes variability of heart rate and blood pressure and endothelial dysfunction and stimulates wakefulness and cortical arousals during sleep; this in turn leads to excessive daytime sleepiness (Narkiewicz, Montano, Cogliati, Van De Borne, Dyken, & Somers, 1998).
Another potential mechanism leading to excessive daytime sleepiness is apnea-related hypoxia. Repeated apneas increase the production of free radicals, known as reactive oxygen species derived from oxygen, by neutrophils, macrophages, and endothelial cells (Dyugovskaya, Lavie, & Lavie, 2002; Schulz et al., 2000). The imbalance between the production of reactive oxygen species (ROS) and ROS detoxification cause oxidative stress (Lavie, 2015; Schulz et al., 2000). The process results in oxidative damage to molecules and proteins. Vgontzas et al. (1997) have found that proinflammatory cytokines such as TNF-α and IL-6 are positively correlated with excessive daytime sleepiness and fatigue; a 2- to 3-fold increase in levels of TNF-α and IL-6 in OSA was associated with excessive daytime sleepiness compared to healthy, non-OSA controls. These findings suggest that hypoxia may increase the production of TNF-α and IL-6 in target sites such as the central nervous system and the consequence of this is the expression of symptoms, including excessive daytime sleepiness and fatigue.

**Symptom Science**

OSA is a major public health concern due to its increasing prevalence, untoward health outcomes and life-altering symptoms such as excessive daytime sleepiness and fatigue. These two symptoms threaten public safety with fatal motor vehicle crashes and impaired quality of life; therefore, studies that seek to understand these symptoms and improve the management of such symptoms are urgently needed (Aldrich, 1989; American Sleep Disorders Association Diagnostic Classification Steering Committee, 1997; Ohayon et al., 1997; The Healthy Sleep Project, 2014).

National Institute of Nursing Research (NINR) seeks to provide a better understanding of chronic disease and improve quality of life across the life-span. NINR has emphasized the importance of the development of new strategies to manage prevalent symptoms in chronic
conditions, such as impaired sleep and fatigue in OSA. The Institute proclaims that research that addresses biological mechanisms underlying symptoms will help support a better understanding of chronic diseases and improve individuals’ health behaviors (National Institute of Nursing Research, 2011). In accordance with the strategic plan of NINR, studies have sought to identify the biological and genetic factors that influence symptom experiences to guide clinical assessment and support interventions that address symptom management (Starkweather, Lyon, & Schubert, 2011; Wang, Lehky, Brell, & Dorsey, 2012). For example, Wang and colleagues (2012) examined the role of biological factors such as cytokines and chemokines in the development of chemotherapy-induced painful peripheral neuropathy. They identified that TNF-α, IL-1β, and IL-6 are involved in the symptom experience. The identification of underlying mechanism(s) of symptoms may contribute insights into the prevention and management of chemotherapy-induced painful peripheral neuropathy.

There is sparse evidence with regard to potential endogenous factors, such as inflammatory biomarkers, that are likely to contribute to excessive daytime sleepiness and fatigue in OSA. Excessive daytime sleepiness and fatigue are common symptoms and are highly prevalent in adults with OSA. These OSA symptoms confer risks including daytime impairment, motor vehicle crashes, poor social relationship development, depression, and anxiety (Ejaz, Khawaja, Bhatia, & Hurwitz, 2011; MacKenzie et al., 2006; Rosenberg & Doghramji, 2009). However, all OSA patients do not have both of these symptoms and some do not have either symptom (Barbé et al., 2001; Young et al., 1993). Based on clinical evidence and the scientific literature, there are different symptom phenotypes of OSA. The biological mechanism(s) for these varied symptom phenotypes are not yet determined. If the biological mechanism(s) of OSA symptom phenotypes is identified, such insight may contribute to early identification of the
symptom phenotype and potentially better aligned treatment recommendations (i.e., precision health) that address not only OSA severity but also the effective management of symptoms.

**Relevance of Symptom Management in OSA to Nursing Science**

Symptoms of OSA, excessive daytime sleepiness and fatigue, are increasingly considered an important health problem due to the adverse consequences including impaired daytime function and increased risk of cardiovascular disease and mortality (Feng et al., 2012; Gooneratne et al., 2011; Pagel, 2009). Despite the recognized significance of OSA symptoms for adverse outcomes, the biological cause of symptom expression, and/or the multiple factors that influence these symptoms, is not well-understood (Vgontzas et al., 1997). Prior studies have identified biologic variations associated with symptoms in patients with OSA (Vgontzas et al., 1997). Further studies are needed to define symptom phenotypes, observable characteristics of an individual’s symptoms combined with endogenous factors associated with the symptoms in OSA (i.e., molecular signatures for OSA).

So far, OSA treatment recommendations including CPAP, upper airway surgical modification, supplemental oxygen, and oral appliances, are prescribed based on AHI alone (Epstein et al., 2009). Excessive daytime sleepiness and fatigue are considered indicators of the disease and responses to treatment (Lau, Eskes, Morrison, Rajda, & Spurr, 2013). Health care providers do not necessarily follow symptom resolution with treatment but rather focus on degree of disease resolution indicated by AHI only (Montserrant et al., 2001). By addressing symptoms of OSA from a symptom management perspective, attention in the discipline of both nursing and medicine will be directed to the importance of not only evaluating efficacy (i.e., by AHI) but also evaluating effectiveness of treatment for symptom resolution.
Nursing science focuses on perceived experiences of individuals and provides a distinct scientific perspective for not only clinical features of symptoms but also biologic mechanisms of symptoms relative to specific diseases (Cashion & Grady, 2015). If the biological cause of symptom expressions, or symptom phenotypes, in OSA is understood, this insight may help health care providers address treatment response at the biological level. NINR’s strategic plan focuses on “symptom management, improving the understanding of the biological mechanisms underlying symptoms and developing new strategies for symptom management that will help individuals with chronic illness by improving their quality of lives.” (National Institute of Nursing Research, 2011, p. 14). This study will contribute to symptom science and symptom management by identifying the biologic mechanism(s) associated with symptoms in OSA and begin to build a foundation that addresses response to treatment(s) that is based on biological understanding of the symptoms.

**Conceptual Framework for the Study**

The middle-range Theory of Unpleasant Symptoms guided the present research. The Theory of Unpleasant Symptoms, which is based on a post-positivist perspective, has been tested in various clinical settings and provides a connection between research and practice (Lenz et al., 1995; Lenz et al., 1997). The major concept of the theory is that multiple symptoms, affected by physiological, psychological, and situational factors, lead to performance outcomes (Lenz et al., 1997). The Theory of Unpleasant Symptoms, originally, had a narrow focus on a single symptom (Lenz et al., 1995). The theory developers conducted research regarding a single symptom; Gift and Cahill (199) studied dyspnea, while Pugh and Milligan (1995) studied fatigue at two different perinatal phases. When the studies about individual symptoms were carried out, the investigators identified the commonalities of dyspnea and fatigue across the experience. The
major considerations when they merged those two symptoms were that both fatigue and dyspnea have a similar nature of the symptom experience as well as similar physiological, psychological, and situational factors (Lenz et al., 1997). Thus, the theory was recognized to potentially address multiple, simultaneous symptoms.

Excessive daytime sleepiness and fatigue are the most common symptoms of OSA (Bardwell et al., 2003). Although these terms are often used interchangeably in the literature and lead to similar consequences such as functional limitations and reduced daytime activities, there are important conceptual differences that must be acknowledged (Lau et al., 2013; Pigeon, Sateia, & Ferguson, 2003; Shen, Barbera, & Shapiro, 2006). Excessive daytime sleepiness is the propensity to fall asleep and is related to sleep fragmentation, frequent arousal, and short sleep duration (Hauri & Fisher, 1985; Laffont et al., 2002; Zammit, 1988). It reflects a physiological need to sleep. To improve or modify excessive daytime sleepiness, total sleep time and sleep architecture needs to be addressed by treatment or intervention (Rosenberg & Doghramji, 2009; Slater & Steier, 2012). Fatigue, on the other hand, is a multidimensional symptom, including physical and psychological factors as well as social influences (Cella, Peterman, Passik, Jacobsen, & Breitbart, 1998; Steptoe, Hamer, & Chida, 2007). Fatigue is defined as a feeling of tiredness, lack of energy, and decreased strength. It is often associated with mood disorders and inflammation, and therefore treatment that improves fatigue necessarily addresses inflammation (Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008).

These two different symptoms, however, often occur in combination in OSA and are interrelated. Excessive daytime sleepiness and fatigue lead to changes in daily performance. For example, 52% of vehicle crashes are related to fatigue and 17.6% of drivers in these crashes also had excessive daytime sleepiness (National Transportation Safety Board, 1995). Factors that
influence the given symptoms may be different; whereas excessive daytime sleepiness is more likely to be related to sleep quality (physiological factor), fatigue is positively correlated with inflammatory cytokine levels (physiological factor) (Bardwell et al., 2003; Dantzer et al., 2008; Hossain et al., 2005; Jackson, Stough, Howard, Spong, Downey, & Thompson, 2011). The interaction of multiple influencing factors can simultaneously affect the symptom experience. Furthermore, the combination of influencing factors is likely to result in a more intense and distressing symptom experience.

The influencing factors, such as sleep fragmentation, intermittent hypoxia, and inflammation in patients with OSA, affect excessive daytime sleepiness and fatigue, and the adverse symptoms are associated with increased risk of cardiovascular morbidity, motor vehicle accidents, poor quality of life, and impaired cognitive function (Ford & Kamerow, 1989; Maggi et al., 1998; Newman, Enright, Manolio, Haponik, & Wahl, 1997; Newman et al., 2000). The following summary will describe the co-occurring symptoms and the influencing factors and how they relate to quality of life, impaired cognitive function, and increased risk of cardiovascular diseases in OSA.

**Major Concept of Theory of Unpleasant Symptoms**

The theory has three major components: 1) symptoms experienced by individuals; 2) the influencing factors, affecting the essence of the symptoms experience; and 3) the outcomes of the symptom experience (Lenz et al., 1997).

**Symptoms.** Symptoms are a central concept in the theory and are measured in terms of intensity, distress, timing, and quality. These four dimensions are separate, but related to each other (Lenz et al., 1997). *Intensity* refers to the dimension that quantifies the severity, strength, or degree of the symptom experienced by individuals. The level of *distress* perceived by individuals
reflects the degree to which individuals are bothered by symptoms, and it is related to the dimension of intensity. The dimension of distress experienced with a symptom contributes to quality of life. *Time* includes the duration of a persistent symptom (i.e. acute vs. chronic), frequency of symptom occurrence, and timing of the symptom in relationship to specific activities. *Quality* describes the nature of the symptom sensation and the degree to which symptoms response to a particular treatment or intervention (Lenz & Pugh, 2003).

Symptoms can occur in isolation but, more frequently, individuals experience multiple symptoms. Most symptoms are experienced as unpleasant (Lenz et al., 1997). As originally proposed by Lenz et al. (1997), the theory focused on subjectively perceived symptoms rather than observable, or objective, symptoms. Kim et al. (2005) argued that the term, symptoms, should be broad enough to include both subjectively perceived symptoms and objectively observable signs, because not all symptoms include just patients’ subjective experiences. Lenz and Pugh (2003) clarified the range of the symptoms as functional changes subjectively perceived by individuals as well as signs that are objectively observable by others.

In the theory, multiple symptoms can occur simultaneously as a result of a single event. In addition, one symptom may result in another symptom. Reishtein (2005) examined the relationship among multiple symptoms experienced by patients with chronic obstructive pulmonary disease (COPD). The multiple symptoms including dyspnea, fatigue, and sleep difficulty occurred simultaneously. The symptom of dyspnea, in particular, was related to worsening other symptoms, such as fatigue and sleep difficulty. In patients with OSA, multiple symptoms, such as fatigue and excessive daytime sleepiness, occur simultaneously. Therefore, in light of previous studies, gaining an understanding of the nature of each symptom associated with OSA may shed light on the interaction among the multiple symptoms of OSA.
Influencing Factor. In the Theory of Unpleasant Symptoms, there are three categories of influencing factors: physiological factors, psychological factors, and situational factors (Lenz et al., 1997). The three categories of factors may interact to influence the symptom experience. For example, the combination and/or interaction of inflammation (physiological), mood disturbance (psychological), and a short sleep duration (situational) may result in a more intense symptom experience than if only one of these factors exists alone.

Physiological factors refer to anatomical, physiological, genetic, and intervention or treatment-related variables (Lenz et al., 1997). Examples include anatomical anomalies, the stage and duration of illness, comorbidities, level of consciousness, race, age, inflammation, and type and duration of treatment/intervention. Various physiological factors may influence the occurrence of a symptom and interact with different other influencing factors (Lenz et al., 1997). According to Pugh and Milligan (1998), the fatigue experienced by breastfeeding mothers is influenced by multiple physiologic factors, including the type of delivery, the duration of labor, the presence of infection, and the quality of sleep. In OSA, the change of sleep architecture due to the sleep fragmentation and frequent arousals is associated with excessive daytime sleepiness and fatigue (Gislason & Sunnergren, 2014; Steirpoulos et al., 2010). Sleep fragmentation and frequent arousals are physiological factors that influence symptoms in OSA.

Psychological factors refer to the affective state or mood of the individual (e.g., the level/degree of depression and anxiety) and the degree of knowledge about the symptoms or diseases. The emotional response to symptoms or to a disease can contribute to perceptions of intensity, distress, timing, and quality of symptoms (Lenz et al., 1997). Pugh and Milligan (1995) have shown that pregnant women with high stress experience more severe fatigue when compared to those with less stress. In epidemiologic studies that examined the effect of OSA on
mood disturbance, the presence of OSA is positively correlated to the incidence of mood disturbances. The depressive disorder is then likely to influence the severity of fatigue and excessive daytime sleepiness in OSA (Ohayon, 2003). A recent study has shown the effect of increased inflammatory cytokines on the relationship between OSA, depression, and cardiovascular disease (Ejaz et al., 2011). Depression, as a psychological factor, is related to inflammation, a physiological factor, and both factors affect the severity of fatigue and excessive daytime sleepiness in OSA (Ejaz et al., 2011).

Situational factors include the social and physical environment that may influence the experience of symptoms. Examples of social environment include access to social support, marital and family status, socioeconomic status, access to health care resources, and lifestyle behaviors. The physical environment (i.e., external conditions, events, and factors surrounding individuals) includes temperature, noise level, and presence of pollutants (Lenz & Pugh, 2003). In OSA, short sleep duration is likely to be the most important situational factor that affects fatigue and excessive daytime sleepiness (Priou et al., 2014). Studies have shown relationships between (1) short sleep duration and increased risk of cardiovascular disease (consequence of symptoms) in OSA; (2) short sleep duration and increased levels of cytokines (physiological factors); and (3) short sleep duration and mood disturbance (psychological factors) (Pilcher & Huffcutt, 1996; Priou et al., 2014; Vgontzas et al., 2004b). It is therefore evident that these influential factors in OSA are interrelated and affect the occurrence of symptoms as well as the consequence of symptoms.

Performance Outcomes. The last element of the theory is performance outcomes. The symptom experience can affect individuals’ performance, such as physical activities and cognitive tasks. Functional performance includes activities of daily living, physical activity, and
work-related roles. Cognitive performance is conceptualized to include concentration, memory, and problem-solving (Lenz & Pugh, 2003). In OSA, excessive daytime sleepiness contributes to adverse consequences such as impaired driving performance with two to three times increased risk of motor vehicle crashes, poor quality of life, and impaired daily functioning (MacKenzie et al., 2006; Rosenberg & Doghramji, 2009). A number of studies have demonstrated a relationship between OSA and poor quality of life and impaired social function (Engleman & Douglas, 2004; Jenkinson, Stradling, & Petersen, 1997; Kales et al., 1985; Smith & Shneerson, 1995). OSA also impacts impaired work performance and interpersonal relationships in severe OSA (Kales et al., 1985). The Theory of Unpleasant Symptoms provides a picture of the relationship between symptom experience and the performance outcomes, as well as between how influential factors influence the symptom experiences and the downstream effects on performance outcomes.

**Relationships among Concepts**

The Theory of Unpleasant Symptoms originally depicted symptoms as occurring in isolation of each other. There are unidirectional relationships between influencing factors and symptom experience, and between symptom experience and performance of symptoms (Lenz et al., 1995). However, the revised version of the theory identified that symptoms tend to occur simultaneously and the three major components of the theory are not unidirectional, but rather reciprocal (Lenz et al., 1997).

Unpleasant symptoms are subjective experiences, which are appraised by individuals (Lenz et al., 1997). Symptoms lead to changes in normal function and may be accompanied by unpleasant sensations (Lenz & Pugh., 2003). This perception-based concept assumes that individuals are aware of their symptoms. The nature of symptoms can be identified by the individual experiencing the symptoms. The unpleasant symptoms of OSA, excessive daytime
sleepiness and fatigue, are subjective symptoms assessed by self-report. Individuals can score how sleepy or fatigued they are by rating their symptoms as, for example, “very sleepy” or “frequent fatigue” (Guilleminault & Brooks, 2001; Johns, 1993; Pagel, 2009). In fact, excessive daytime sleepiness and fatigue in patients with OSA occur simultaneously, not in isolation of each other. These two symptoms are often significantly related each other (Chervin, 2000; Merkelbach et al., 2011; Mills, Kim, Bardwell, Hong, &Dimsdale, 2008). The American Academy of Sleep Medicine (2005) recommends that both excessive daytime sleepiness and daytime fatigue should be evaluated for the diagnosis of OSA in addition to measuring the severity of OSA.

Sleep fragmentation and arousals (physiological factors) are also related to the occurrence and the severity of excessive daytime sleepiness and fatigue (Pagel, 2009). The unpleasant symptoms in OSA are significantly improved with CPAP treatment by reducing arousals and fragmentation of sleep, and consequently, OSA treatment results in improved quality of life (Tomfohr, Ancoli-Israel, Loredo, & Dimsdale, 2011). Yet, in spite of the recognized impact of repetitive hypoxia on inflammation in OSA, there is a limited understanding of the relationship between inflammation and everyday symptoms, such as excessive daytime sleepiness and fatigue. Furthermore, there are variations in the expression of symptoms in OSA; not all OSA patients have sleepiness and/or fatigue (Barbé et al., 2001; Young et al., 1993), even when OSA severity is controlled. This suggests that OSA symptom phenotypes may exist. To date, there is a substantial body of research that examines the interaction of fatigue and inflammation in other chronic diseases such as cancer and bowel disease (Bower, 2007; Meyers, Albitar, & Estey, 2005; Pellino et al., 2014; Reyes-Gibby, Wang, Spitz, Wu, Yennurajalingam, & Shete, 2013). Such studies have identified that fatigue severity is correlated with increased levels of the
inflammatory cytokines in terms of IL-6 and TNF-α (Meyers et al., 2005). It is, therefore, possible that OSA molecular signatures may influence varied symptom phenotypes in OSA; a study identifying the interaction and/or relationship between inflammation and excessive daytime sleepiness and fatigue in OSA is therefore needed.

**Hypothesized Models for Understanding the Relationship between Inflammation, Excessive Daytime Sleepiness, and Fatigue in OSA**

An adapted conceptual model that combines a physiologic framework and the Theory of Unpleasant Symptoms was employed in this research. The physiological framework is merged with the Theory of Unpleasant Symptoms in order to understand the mechanism of three categories of influencing factors impact on the symptom experience as well as the consequence of symptoms (Figure 2.3).

The distinct attributes of airway muscle play a crucial role in the pathophysiology of OSA. Patients with OSA experience snoring and partial or total airway collapse during sleep (American Academy of Sleep Medicine, 2005). Reduced activity of pharyngeal muscles at sleep onset predisposes the upper airway to collapse and obstruction. The upper airway collapse is less likely to occur in healthy adults (i.e., non-OSA) since they maintain a consistent tension of upper airway muscles. If airway collapse occurs in healthy adults, it is re-opened by activation of the respiratory drive and normoxia is maintained (Fogel, Malhotra, & White, 2004). In OSA however, the apnea episodes that occur lead to hypoxia. Repetitive hypoxia during sleep leads to the activation of the sympathetic nervous system and oxidative stress, and consequently, to systemic inflammation (May & Mehra, 2014). The respiratory stimuli to re-open the airway induce frequent arousals with a resultant sleep fragmentation and thereby curtailed sleep duration (Chamberlin, 2013). Sleep fragmentation and repetitive hypoxia leads to release of
neurotransmitters such as serotonin, norepinephrine, and gamma-aminobutyric acid (GABA), which also play a crucial role in the regulation of mood (Ejaz et al., 2011; Ishman, Cavey, Mettel, & Gourin, 2010).

OSA is a chronic disease as individuals with OSA report the development of multiple symptoms, such as fatigue and excessive daytime sleepiness, with untreated OSA (American Academy of Sleep Medicine, 2005; Calverley, 1997; Chervin, 2000; Young et al., 1993). Patients with OSA, therefore, require ongoing and long-term management of their OSA and symptoms. This approach is recommended based on the consequences of untreated OSA and persistent symptom experience. Studies have demonstrated that OSA with more severe symptoms results in adverse consequences such as impaired functional status, poor quality of life, and reduced physical performance (Fawcett, Tulman, & Myers, 1988; Graydon, Ross, Webster, Goldstein, & Avendano, 1995; Pugh & Milligan, 1995).

The adverse consequences of the OSA symptoms of excessive daytime sleepiness and fatigue are associated with sleep fragmentation (physiological factor), mood disturbance (psychological factor), and short sleep duration (situation factor) (Gislason & Sunnergren, 2014; Ohayon, 2003; Priou et al., 2014; Steirpoulos et al., 2010). However, the relationship of inflammation and symptoms (i.e., excessive daytime sleepiness and fatigue) in OSA has not been clearly explicated. Furthermore, it is not clear if diurnal variation of inflammation plays a role in the expression of symptoms, or symptom phenotypes, when excessive daytime sleepiness and fatigue are considered. Therefore, the combined conceptual model underpins the present research and supports the research questions.
Chapter 2 Summary

A critical review of the extant literature has identified that the relationship between diurnal variation of inflammatory biomarker levels and OSA and between inflammatory biomarker levels and excessive daytime sleepiness and fatigue is not well explicated. The combination of the pathophysiological framework and the Theory of Unpleasant Symptoms that form the research framework for the present study may improve the understanding of the biological mechanisms underlying symptoms and begin to address symptom phenotypes of OSA. The extant literature shows that inflammatory biomarkers, a physiologic mechanism, are associated with specific symptoms. The present study intended to examine OSA symptoms and the relationship of these symptoms to potential biological causal mechanisms such as inflammation. The results of this study will potentially allow the field to develop strategies for the effective management of common OSA symptoms in tandem with highly efficacious treatment decisions for OSA.
Figure 2.3. Hypothesized Model

- Decreased pharyngeal muscle activity
- Sleep onset
- Airway obstruction
- Hypoxia
- Apnea
- Excessive daytime sleepiness
- Fatigue
- Poor quality of life

Factors:
- Age
- Obesity
- Type 2 diabetes mellitus
- Cardiovascular disease

Influences:
- Inflammation
  - Arousals during sleep
  - Sleep fragmentation
- Mood disturbance
- Short sleep duration

Interaction:
- Reciprocal influence on factor or symptoms
Chapter 3

Method

This study examined inflammatory biomarkers, including proinflammatory cytokines, anti-inflammatory cytokines, and CRP as a physiological factor, affecting the symptom experience in OSA. Hypoxia and arousals induced by OSA lead to changes in molecular processes, and accordingly, a molecular signature(s) for OSA can be identified. Patients with OSA experience frequent sleep fragmentation, which affects daytime function. Inflammatory cytokines are also involved in sleep regulation and demonstrate a diurnal pattern of expression. To date, little is known about the relationship between inflammation and excessive daytime sleepiness and fatigue in OSA and, in particular, about the relationship between the diurnal variation of inflammatory biomarkers and daytime symptoms in OSA. The overall objective, therefore, is to describe the molecular signature of OSA and symptom phenotypes in adults with OSA. The research questions are: 1) what is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA, and is there a significant relationship between diurnal variation and AHI; 2) is there an association between inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP and everyday symptoms, including excessive daytime sleepiness and fatigue, in adults with OSA; 3) is diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP associated with symptoms in adults with OSA; and 4) what is symptom phenotype in adults with OSA.

Design of the Study

A cross-sectional cohort study was conducted to address the research questions. The protocol was approved by the Institutional Review Board (IRB) at Penn State Hershey Medical
Center (PSHMC). The cohort consisted of adults with suspected OSA recruited from a clinical sleep center. After informed consent, participants completed a diagnostic polysomnography with symptoms and inflammatory biomarkers measures assessed pre- and post-polysomnography. All measures were collected in a single-night, but with two data points which permits diurnal variation to be examined. This design supported the acquisition of data at the protocol periods necessary to address the research questions.

Sample and Setting

Study Population

The target population for this study was adults (≥18 years) with suspected OSA, referred for diagnosis at the PSHMC’s sleep center and undergoing in-laboratory diagnostic polysomnography. Potential participants were initially approached by a polysomnography technologist at the sleep center to elicit their interest in speaking with a researcher about study participation. The principal investigator met with interested study participants to provide: a full description of the study; risks and benefits of participation; an explanation of voluntariness of study participation; and the option to withdraw at any time during study participation.

Sample Size Calculation

Sample size calculations for the present study were based on achieving a certain level of precision around an estimate of partial correlation, which permits evaluation of confounding factors for the relationships under study. There is sparse evidence that addresses the diurnal variation of inflammatory biomarkers in OSA. Based on relatively small preliminary studies and assumptions for the current study, an estimated mean difference, pre- to post-polysomnography, in biomarker levels is estimated at 0.3. In order to have a level of precision ±0.3 for a 95% CI around an estimate of partial correlation, a sample size of 32 subjects was required. This
calculation assumed 15% withdrawal, and a level of correlation around 0.5. Based on the 0% attrition rate, the required sample size was 28 (Table 3.1).

**Table 3.1. Sample Size Estimation**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Precision for 95% CI</th>
<th>Sample</th>
<th>Alpha</th>
<th>Attrition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial correlation among variables</td>
<td>±0.3</td>
<td>32</td>
<td>0.05</td>
<td>15%</td>
</tr>
<tr>
<td>Partial correlation among variables</td>
<td>±0.3</td>
<td>28</td>
<td>0.05</td>
<td>0%</td>
</tr>
</tbody>
</table>

Over the study period there was no withdrawal; however, 62.3% of enrolled study participants either did not have OSA or underwent a split-night polysomnography study (n=38). The final sample size (n=22) may not provide sufficient power to detect statistically significant differences based on the *a priori* sample size estimate.

**Inclusion and Exclusion Criteria**

To be eligible to participate in the study, the following study inclusion criteria were met:

1) OSA diagnosed by polysomnography with the AHI ≥5 events/hour; 2) males and females ≥18 years of age; 3) able to read and speak English. Participants were excluded for the following reasons: 1) participation in a split-night study (i.e., diagnostic polysomnography followed by CPAP titration during polysomnography on the same night if OSA is indicated during the diagnostic portion of the polysomnography); 2) any treatment of OSA within previous one year or any current treatment of OSA (i.e., CPAP, oral appliances, upper airway surgery for OSA, and bariatric surgery); 3) comorbid inflammatory conditions including current and/or recent acute infections and any pre-existing autoimmune diseases diagnosed within the past 3 months; 4) use of antibiotics and/or immunosuppressive medication (e.g., corticosteroids) within the previous 3 months or current use; 5) concurrent regular use (> 3 nights/week) of sedative/hypnotics; 6)
catecholamine therapy within 1 month prior to enrollment and 7) diagnosis of another sleep disorder, including insomnia or narcolepsy, in addition to OSA, based on polysomnography, multiple sleep latency test, and/or sleep diary records.

**Recruitment Process**

On the night of diagnostic polysomnography, patients referred for a polysomnography to diagnose OSA were queried by a sleep center polysomnography technologist about their willingness to speak with a researcher about participating in a one-night research protocol. Clinical patients arrived at the sleep center approximately 2-3 hours prior to the start of polysomnography for acclimatization to the sleep environment in the laboratory and study hook-up. At the time of checking-in for polysomnography and baseline vital sign collection, patients were asked by the polysomnography technologist for willingness to speak to a researcher. With a positive response from adult patients (≥18 years), the principal investigator was introduced to the patient for full description of the study protocol and informed consent. Participants completed the Berlin Questionnaire to pre-screen for likelihood of OSA. As this study necessitates participants have OSA with diagnostic polysomnography AHI ≥5 events/hour, the use of a pre-diagnosis screening questionnaire reduced the risk of enrolling participants who were not likely to have OSA.

**Study Measures**

All measures for this study are summarized in Table 3.2.

**Demographic Questionnaire**

A 16-item demographic questionnaire was used to acquire self-reported baseline characteristics. Items address gender, marital status, income, education, employment, marital
status, presenting sleep symptoms, referral source, work schedule, and non-prescription medications.

**Berlin Questionnaire**

The Berlin questionnaire was used for screening of obstructive sleep apnea (Netzer, Stoohs, Netzer, Clark, & Strohl, 1999). The questionnaire includes 11 items divided into three categories. Category 1 comprises 5 questions regarding the presence of snoring, the intensity and frequency of snoring, and witnessed apneas. Category 2 comprises four questions regarding fatigue and daytime sleepiness. The last category comprises two questions regarding the presence of high blood pressure and current BMI (cutoff values is a BMI of >30kg/m²) (Netzer et al., 1999). The questionnaire can be completed in less than five minutes (Morgenstern, Wang, Beatty, Batemarco, Sica, & Greenberg, 2014). Study subjects are scored either as being at high risk of OSA or low risk of OSA. High risk of OSA is determined if two or more categories are scored as positive; low risk of OSA is determined if less than two categories are scored as positive. The internal consistency of the questionnaire is high with Cronbach’s α of 0.86 to 0.92 (Netzer et al., 1999). The sensitivity ranges from 54% to 85% and the specificity ranges from 43% to 87% (Ahmadi, Chung, Gibbs, & Shapiro, 2008; Netzer et al., 1999; Weinreich, Plein, Teschler, Resler, & Teschler, 2006).

**Epworth Sleepiness Scale (ESS)**

The ESS was used for measuring lasting symptoms of daytime sleepiness in adults with OSA. The ESS asks the degree to which individuals appraise eight different situations in their chances of dozing off (Johns, 1993). The respondents can rate the degrees of sleepiness on a scale of 0 to 3 (0 indicates “would never doze”; 3 indicates “high chance of dozing”). The total scores of ESS range from 0 to 24; higher ESS scores suggest more severe daytime sleepiness. A
total score less than 10 is defined as normal, whereas a total score greater than or equal to 10 is considered to be an indicator of excessive daytime sleepiness (Johns, 1993). The ESS has been validated in OSA (Johns 1992; Johns 2000) with high internal consistency ($\alpha = 0.88$; Johns 1992).

**Stanford Sleepiness Scale (SSS)**

This instrument measures subjective sleepiness levels at the time of evaluation, or at the present moment (i.e., momentary symptom), on a scale of one to seven: a rating of 1 is described as “feeling active, vital, alert, or wide awake,” and a rating of 7 is described as “Almost in reverie, cannot stay awake, sleep onset appears imminent.” A score of 4 or above indicates that respondents are subjectively sleepy (Hoddes, Zarcone, & Dement, 1972). The single-item survey can be completed in one minute (Chang, Chen, Wu, Hsu, Liu, & Hsu, 2014). Convergent validity of SSS using the Wilkinson addition and vigilance tests show a correlation of 0.68 and test-retest reliability correlation coefficient is 0.88 (Hoddes, Zarcone, Smythe, Philliphs, & Dement, 1973).

**Lee’s Fatigue and Energy Scales**

This instrument was initially developed for adults with sleep disorders (Lee, 1993). The scale includes two subscales including 13 fatigue items (items 1-5 and 11-18) and 5 energy items (items 6-10) to measure momentary fatigue and energy levels. Each item is rated on a 0 to 10 numeric rating scale. The cut point score for morning fatigue is $\geq 3.2$ (i.e., greater than equal to 3.2 indicates fatigue) and for evening fatigue is $\geq 5.6$ (i.e., greater than equal to 5.6 indicates fatigue); the cut point score for morning energy is $\leq 6.0$ for morning energy (i.e., less than equal to 6.0 indicates low levels of energy) and for evening energy is $\leq 3.5$ (i.e., less than equal to 3.5 indicates low levels of energy) (Lee, Hicks, & Nino-Murcia, 1991). The scale can be completed in less than two minutes (Lerdal, Kottorp, Gay, & Lee, 2013). The internal consistency of the
scale is high, ranging from $\alpha = 0.94$ to 0.96 (Lee et al., 1991). Concurrent validity with Stanford Sleepiness Scale (SSS) and Profile of Mood States Scale (POMS) has been established (Lee et al., 1991).

**Profile of Mood States (POMS) 2nd Edition-Adult Short**

The Profile of Mood States 2nd Edition-Adult Short (POMS 2-A Short), was used for the assessment of lasting mood states as a psychological factor. The scale consists of 35 questions with descriptive words of emotion and mood; the measure assesses current mood state (i.e., at time of measurement). Each item is rated on a Likert scale of 0 (not at all) to 4 (extremely) (McNair, Lorr, & Droppleman, 1992). Eight subscales of the POMS include Anger-Hostility (AH), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Activity (VA), Friendliness (F), and Total Mood Disturbance (TMD). A total mood disturbance score (TMD) is calculated with this formula:

$$TMD=(AH+CB+DD+FI+TA)-VA$$

(Lin, Hsiao, & Wang, 2014). Higher scores indicate more negative mood states. The POMS 2 short version can be completed within three to five minutes (Lin et al., 2014). The internal consistency of the POMS 2 ranged from .80 to .95 and a test-retest reliability coefficient range from .52 to .80 (Lin et al., 2014). For the purposes of this study, the TMD score will be used in symptom analyses.

**Quebec Sleep Questionnaire (QSQ)**

The QSQ was used for measuring lasting quality of life, a consequence of excessive daytime sleepiness and fatigue in OSA. QSQ is a disease-specific questionnaire to assess health-related quality of life in patients with OSA. The instrument includes 32 items with five domains: hypersononolence, diurnal symptoms, nocturnal symptoms, emotions, and social interactions. Each item is scored on a 7-point Likert scale, with higher scores indicating better quality of life.
The questionnaire takes approximately 10-15 minutes to complete (Shahid, Wilkinson, Marcu, & Shapiro, 2011). The internal consistency ranged from .68 to .94. The test-retest reliability is high, ranging from .82 to .91 (Lacasse, Bureau, & Series, 2004).

**Perceived Stress Scale (PSS)**

The Perceived Stress Scale was used to measure how much individuals express situations in their lives as stressful over the past month (i.e., lasting stress). The scale consists of 10 brief questions. Each item is rated on a Likert scale of 0 (never) to 4 (very often) (Cohen, Kamarck, & Mermelstein, 1983). The total scores can range from 0 to 40, along with higher scores which indicate greater likelihood for perceived stress. According to the scoring manual, PSS’s scores ranging from 0 to 7 indicate much lower than average perceived stress levels; PSS’s scores ranging from 8 to 11 are considered slightly lower than average perceived stress levels; PSS’S scores ranging from 12 to 15 indicate average perceived stress levels; PSS’s scores ranging from 16 to 20 are associated with slightly higher than average perceived stress levels; and PSS’s scores of 21 and over indicate much higher than average perceived stress levels (Cohen et al., 1983). Out of 10 items, four perceived coping scores (items 4, 5, 7, and 8) were obtained; six perceived distress scores (items 1, 2, 3, 6, 9, and 10) were reobtained by reversing responses (i.e., 0=4, 1=3, 2=2, 3=1 & 4=0). Cronbach’s alpha for assessing the internal consistency of the PSS was 0.75 (Cohen et al., 1983). Cohen and Williams (1988) examined the relationship the sleep quality and PSS scores; the negative correlation was small, but statistically significant (r=0.08, p<0.0001).

**Beck’s Depression Inventory (BDI)**

The Beck Depression Inventory is one of the most widely-used instruments for quantifying levels of lasting depression (Beck & Beamesderfer, 1974). The BDI consists of 21
items; respondents can rate each symptom item with a four-point scale, ranging from 0 (absent) to 3 (severe). The total scores range from 0 to 63; a higher score indicates greater likelihood for depression. The BDI scores of 0 to 13 are considered within the normal range; the BDI scores of 14 to 16 indicate mild depression; the scores of 17 to 20 are considered moderate depression; and the score of 21 or more indicates the high possibility of severe depression (Nielson, 1980). The internal consistency for the BDI is moderate to high with Cronbach’s α of 0.73 to 0.92 (Beck, Steer, & Garbin, 1988).

**Polysomnography (PSG)**

All subjects underwent overnight, in-laboratory diagnostic polysomnography, using standard techniques according to the standards of the American Academy of Sleep Medicine (2007) and scoring criteria for sleep stages and arousals from sleep, including hypopnea event desaturation level of ≥3% (Iber, Ancoli-Israel, Cherson, & Quan, 2007). Polysomnography includes simultaneous recording of electroencephalography (EEG), electrooculography (EOG), submental electromyography (EMG), oronasal airflow by an airflow pressure transducer and thermistor, electrocardiography (ECG), chest and abdominal respiratory efforts by plethysmography, arterial oxyhemoglobin saturation (SpO₂) by pulse oximetry, and body position (American Academy of Sleep Medicine Task Force, 1999). The polysomnographic parameters derived from polysomnography include apnea hypopnea index (AHI), oxidative desaturation index (ODI), lowest arterial oxyhemoglobin saturation (lowest SpO₂ nadire), mean arterial oxyhemoglobin saturation (mean SpO₂), arousal index, total sleep time (TST), sleep stages including N1 (non-rapid eye movement [NREM] 1), N2 (NREM 2), N3 (NREM 3 or slow wave sleep [SWS]), and stage R (rapid eye movement [REMI]), respiratory event related arousals (RERA), non-respiratory arousals or spontaneous arousals (ArI), sleep latency (SL), and sleep
efficacy (%TST/time in bed) (American Sleep Disorders Association, 1992; American Academy of Sleep Medicine, & Iber, 2007).

**Inflammatory Biomarkers**

Both inflammatory biomarkers including CRP, IL-1β, IL-6, IL-8, IL-10, and TNF-a and cortisol were obtained from a venous blood sample via an antecubital vein. A previous study identified diurnal variation of serum IL-6 levels between 8:00 p.m. and 10:00 p.m. (before sleep) and between 6:00 a.m. and 7:00 a.m. (after sleep) in OSA adults (Maeder et al., 2015). In accordance with these findings, the blood samples in the current study were acquired before (i.e., 8-10p.m.) and after (i.e., 6-7a.m.) sleep. Ten milliliters of blood sample were drawn by a qualified professional (i.e., principal investigator) for inflammatory biomarker assessment. Blood was collected in two tubes, one containing ethylenediaminetetraacetic acid (EDTA) and the other containing sodium heparin (EDTA samples were on ice). Samples were then centrifuged (3000 rpm for 10 min), the supernatant (plasma) was removed by pipette, and 300μL of aliquots were stored at -80°C in 1.5ml Eppendorf tubes. Blood samples were measured using either enzyme-linked immunosorbent assay (ELISA) or multiplex assay.

**Enzyme-Linked Immunosorbent Assay (ELISA)**

CRP levels in serum were measured using the enzyme-linked immunosorbent assay (ELISA) kits (Item NO. 10011236, Cayman Chemical Company, Ann Arbor, MI, USA). 100μl of 1:4,000 diluted samples were added to a 96 well pre-coated plate and incubated for one hour at room temperature with continuous shaking. Then plate was washed four times. 100μl pre-diluted conjugate was added and incubated for 30 minutes at room temperature with continuous shaking. Then the plate was washed four times. Color development was performed using 100μl substrate solution for five minutes then stopped using 100μl stop solution. The optical density
(OD) values were determined at 450 nm on an ELISA plate reader. CRP concentrations were quantified by comparison to the standard curves. The sensitivity for CRP assay ranged from 46.9pg/ml to 3,000pg/ml. The intra-assay coefficient of variation ranged from 1.9% to 7% (Cayman Chemical, 2015).

**Multiplex Assays**

Levels of IL-1β, IL-6, IL-8, IL-10, and TNF-a in serum were measured using a Luminex Human MMP 5-Plex Panel (Invitrogen Corporation, Carlsbad, CA, USA). Microarray beads were incubated with 100μl of sample and 50μl of incubation buffer for 2 hours at room temperature with continuous shaking. The beads were washed two times. 100μl pre-diluted biotinylated antibodies were added and incubated for 1 hour at room temperature with continuous shaking. After washing two times, 100μl pre-diluted R-phycoerythrin–labeled streptavidin (SA-RPE) was added and incubated for 30 minutes at room temperature with continuous shaking. The beads were washed three times and re-suspended in 125μl wash solution. The plates were analyzed using a Luminex MAGPIX instrument. The sensitivity for the Multiplex assay ranged from less than 0.5pg/ml to less than 5pg/ml for study cytokines. The intra-assay coefficient of variation was less than 10%. The inter-assay coefficient of variation ranged from 7% to 9.8% (Invitrogen Corporation, 2010).
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Instrument Description &amp; Purpose</th>
<th>Variables/Outcomes</th>
<th>Time of Measure</th>
</tr>
</thead>
</table>
| Berlin Questionnaire | • 10-item questionnaire  
• Pre-diagnosis risk estimation of OSA | Variable(s): Pre-enrollment criteria | Pre-enrollment |
| Medical History | • Established diagnoses and prescription medications  
• EMR extraction | Exclusion Criteria | Evening before PSG |
| Patient Characteristics Form | • 16-item questionnaire  
• Self-reported demographic characteristics; baseline characteristic | Variable(s): Gender, Race/ethnicity, Marital status, Education, Employment, Sleep symptoms, Non-prescription medications | Evening before PSG |
| Physical Examination | • Standard of Care procedure conducted by PSG technologists at study registration  
• Baseline characteristics | Variable(s): Height, Weight, BMI, Blood pressure, Heart rate, Respiration rate, Body temperature, and resting awake SpO₂ Date of birth (age calculation) | Evening before PSG |
| Diagnostic PSG or Sleep Study | • Validated diagnostic procedure for OSA  
• Inclusion criteria; sample description; prognostic variable | Variable(s): apnea hypopnea index (AHI), oxidative desaturation index (ODI), lowest arterial oxyhemoglobin saturation (lowest SpO₂), mean arterial oxyhemoglobin saturation (mean SpO₂), arousal index, total sleep time (TST), sleep stages including N1, N2, N3, and R, respiratory event related arousals, non-respiratory arousals, sleep latency, and sleep efficacy (% TST/time in bed) | Continuous measurement over 1-night |
| Epworth Sleepiness Scale (ESS) | • 9-item questionnaire  
• Valid, reliable measure of the persistent sleepiness in OSA | Variable(s): Subjective sleepiness | Evening & morning |
| Stanford Sleepiness Scale(SSS) | • Single item rated on 7-point scale  
• Validated measure of the momentary sleepiness in sleep disorders | Variable(s): Subjective sleepiness | Evening & morning |
| Lee’s Fatigue and Energy Scales | • 18-item questionnaire  
• Valid, reliable measure of the momentary fatigue in OSA | Variable(s): Subjective fatigue | Evening & morning |
| Profile of Mood States Inventory (POMS) | • 35-item questionnaire  
• Valid, reliable measure of mood and energy state | Variable(s): Subjective mood (Total Mood Disturbance) | Evening & morning |
| Quebec Sleep Questionnaire (QSQ) | • 32-item questionnaire  
• Valid, reliable measure of QOL in OSA | Variable(s): Subjective health-related quality of life | Evening & morning |
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Instrument Description &amp; Purpose</th>
<th>Variables/Outcomes</th>
<th>Time of Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perceived Stress Scale (PSS)</td>
<td>• 10-item questionnaire • Valid, reliable measure of perceived stress in OSA</td>
<td>Variable(s): Subjective perceived stress</td>
<td>Evening &amp; morning</td>
</tr>
<tr>
<td>Beck Depression Inventory (BDI)</td>
<td>• 21-item questionnaire • Validated measure of depressive symptoms in sleep disorders</td>
<td>Variable(s): Subjective depressive symptoms</td>
<td>Evening &amp; morning</td>
</tr>
<tr>
<td>Inflammatory Biomarker</td>
<td>• To identify the molecular signature for OSA</td>
<td>Variable(s): IL-1β, IL-6, IL-8, IL-10, and</td>
<td>Evening &amp; morning</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-α</td>
<td></td>
</tr>
</tbody>
</table>

**Procedures**

Participants were recruited at the PSHMC sleep center; adult patients referred to the sleep center for suspected OSA were the target population. Patients underwent clinical polysomnography at the PSHMC sleep center to establish diagnosis of OSA (AHI ≥5 events/hour).

*Phase I (Evening prior to polysomnography)*: Eligible participants were provided informed consent and enrolled in the study. At enrollment, participants completed baseline measures including a patient characteristics questionnaire. Before full-night diagnostic study was conducted, participants completed a battery of questionnaires including Epworth Sleepiness Scale, Stanford Sleepiness Scale, Lee’s Fatigue and Energy Scales, Profile of Mood States Inventory, Quebec Sleep Questionnaire, Perceived Stress Scale, and Beck’s Depression Inventory and had blood drawn for inflammatory biomarker assessment between 8:00 and 10:00 p.m. Ten milliliters of blood were drawn by a qualified professional (i.e., principal investigator). Blood was collected in two tubes, one containing EDTA and the other containing sodium heparin (EDTA samples were on ice). Samples were then centrifuged (3000 rpm for 15 min), the supernatant (plasma) was removed by pipette, and 300μL of aliquots was stored at -80°C in 1.5ml Eppendorf tubes. Participants received standard of care (usual care) education pre-
polysomnography from sleep technologists as assigned. Diagnostic polysomnography procedures per standard practice were then begun at approximately 10:30-11:00 p.m. (American Academy of Sleep Medicine Task Force, 1999).

**Phase II (In morning, immediately after awakening):** Polysomnography devices were removed between 05:00-06:00 by polysomnography technologist. As sleep inertia, or the feeling of grogginess and disorientation that can come with awakening from a deeper stage of sleep such as SWS or REM (Howard, 2005), potentially confound subjective symptoms measured immediately upon awakening, a waking interval of 15-30 minutes was permitted before administration of measures of subjective symptoms was conducted. Participants completed questionnaires including Stanford Sleepiness Scale and Lee’s Fatigue and Energy Scales after polysomnography equipment removal and the awaking interval. This subjective symptom measurement interval (primary symptom outcome[s]) was employed concurrent with the proinflammatory biomarker measurement interval. Ten milliliters of blood were drawn by a qualified professional (principal investigator). Blood was collected in two tubes, one containing EDTA (EDTA samples were on ice) and the other containing sodium heparin. Samples were then centrifuged (3000 rpm for 15 min), the supernatant (plasma) was removed by pipette, and 300μL of aliquots was stored at -80°C in 1.5ml Eppendorf tubes.

**Phase III (In morning, 1 hour after awakening):** Repeated symptom measures with questionnaires including Stanford Sleepiness Scale and Lee’s Fatigue and Energy Scales after being awake for one hour (secondary symptom outcome interval) were planned. However, study participants were not consistently willing to wait one hour for Phase III for several reasons: 1) get to work on time; 2) go home to take children to school; and 3) other schedules or appointments. Therefore, the secondary symptom measures were not completed. After each
participant was debriefed, the protocol was completed. Blood samples were transferred on dry ice to a University Park laboratory for storage and analysis according to institutional policy by trained study personnel (Figure 3.1. Study Protocol).

**Figure 3.1. Study Protocol**

- **Data Collection and Management**

  **Maintaining Confidentiality and Data Security**

  All participants were assigned a randomly-generated study identification (ID) number. All study-related documents were labeled by study ID only. A master list of participants’ names linked to identification number was maintained by the principal investigator and stored in a locked, fire-proof file at the sleep center. Only the principal investigator and advisor had access
to the master list. The list was destroyed once all data were collected and entered/locked in the study database.

*Storage of and Access to Data and/or Specimens*

All hardcopy data collection forms were stored in a locked, fire-proof filing cabinet at the sleep center during data collection, accessible to the principal investigator and advisor only. All electronic data collection forms and source documents were stored on the IRB approved sleep server at the Hershey Center for Applied Research. De-identified blood samples were labeled with the date of sample collection, sample number, and study ID and were shipped and stored in the laboratory of Dr. Christopher Engeland (Room 147, HHD building, Penn State University at University Park) until analyzed. Only the principal investigator, advisor, and Dr. Engeland’s immediate laboratory staff had access to the blood samples. All electronic study files were secured on the sleep server in a study-specific folder protected by permissions established by the mentor (Dr. Sawyer) of the principal investigator. Other study investigators only had access to the de-identified data. Personal identifiers were removed from the database and stored as a de-identified database. There is no data sharing plan for the study; data was not/will not be shared or transferred to outside investigators.

*Transferring Data and/or Specimens*

Specimens of blood were placed in an IRB and biohazard approved container which prevents leakage during transport from PSHMC sleep center to Dr. Engeland’s laboratory at University Park. The container for transport was labeled with an orange or orange-red biohazard emblem and closed prior to being transported. Specimens were transported on dry ice by a study team member.
Privacy

Only the principal investigator and advisor had access to the master list of participant’s names that are linked to study identification numbers. All electronic data and hard copy data collection forms and source documents were labeled only by participant identification number. Participants were made aware that there was no identifiable information used when reporting results. Only aggregate demographic information (i.e., age, gender, and ethnicity) and study data were used when reporting results.

Protection of Human Subjects

Consenting Process: Obtaining Informed Consent

All participants were provided a consent form that fully described the study. Informed consent procedures took place prior to any study-related activities. Participants read the informed consent form that describes their rights as a research participant, including their right to withdraw and discontinue participation in the research at any time. Potential participants were informed at the time of informed consent that their clinical care at the sleep center was in no way associated with their decision to participate or not participate in the study.

Consent Documentation

All participants signed an informed consent form prior to participating in the research study. All participants received a copy of the informed consent for their records. A copy of the signed consent form and the IRB-approved study abstract was scanned to the electronic medical records (EMR). Original signed consent form was maintained in study participant records (hard copy) and stored during data collection at the sleep center in a locked, fire-proof filing cabinet accessible to study personnel only.
Plan for Recording and Managing Any Adverse Events

Adverse Events

This study involved drawing blood specimens. The risks of the blood draw were discomfort during blood draw procedure, bleeding/bruising at the blood draw site, and infection at the blood draw site. To prevent against the risks, aseptic technique during blood draw was used and pressure to the blood draw site was applied immediately after completion of the blood draw. There were minimal risks associated with completion of questionnaires employed in the study. Psychological stress might result from responding to questionnaires; the questionnaires used in this study include relatively benign items, assessing function, quality of life, sleepiness and fatigue. No adverse events, unexpected events, or reportable events occurred during the conduct of the study. There was also a risk of loss of confidentiality, which means that information collected about a participant in this research could become known by others outside of the research team. To keep this from happening, participants’ information was assigned a code number (i.e., de-identified) and held in confidence with IRB-approved confidentiality protection measures.

Data Analysis

All research variables were descriptively analyzed to examine distribution and assess for outliers. Graphical tests such as histograms and box-whisker plots were examined. Outliers defined as a number which is less than the lower quartile (Q1) (i.e., any numbers less than $1.5 \times$ IQR; the formula is $Q1-(1.5 \times \text{IQR})$) or greater than the upper quartile (Q3) (i.e., any numbers greater than $1.5 \times$ IQR; the formula is $Q3+(1.5 \times \text{IQR}$). Data sets were analyzed using the Kolmogorov-Smirnov test. After removing the identified outliers, data sets were repeatedly analyzed using the Kolmogorov-Smirnov test. The data sets were normally distributed after
removing the outliers (i.e., p>0.05). Normality is not required for non-parametric tests but exploration of the data, including distribution of data per variable, was undertaken as an exploratory analytic opportunity. Data that were not normally distributed regardless of outliers were analyzed using appropriate non-parametric tests. Missing data was assumed missing at random, no imputation procedure was planned. Statistical significance was analyzed with the Wilcoxon Rank Sum Test; the Spearman partial correlation coefficients were used to control for potential confounders including age, BMI, cardiovascular diseases, type II DM. Statistical significance level was defined at p <0.05.

**Research Question 1:** *What is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA, and is there a significant relationship between diurnal variation and AHI?*

Diurnal variation was defined as the median difference of inflammatory biomarkers levels between evening and morning as response variables using Wilcoxon Singed-Rank test; all biomarker data was continuous level data. Partial correlations were employed to evaluate the relationship between diurnal variations of inflammatory biomarkers and sleep apnea-related outcomes, which were also a continuous variable. Partial correlations adjusted for the effects of age (continuous variable), BMI (continuous variable, reflects obesity as a confounding factor), cardiovascular disease, and type 2 diabetes mellitus (binary variables; 0=no, 1=yes).

**Research Question 2:** *Is there an association between inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP and everyday symptoms, including excessive daytime sleepiness and fatigue, in adults with OSA?*

The partial correlation was employed to identify the relationship between each inflammatory biomarker (continuous variables) and each of the symptoms (continuous variables)
at two different time points (pre-sleep polysomnography and post-sleep polysomnography). The partial correlation was used to identify the relationship between individual inflammatory biomarkers and sleepiness and fatigue, individually. The sleepiness and fatigue as response variables were analyzed as continuous variables. Each inflammatory biomarker (continuous variables) was separately analyzed as predictor variable. Data for both evening and morning measurement periods were considered separately. Confounding factors including age, BMI, type 2 diabetes and cardiovascular disease were controlled.

**Research Question 3:** Is diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP associated with symptoms in adults with OSA?

Partial correlation was employed to evaluate the relationship between the median difference each of inflammatory biomarkers (continuous variable) and symptoms (continuous variable) measured on awakening. Partial correlation adjusted for the effects of age (continuous variable), BMI (continuous variable, reflects obesity as a confounding factor), and cardiovascular disease and type 2 diabetes mellitus (binary variables; 0=no, 1=yes).

**Research Question 4:** What is symptom phenotype in adults with OSA?

Exploratory cluster analysis was performed to identify symptom phenotype of OSA. The cosine function was used to measure similarity between two vectors A and B and qualified as the basis for a hierarchical clustering of patients. Cosine similarity, $\cos(\theta)$ was calculated as the cosine of the angle between non-zero vectors and represented using a dot product with the formula:

$$\text{similarity} = \cos \theta = \frac{A \times B}{\|A\| \|B\|}$$
If \(d_1\) and \(d_2\) are two vectors, then

\[
\cos(d_1, d_2) = \frac{D_1 \times D_2}{\sqrt{\sum_{i=1}^{n} D_{1i}^2} \times \sqrt{\sum_{i=1}^{n} D_{2i}^2}}
\]

The cosine similarity between all of the symptom variables, including sleepiness (evening and morning), fatigue (evening and morning), energy (evening and morning), mood disturbance (evening [baseline]), perceived stress (evening [baseline]), and depression (evening [baseline]) were calculated. Based on the literature, a cosine cutoff of greater than 0.9 evaluates similar attributes between two vectors; this cosine cutoff was applied (Dumais et al., 1988). After calculating the cosine similarity between variables, the networks between symptoms were graphically visualized with yED Graph Editor (yWorks GmbH, Tübingen, Germany, http://www.yworks.com/en/products_yed_about.html). Network graphs showed natural symptom clusters and the levels of interaction between symptoms.

**Chapter 3 Summary**

A cross-sectional cohort study was conducted to identify the relationships between inflammatory biomarkers and everyday symptoms in OSA and between the diurnal variation of inflammatory biomarkers and everyday symptoms in OSA. In addition, a preliminary exploration of the symptom phenotype of OSA was intended. Scientific literature suggests confounders will likely affect the causal relationship between inflammatory biomarkers and everyday symptoms in OSA. Therefore, the partial correlation analysis, adjusting for previously identified confounders, will enable the detection of the causal mechanism between exposure (increased inflammatory biomarkers) and outcomes (everyday symptoms) in OSA. Clustering, to place everyday symptoms into groups, will support the identification of OSA symptoms phenotype.
The results of the present study will contribute insightful and hypothesis-generating knowledge to support subsequent symptom science in OSA.
Chapter 4

The purpose of this study was to identify and explore diurnal variation of inflammatory biomarkers and their relationship with everyday symptoms among adult patients with OSA. Data from inflammatory biomarkers and questionnaires measured pre-sleep bout (8pm; Time 1) and post-sleep bout (6am; Time 2), and overnight polysomnography were analyzed to address the following research questions.

1. What is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA, and is there a significant relationship between diurnal variation and AHI;
2. Is there an association between inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP and everyday symptoms, including excessive daytime sleepiness and fatigue, in adults with OSA;
3. Is diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP associated with symptoms in adults with OSA; and
4. What is symptom phenotype in adults with OSA.

Sample Recruitment

Study participants were recruited from a clinical sleep center at a suburban, tertiary academic medical center. The study was open for enrollment from August 2016 to December 2016. Clinical patients referred to OSA diagnostics were approached to participate in this study (n=72). Using the Berlin Questionnaire prior to enrollment/consent, eleven participants had a negative Berlin questionnaire (i.e., less than two categories scored positive) and were not enrolled (15.3%). Sixty-one (84.7%) study subjects agreed to participate in the study, met screening criteria and received informed consent. With polysomnography completion, 23
participants had OSA (AHI ≥ 5 events/hour); one subject had total sleep time of 45 minutes on polysomnography and was excluded from analysis due to short sleep duration as potential confounder. Of the remaining 38 enrolled participants, 16 had split-night studies (not eligible to continue) and 22 did not meet AHI criteria for OSA. Although 43 participants completed the study, 22 evaluable sample units were included in the current data analysis because this study aimed to assess diurnal variation of inflammatory biomarkers and resultant symptom expression in OSA (Figure 4.1) in a single cohort designed study.

**Figure 4.1. Enrollment and Evaluable Data Flowchart**

Notes. PSGs, polysomnography; OSA, obstructive sleep apnea; Non-OSA, non-obstructive sleep apnea
The results of the data analysis with a convenience sample size of 22 are presented in this chapter.

**Characteristics of Study Sample**

As shown in Table 4.1., the study participants included an equal ratio of male and female adults; the median age was 51 years. The majority of the study participants were obese (77.8%) and none were within normal weight categorization. Study participants were predominantly non-Hispanic (86.4%) and married (68.2%). Reasons for referral to the sleep center included snoring (77.3%), restless sleep (63.6%), and sleepiness (54.5%). Half of the sample was college-educated, employed full-time (82.0%), with few night shift workers (n=2; 9.0%). Medications administered within 24 hours of first blood sampling included Aspirin® (acetylsalicylic acid [ASA]) (18.2%), Ibuprofen® (a nonsteroidal anti-inflammatory drug [NSAIDs]) (13.6%), and Tylenol® (acetaminophen) (13.6%). The most prevalent co-morbidities were cardiovascular disease (54.5%) and type 2 diabetes mellitus (T2DM; 13.6%). The descriptive analyses of all study variables are provided in the Tables 4.2, 4.3, and 4.4.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>22</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>49(17.12)</td>
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<tr>
<td>Median (IQR)</td>
<td>51(36, 61)</td>
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<td>Female</td>
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<td>Body Mass Index, kg/m²</td>
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<tr>
<td>Median (IQR)</td>
<td>34.10(30.2, 41.4)</td>
</tr>
<tr>
<td>Normal Weight (18.5-24.9 kg/m²)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Overweight (25.0-29.9 kg/m²)</td>
<td>4(22.2%)</td>
</tr>
<tr>
<td>Obese (≥30.0 kg/m²)</td>
<td>18(77.8%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3(13.6%)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>19(86.4%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>19(86.4%)</td>
</tr>
<tr>
<td>African-American</td>
<td>3(13.6%)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>15(68.2%)</td>
</tr>
<tr>
<td>Single</td>
<td>4(18.2%)</td>
</tr>
<tr>
<td>Income</td>
<td></td>
</tr>
<tr>
<td>&lt;$52,000</td>
<td>11(50.0%)</td>
</tr>
<tr>
<td>≥$52,000</td>
<td>11(50.0%)</td>
</tr>
<tr>
<td>Sleep Problem</td>
<td></td>
</tr>
<tr>
<td>Snoring</td>
<td>17(77.3%)</td>
</tr>
<tr>
<td>Breathing Stop</td>
<td>5(22.7%)</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>12(54.5%)</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>3(13.6%)</td>
</tr>
<tr>
<td>Restless Sleep</td>
<td>14(63.6%)</td>
</tr>
<tr>
<td>Can’t Fall Asleep</td>
<td>7(31.8%)</td>
</tr>
<tr>
<td>Referral Source</td>
<td></td>
</tr>
<tr>
<td>Primary Care Provider</td>
<td>15(68.2%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>High School or less</td>
<td>12(50.0%)</td>
</tr>
<tr>
<td>College or more</td>
<td>12(50.0%)</td>
</tr>
<tr>
<td>Working Full-Time</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18(82.0%)</td>
</tr>
<tr>
<td>Working Night Shift</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2(9.0%)</td>
</tr>
<tr>
<td>Medication Related to Anti-Inflammation (last 24 hours)</td>
<td></td>
</tr>
<tr>
<td>Characteristics</td>
<td>n (%)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>3(13.6%)</td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
<td>12(54.5%)</td>
</tr>
</tbody>
</table>

*Notes. SD, standard deviation; IQR, interquartile range*

Description of Research Variables: Inflammatory Biomarkers, Symptoms, and Polysomnography Variables

**Inflammatory Biomarkers:**

*Description of Inflammatory Biomarkers*

The description of each inflammatory biomarker measured at two different time points (evening; Time 1 and morning; Time 2) and differences (Time 2-Time 1) is provided in Table 4.2. The mean and median values of IL-10 evening and TNF-α were the same, or similar, indicating symmetric distribution of the variables (also refer to graphical analysis). The diurnal variation of IL-6 had a negative value (i.e., higher level of IL-6 in the evening) which contrasts with all other inflammatory biomarkers. The graphical analysis of the distribution of inflammatory biomarkers (pre-sleep bout and post-sleep bout) is provided in Figure 4.2.
Table 4.2. Description of Inflammatory Biomarkers

<table>
<thead>
<tr>
<th>Variables</th>
<th>M(SD)</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10E</td>
<td>0.16(0.09)</td>
<td>0.16</td>
<td>0.10, 0.20</td>
</tr>
<tr>
<td>IL-10M</td>
<td>0.19(0.12)</td>
<td>0.14</td>
<td>0.08, 0.28</td>
</tr>
<tr>
<td>IL-10D</td>
<td>0.025(0.122)</td>
<td>0.015</td>
<td>-0.05, 0.08</td>
</tr>
<tr>
<td>IL-6E</td>
<td>1.02(1.22)</td>
<td>0.63</td>
<td>0.59, 1.06</td>
</tr>
<tr>
<td>IL-6M</td>
<td>1.03(1.11)</td>
<td>0.55</td>
<td>0.35, 1.02</td>
</tr>
<tr>
<td>IL-6D</td>
<td>0.011(1.28)</td>
<td>-0.135</td>
<td>-0.310, 0.290</td>
</tr>
<tr>
<td>IL-8E</td>
<td>3.38(1.676)</td>
<td>2.96</td>
<td>2.29, 4.44</td>
</tr>
<tr>
<td>IL-8M</td>
<td>4.057(1.918)</td>
<td>3.69</td>
<td>2.46, 4.97</td>
</tr>
<tr>
<td>IL-8D</td>
<td>0.672(2.125)</td>
<td>0.365</td>
<td>-0.14, 1.58</td>
</tr>
<tr>
<td>TNF-aE</td>
<td>1.58(0.74)</td>
<td>1.31</td>
<td>1.03, 2.02</td>
</tr>
<tr>
<td>TNF-aM</td>
<td>1.95(0.82)</td>
<td>1.81</td>
<td>1.39, 2.40</td>
</tr>
<tr>
<td>TNF-aD</td>
<td>0.371(0.502)</td>
<td>0.390</td>
<td>0.05, 0.66</td>
</tr>
<tr>
<td>CRPE</td>
<td>0.96(1.44)</td>
<td>0.54</td>
<td>0.17, 0.93</td>
</tr>
</tbody>
</table>

Notes. M(SD), mean(standard deviation); IQR, interquartile range; IL-10E, interleukin-10 measured in the evening; IL-10M, interleukin-10 measured in the morning; IL-10D, changes of IL-10 between evening and morning; IL-6E, interleukin-6 measured in the evening; IL-6M, interleukin-6 measured in the morning; IL-6D, changes of IL-6 between evening and morning; IL-8E, interleukin-8 measured in the evening; IL-8M, interleukin-8 measured in the morning; IL-8D, changes of IL-8 between evening and morning; TNF-aE, tumor necrosis factor (TNF)-α measured in the evening; TNF-aM, tumor necrosis factor (TNF)-α measured in the morning; TNF-aD, changes of tumor necrosis factor (TNF)-α between evening and morning; CRPE, C-reactive protein measured in the evening

**Distribution of Inflammatory Biomarkers**

The box-whisker plot represents the interquartile range (IQR; the 25th to 75th percentiles); the line in the middle of the box represents the median value and the dot inside the box shows the mean value (Figure 4.2). The whiskers show the non-outlier range (the upper whisker shows the largest value within $1.5 \times IQR$; the lower whisker shows the smallest value within $1.5 \times IQR$). The dot outside the box indicates outlier values. The side-by-side boxplot of
IL-10 showed that the median of evening IL-10 was higher than the morning of IL-10, but the IQR was larger for the morning of IL-10, indicating more variation in morning of IL-10. The median of IL-6 in the evening was slightly higher than the median of IL-6 in the morning; there were outliers in both morning and evening of IL-6. The median of both IL-8 and TNF-α measured in the morning were higher than those in the evening for IL-8 and TNF-α. IL-1β had an undetectable value in most of study participants; so, IL-1β analyses are not reported. In summary, there were identified outliers with evening IL-10, evening and morning IL-6, and morning IL-8.
Figure 4.2. Distribution of the Evening and Morning Inflammatory Biomarkers in OSA

**Notes.** IL10E, interleukin-10 measured in the evening; IL10M, interleukin-10 measured in the morning; IL6E, interleukin-6 measured in the evening; IL6M, interleukin-6 measured in the morning; IL8E, interleukin-8 measured in the evening; IL8M, interleukin-8 measured in the morning; TNFAE, tumor necrosis factor (TNF)-α measured in the evening; TNFAM, tumor necrosis factor (TNF)-α measured in the morning
Symptoms:

Description of Symptom Variables

The description of momentary symptoms and lasting symptoms is provided in Table 4.3. Momentary symptoms measured the current experience of sleepiness, fatigue, and energy with Stanford Sleepiness Scale and Lee Fatigue and Energy Scale. The mean sleepiness in the morning was higher than those in the evening, indicating no recovery of sleepiness through the sleep period. According to the scoring manual for Lee Fatigue and Energy Scale (Lee et al., 1991), evening fatigue of $\geq 5.6$ and morning fatigue of $\geq 3.2$ and morning energy of $\leq 6.0$ and evening energy of $\leq 3.5$ are considered clinically significant symptoms. The present study showed that the mean and median fatigue levels in the evening were lower than 5.6, or not clinically significant; whereas, the mean and median fatigue levels in the morning were higher than 3.2, or clinically significant. Because all morning symptoms in terms of sleepiness, fatigue, and energy were above the cutoff values, and all evening symptoms were below the cutoff values; only morning symptoms were clinically significant.

Lasting symptoms, including daytime sleepiness, mood disturbances, diurnal symptoms, social interactions, nocturnal symptoms, emotions, perceived stress, and depressive symptoms were measured by asking study participants to reflect over the past week or longer. The mean and median lasting daytime sleepiness was higher than the cutoff value of 10 (Johns, 1993). In terms of mood disturbance, the mean value was higher than the median value and the IQR was 0 to 38. The depression score (BDI) mean (14.86) indicated mild depression in the sample.
### Table 4.3. Description of Symptom Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>M(SD)</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Momentary Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleepiness E(^a)</td>
<td>3.40(1.36)</td>
<td>3.00</td>
<td>2, 4</td>
</tr>
<tr>
<td>Sleepiness M(^a)</td>
<td>3.54(1.43)</td>
<td>3.00</td>
<td>3, 4</td>
</tr>
<tr>
<td>Sleepiness D(^a)</td>
<td>0.13(1.60)</td>
<td>0.00</td>
<td>0, 1</td>
</tr>
<tr>
<td>Fatigue E(^b)</td>
<td>4.71(2.26)</td>
<td>5.23</td>
<td>3.38, 5.76</td>
</tr>
<tr>
<td>Fatigue M(^b)</td>
<td>4.21(1.91)</td>
<td>4.88</td>
<td>3.00, 5.53</td>
</tr>
<tr>
<td>Fatigue D(^b)</td>
<td>-0.50(2.56)</td>
<td>0.15</td>
<td>-2.92, 1.00</td>
</tr>
<tr>
<td>Energy E(^b)</td>
<td>3.89(1.68)</td>
<td>3.80</td>
<td>2.80, 4.60</td>
</tr>
<tr>
<td>Energy M(^b)</td>
<td>3.75(2.02)</td>
<td>4.00</td>
<td>2.0, 5.0</td>
</tr>
<tr>
<td>Energy D(^b)</td>
<td>-0.13(2.19)</td>
<td>-0.40</td>
<td>-2.2, 1.2</td>
</tr>
<tr>
<td><strong>Lasting Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime Sleepiness(^d)</td>
<td>11.50(5.46)</td>
<td>13.00</td>
<td>7, 15</td>
</tr>
<tr>
<td>Mood Disturbance(^d)</td>
<td>20.13(25.53)</td>
<td>11.00</td>
<td>0, 38</td>
</tr>
<tr>
<td>Diurnal Symptoms(^e)</td>
<td>3.64(1.67)</td>
<td>3.58</td>
<td>2.20, 5.10</td>
</tr>
<tr>
<td>Social Interactions(^e)</td>
<td>5.10(1.80)</td>
<td>5.37</td>
<td>3.50, 6.75</td>
</tr>
<tr>
<td>Nocturnal Symptoms(^e)</td>
<td>3.61(1.41)</td>
<td>3.50</td>
<td>2.71, 4.14</td>
</tr>
<tr>
<td>Emotions(^e)</td>
<td>4.43(1.58)</td>
<td>4.50</td>
<td>3.0, 5.8</td>
</tr>
<tr>
<td>Hypersomnia(^e)</td>
<td>4.35(1.42)</td>
<td>4.66</td>
<td>3.50, 5.33</td>
</tr>
<tr>
<td>Perceived Stress(^f)</td>
<td>22.18(5.45)</td>
<td>23.00</td>
<td>19, 27</td>
</tr>
<tr>
<td>Depressive Symptoms(^g)</td>
<td>14.86(9.33)</td>
<td>13.00</td>
<td>8, 19</td>
</tr>
</tbody>
</table>

**Notes.** M(SD), mean(standard deviation); IQR, interquartile range; Sleepiness E, sleepiness measured in the evening; Sleepiness M, sleepiness measured in the morning; Sleepiness D, changes of sleepiness between evening and morning; Fatigue E, fatigue measured in the evening; Fatigue M, fatigue measured in the morning; Fatigue D, changes of fatigue between evening and morning; Energy E, energy measured in the evening; Energy M, energy measured in the morning; Energy D, changes of energy between evening and morning
\(^a\) measured by Stanford Sleepiness Scale; \(^b\) measured by Lee Fatigue and Energy Scale; \(^c\) measured by Epworth Sleepiness Scale; \(^d\) measured by Profile of Mood States; \(^e\) measured by Quebec Sleep Questionnaire; \(^f\) measured by Perceived Stress Scale; \(^g\) measured by Beck’s Depression Inventory
**Distribution of Evening and Morning Symptoms**

The distribution of momentary symptoms by graphical analysis is provided in Figure 4.3. The side-by-side boxplot for sleepiness show similar median values; but sleepiness had different distributions, including two outliers for morning sleepiness and a comparatively tall box for evening sleepiness. The median and mean values for morning fatigue were slightly lower than median/mean values for evening fatigue. Energy in post-sleep bout showed more spread distribution compared to those in pre-sleep bout.

**Figure 4.3. Distribution of Momentary Symptoms in OSA**

**Notes.** SLEEPE, sleepiness measured in the evening; SLEEPM, sleepiness measured in the morning; FATIGUEE, fatigue measured in the evening; FATIGUEM, fatigue measured in the morning; ENERGYE, energy measured in the evening; ENERGYM, energy measured in the morning
**Sleep-Related Variables:**

**Description of Sleep-Related Variables**

The descriptive analysis of polysomnography variables is provided in Table 4.4. Among the participants diagnosed with OSA (AHI ≥ 5 events/hour), 68.2% (n=15) had mild OSA (AHI ≥5-15 events/hour), while 31.8% (n=7) had either moderate (AHI >15-30 events/hour) or severe OSA (AHI >30 events/hour). The median apnea index was 3.0 events/hour (IQR, 0.8 -7.1 events/hour); the median hypopnea index was 8.0 events/hour (IQR, 4.8 -12.6 events/hour).

Beyond the apnea-hypopnea index, four other OSA severity variables were examined including:

1. total sleep time spent with O₂ saturations between 80% and 90% during sleep (OSD 80%);
2. total sleep time spent with O₂ saturations less than or equal to 88% (TS 88%);
3. lowest oxygen saturation (O₂ nadir); and
4. average oxygen saturation during sleep (Average O₂).

Median total sleep time was 380.25 minutes; median oxygen desaturation time between 80% and 90% was 23.75 minutes (IQR 9.3-46.6 minutes); TS88%, a clinically-used desaturation variable from polysomnography, was median of 6 minutes (IQR 1.7-11.6 minutes). Both OSD 80% and TS 88% had large differences in the mean and the median, indicating non-normality. The oxygen saturation nadir median was 83.5% (IQR 80-85%); the average oxygen saturation over the sleep period was 93.4% (IQR 92.5-94.8%).

Other sleep architecture variables relative to OSA were also examined. The median arousal index was 20.5 events/hour (IQR 12.1-27.5 events/hour). Respiratory effort related arousal index (RERA index) was median 2.25 events/hour [IQR 1.30-4.20 events/hour]. The summary polysomnography results indicate an expected level and severity of oxygen desaturation over the sleep period, along with the sleep fragmentation based on the arousal index and RERA index.
Table 4.4. Description of Sleep Related Variables in OSA

<table>
<thead>
<tr>
<th>Variables</th>
<th>M(SD)</th>
<th>Median</th>
<th>IQR or N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apnea-Hypopnea Index (AHI), events/hour</td>
<td>15.32(12.89)</td>
<td>12.85</td>
<td>7.8, 15.8</td>
</tr>
<tr>
<td>Mild AHI (n=15)</td>
<td>9.40(3.17)</td>
<td>9.1</td>
<td>68.2%</td>
</tr>
<tr>
<td>Moderate AHI (n=5)</td>
<td>18.72(6.27)</td>
<td>16.0</td>
<td>22.7%</td>
</tr>
<tr>
<td>Severe AHI (n=2)</td>
<td>51.2(5.93)</td>
<td>51.2</td>
<td>9.1%</td>
</tr>
<tr>
<td>Apnea Index, events/hour</td>
<td>5.25 (6.81)</td>
<td>3.0</td>
<td>0.8, 7.1</td>
</tr>
<tr>
<td>Hypopnea Index, events/hour</td>
<td>10.00 (10.60)</td>
<td>8.0</td>
<td>4.8, 12.6</td>
</tr>
<tr>
<td>Total Sleep Time, min</td>
<td>368.8(61.29)</td>
<td>380.25</td>
<td>328.50, 411.00</td>
</tr>
<tr>
<td>OSD 80%, min</td>
<td>63.05 (107.2)</td>
<td>23.75</td>
<td>9.30, 46.60</td>
</tr>
<tr>
<td>TS 88%, min</td>
<td>28.74 (61.80)</td>
<td>6.0</td>
<td>1.7, 11.6</td>
</tr>
<tr>
<td>O₂ Nadir, %</td>
<td>82.50 (4.49)</td>
<td>83.50</td>
<td>80.0, 85.0</td>
</tr>
<tr>
<td>Average O₂, %</td>
<td>93.45 (2.27)</td>
<td>93.10</td>
<td>92.5, 94.8</td>
</tr>
<tr>
<td>Arousal Index, events/hour</td>
<td>22.50 (14.81)</td>
<td>20.50</td>
<td>12.1, 27.5</td>
</tr>
<tr>
<td>RERA Index, events/hour</td>
<td>4.20 (5.31)</td>
<td>2.25</td>
<td>1.30, 4.20</td>
</tr>
</tbody>
</table>

Notes. M(SD), mean (standard deviation); IQR, interquartile range; n, number; AHI, apnea-hypopnea index; OSD 80%, total sleep time spent with O₂ saturations between 80% and 90%; TS 88%, total sleep time spent with O₂ saturations less than or equal to 88%; RERA Index, respiratory effort related arousal index

Distribution of Sleep-Related Variables

The distribution of sleep-related variables is provided in Figure 4.4. Different severity of OSA exhibited in the box-whisker plot as AHI, with the separation of apnea index (AI) and hypopnea index (HI). The mean values both OSD80%, indicating total sleep time spent with O₂ saturations between 80% and 90% and TS88%, indicating total sleep time spent with O₂ saturations less than or equal to 88% were higher than median, which means a distribution that is skewed to the right. Similarly, the mean value of RERA index was greater than median with outliers.
Figure 4.4. Distribution of Sleep-Related Variables
Research Question 1. What is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA, and is there a significant relationship between diurnal variation and AHI?

Research Question 1.1. What is the diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP in adults with OSA?

Figure 4.5 shows paired evening and morning inflammatory biomarkers for the sample (n= 22). Diurnal variation of cytokines was tested using the Wilcoxon Signed-Rank test due to skewed data. Table 4.5 reports median and interquartile ranges (IQRs) and test of differences results for evening morning cytokines. CRP was measured in the evening only since it has a stable pattern over 24 hours; therefore, diurnal variation of CRP was not reported in the study. Both IL-10 and IL-6 were not significantly different between the evening and the morning. There is an increasing trend of IL-8 (i.e., lower IL-8 levels in the evening and higher IL-8 levels in the morning), but it was not statistically significant (p=0.095). Morning TNF-α was significantly higher than evening TNF-α (p=0.0012).
**Figure 4.5.** Evening to Morning Inflammatory Biomarkers in OSA

**Notes.** IL-10, interleukin-10; IL10E, interleukin-10 measured in the evening; IL10M, interleukin-10 measured in the morning; IL-6, interleukin-6; IL6E, interleukin-6 measured in the evening; IL6M, interleukin-6 measured in the morning; IL-8, interleukin-8; IL8E, interleukin-8 measured in the evening; IL8M, interleukin-8 measured in the morning; TNF-α, tumor necrosis factor-α; TNFAE, tumor necrosis factor (TNF)-α measured in the evening; TNFAM, tumor necrosis factor (TNF)-α measured in the morning
Table 4.5. Pre-Sleep and Post-Sleep Bout Inflammatory Biomarkers in OSA

<table>
<thead>
<tr>
<th></th>
<th>Evening</th>
<th>Morning</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>0.16(0.10-0.20)</td>
<td>0.14(0.08-0.28)</td>
<td>0.3972</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.63(0.59-1.06)</td>
<td>0.55(0.35-1.02)</td>
<td>0.6261</td>
</tr>
<tr>
<td>IL-8</td>
<td>2.96(2.29-4.44)</td>
<td>3.69(2.46-4.97)</td>
<td>0.0950</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.31(1.03-2.02)</td>
<td>1.81(1.39-2.40)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

Notes: Data are presented as median (min-max) values; Wilcoxon Signed Rank test
IL-10, interleukin-10; IL-6, interleukin-6; IL-8, interleukin-8; TNF-α, tumor necrosis factor (TNF)-α

Research Question 1.2. Is there a significant relationship between diurnal variation and AHI?

The association between diurnal variation of inflammatory biomarkers and AHI was assessed using the Spearman partial rank correlation coefficient, adjusted for four confounding variables that included age, BMI, cardiovascular disease, and type II DM (Table 4.6).

Table 4.6. Partial Correlations Between Diurnal Variation of Inflammatory Biomarkers and AHI, AI, and HI Including Outliers

<table>
<thead>
<tr>
<th></th>
<th>IL-10D</th>
<th>IL-6D</th>
<th>IL-8D</th>
<th>TNF-αD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI</td>
<td>0.33</td>
<td>-0.08</td>
<td>0.05</td>
<td>0.41</td>
</tr>
<tr>
<td>AI</td>
<td>0.24</td>
<td>-0.03</td>
<td>0.25</td>
<td>-0.01</td>
</tr>
<tr>
<td>HI</td>
<td>0.01</td>
<td>-0.25</td>
<td>-0.22</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes
IL-10D, diurnal variation of interleukin-10; IL-6D, diurnal variation of interleukin-6; IL-8D, diurnal variation of interleukin-8; TNF-αD, diurnal variation of tumor necrosis factor(TNF)-α; AHI, apnea-hypopnea index; AI, apnea index; HI, hypopnea index
Among the sample of mild OSA adults, apnea-hypopnea index, or apnea index, or hypopnea index, were not significantly related to diurnal variation of any inflammatory biomarkers. There was a positive correlation between diurnal variation of TNF-α and AHI but it did not reach statistical significance (r=0.41, p=0.09).

**Exploratory Analyses: Partial Correlations Between Diurnal Variation of Inflammatory Biomarkers and Other Sleep-Related Variables**

Although AHI is the most common indicator of OSA severity, other PSG-derived variables may be important to consider relative to inflammation in OSA. Though frequency of apneic/hypopneic events is of importance, other sleep-related phenomena are hypothesized to contribute to the mechanism(s) of inflammation in OSA. Therefore, the relationship between other PSG-derived variables and inflammatory biomarkers was also explored (Table 4.7).

**Table 4.7. Partial Correlations Between Diurnal Variation and Other Sleep Related Variables**

<table>
<thead>
<tr>
<th></th>
<th>IL-10D</th>
<th>IL-6D</th>
<th>IL-8D</th>
<th>TNF-αD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST</td>
<td>0.11</td>
<td>-0.52*</td>
<td>0.66**</td>
<td>0.13</td>
</tr>
<tr>
<td>Arousal Index</td>
<td>0.21</td>
<td>0.01</td>
<td>-0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>RERA index</td>
<td>0.28</td>
<td>0.06</td>
<td>-0.17</td>
<td>0.32</td>
</tr>
<tr>
<td>OSD 80%</td>
<td>-0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>TS 88%</td>
<td>-0.01</td>
<td>-0.08</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Average O₂</td>
<td>0.13</td>
<td>-0.16</td>
<td>-0.01</td>
<td>-0.14</td>
</tr>
<tr>
<td>O₂ Nadir</td>
<td>-0.03</td>
<td>0.18</td>
<td>-0.27</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05; **p-value: ≤0.01

**Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes**

IL-10D, changes of IL-10 between evening and morning; IL-6D, changes of IL-6 between evening and morning; IL-8D, changes of IL-8 between evening and morning; TNF-αD, changes of TNF-α between evening and morning; AHI, apnea-hypopnea index; A1, apnea index; HI, hypopnea index; TST, total sleep...
time; RERA Index, the respiratory effort related arousal index; OSD 80%, total sleep time with O_2 saturations between 80% and 90%; TS 88%, total sleep time with O_2 saturations less than or equal to 88%; O_2 nadir, lowest oxygen saturation during sleep

Some statistically significant relationships were detected between diurnal variation of pro-inflammatory biomarkers and sleep quality. Total sleep time was negatively correlated to the diurnal variation of IL-6 (r=-0.52, p=0.026) and positively correlated to the diurnal variation of IL-8 (r=0.66, p=0.002), which follows the normal diurnal pattern of IL-6 and IL-8 (i.e., decreasing trend over sleep period and increasing trend over sleep period, respectively).

Surprisingly, there was no relationship between oxygenation during sleep and diurnal variation of inflammatory biomarkers. The analyses were repeated after removal of the identified outliers in evening IL-10, evening and morning IL-6, and morning IL-8 (Table 4.8).

**Table 4.8. Partial Correlations Between Diurnal Variation and Other Sleep Related Variables**

*After Excluding Outliers*

<table>
<thead>
<tr>
<th></th>
<th>IL-10D (n=20)</th>
<th>IL-6D (n=18)</th>
<th>IL-8D (n=19)</th>
<th>TNF-αD (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI</td>
<td>0.26</td>
<td>0.26</td>
<td>0.15</td>
<td>0.41</td>
</tr>
<tr>
<td>AI</td>
<td>0.11</td>
<td>0.43</td>
<td><strong>0.51</strong>*</td>
<td>-0.01</td>
</tr>
<tr>
<td>HI</td>
<td>0.15</td>
<td>-0.25</td>
<td>-0.28</td>
<td>0.23</td>
</tr>
<tr>
<td>TST</td>
<td>-0.15</td>
<td>-0.30</td>
<td>0.48</td>
<td>0.13</td>
</tr>
<tr>
<td>Arousal Index</td>
<td>0.18</td>
<td>-0.39</td>
<td>0.15</td>
<td>0.23</td>
</tr>
<tr>
<td>RERA index</td>
<td>0.31</td>
<td>-0.37</td>
<td>-0.11</td>
<td>0.32</td>
</tr>
<tr>
<td>OSD 80%</td>
<td>0.04</td>
<td><strong>0.54</strong>*</td>
<td>0.01</td>
<td>0.24</td>
</tr>
<tr>
<td>TS 88%</td>
<td>0.04</td>
<td><strong>0.64</strong>*</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Average O_2</td>
<td>-0.04</td>
<td>-0.49</td>
<td>0.05</td>
<td>-0.14</td>
</tr>
<tr>
<td>O_2 Nadir</td>
<td>0.05</td>
<td><strong>-0.51</strong>*</td>
<td>-0.13</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes
IL-10D, change of IL-10 between evening and morning; IL-6D, change of IL-6 between evening and morning; IL-8D, change of IL-8 between evening and morning; TNF-αD, change of TNF-α between evening and morning; AHI, apnea-hypopnea index; AI, apnea index; HI, hypopnea index; TST, total sleep time; RERA Index, respiratory effort related arousal index; OSD 80%, total sleep time spent with O₂ saturations between 80% and 90%; TS 88%, total sleep time spent with O₂ saturations less than or equal to 88%; O₂ nadir, lowest oxygen saturation

With removal of outliers, the strength of the correlation between the diurnal variation of IL-8 and total sleep time decreased and was no longer significant (r = 0.48, p=0.07). A moderate positive relationship was identified between diurnal variation of IL-8 and the apnea index (r=0.51, p=0.049). Interestingly, no relationship was identified between diurnal variation of IL-8 and hypopnea index (r= -0.28; p=0.308). Notably, there were significant relationships identified between diurnal variation of IL-6 and several exploratory OSA severity markers, including OSD 80%, TS 88%, average oxygen saturation, and oxygen saturation nadir. The more oxygen desaturation, either between 80% and 90% or less than 88%, the more difference there was between morning IL-6 and evening IL-6, and vice versa ([OSD 80%; r=0.54, p=0.04], [TS 88%; r=0.64, p=0.01]). Furthermore, both the average oxygen saturation and the oxygen saturation nadir were negatively correlated to the diurnal variation of IL-6 (r=-0.49, p=0.07; r=-0.51, p=0.05, respectively).

Exploratory Analyses: Partial Correlations Between Morning Inflammatory Biomarkers and Other Sleep-Related Variables

Beyond the relationship of diurnal variation of inflammatory biomarkers and sleep-related variables, an exploratory analysis of the partial correlation between morning inflammatory biomarkers and sleep-related variables was repeated. Some extreme values were observed in the data set; therefore, the partial correlation analysis was conducted after removing
the outliers, which identified with evening IL-10, evening and morning IL-6, and morning IL-8 (Table 4.9). There were no significant relationships between sleep-related variables and morning IL-10 or TNF-α. However, morning IL-6 had significant relationships with the arousal index and oxygen desaturation variables. The morning IL-6 was negatively correlated with both the arousal index \( (r=-0.68, p=0.003) \) and the RERA index \( (r=-0.58, p=0.02) \). The total sleep time spent with \( O_2 \) saturations less than or equal to 88\% was positively correlated to morning IL-6 \( (r=0.61, p=0.012) \), and the oxygen saturation nadir had a negative relationship with the morning IL-6, though this did not reach statistical significance \( (r=-0.47, p=0.07) \).

Table 4.9. Partial Correlations Between Morning Cytokines and Sleep-Related Variables After Excluding Outliers

<table>
<thead>
<tr>
<th></th>
<th>IL-10M (n=21)</th>
<th>IL-6M (n=19)</th>
<th>IL-8M (n=21)</th>
<th>TNF-αM (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI</td>
<td>0.07</td>
<td>-0.26</td>
<td>0.23</td>
<td>-0.11</td>
</tr>
<tr>
<td>AI</td>
<td>-0.09</td>
<td>-0.14</td>
<td>0.21</td>
<td>-0.34</td>
</tr>
<tr>
<td>HI</td>
<td>-0.18</td>
<td>-0.35</td>
<td>-0.12</td>
<td>-0.08</td>
</tr>
<tr>
<td>TST</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
<td>0.12</td>
</tr>
<tr>
<td>Arousal Index</td>
<td>-0.28</td>
<td>-0.68**</td>
<td>0.06</td>
<td>-0.12</td>
</tr>
<tr>
<td>RERA index</td>
<td>-0.21</td>
<td>-0.58*</td>
<td>-0.02</td>
<td>-0.03</td>
</tr>
<tr>
<td>OSD 80%</td>
<td>0.16</td>
<td>0.43</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>TS 88%</td>
<td>0.28</td>
<td>0.61*</td>
<td>0.19</td>
<td>0.03</td>
</tr>
<tr>
<td>Average O2</td>
<td>-0.12</td>
<td>-0.32</td>
<td>-0.29</td>
<td>-0.25</td>
</tr>
<tr>
<td>O2 Nadir</td>
<td>-0.32</td>
<td>-0.47</td>
<td>-0.16</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05; **p-value: ≤0.01

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

IL-10M, the level of morning IL-10; IL-6M, the level of morning IL-6; IL-8M, the level of morning IL-8; TNF-αM, the level of morning TNF-α; AHI, apnea-hypopnea index; AI, apnea index; HI, hypopnea index; TST, total sleep time; RERA Index, respiratory effort related arousal index; OSD 80%, total sleep time spent with \( O_2 \) saturations between 80\% and 90\%; TS 88\%,
total sleep time spent with $O_2$ saturations less than or equal to 88%; $O_2$ nadir, lowest oxygen saturation

**Exploratory Analyses: Partial Correlations Between Arousal and RERA and Inflammatory Biomarkers**

Supplemental partial correlation analyses were conducted to explore the relationship between arousal and respiratory effort related arousal (RERA) and inflammatory biomarkers (Table 4.10).

**Table 4.10. Partial Correlations Between Morning Between Arousal and RERA and Inflammatory Biomarkers**

<table>
<thead>
<tr>
<th></th>
<th>IL-10M</th>
<th>IL-10D</th>
<th>IL-6M</th>
<th>IL-6D</th>
<th>IL-8M</th>
<th>IL-8D</th>
<th>TNFA-M</th>
<th>TNFA-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Arousal (n=11)</td>
<td>0.29</td>
<td>0.30</td>
<td>0.11</td>
<td>0.74</td>
<td>0.18</td>
<td>-0.13</td>
<td>0.31</td>
<td>0.92*</td>
</tr>
<tr>
<td>High Arousal (n=11)</td>
<td>-0.42</td>
<td>-0.69</td>
<td>-0.08</td>
<td>-0.14</td>
<td>-0.32</td>
<td>-0.11</td>
<td>-0.19</td>
<td>0.39</td>
</tr>
<tr>
<td>Low RERA (n=11)</td>
<td>0.01</td>
<td>0.23</td>
<td>-0.55</td>
<td>-0.25</td>
<td>-0.06</td>
<td>0.41</td>
<td>-0.27</td>
<td>0.87*</td>
</tr>
<tr>
<td>High RERA (n=11)</td>
<td>-0.18</td>
<td>-0.43</td>
<td>0.39</td>
<td>-0.02</td>
<td>-0.29</td>
<td>-0.73</td>
<td>0.26</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*p-value: ≤0.01

**Notes.** Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

*IL-10M, the level of morning IL-10; IL-10D, change of IL-10 between evening and morning; IL-6M, the level of morning IL-6; IL-6D, change of IL-6 between evening and morning; IL-8M, the level of morning IL-8; IL-8D, change of IL-8 between evening and morning; TNF-αM, the level of morning TNF-α; TNF-αD, change of TNF-α between evening and morning; RERA Index, respiratory effort related arousal index*

Arousal index was dichotomized as low/high based on median value for the current sample (arousal index median 20.5 events/hour); low arousal index was ≤20.5 and high arousal
index was >20.5. Likewise, RERA variable was dichotomized as low/high; the median RERA index for the current sample was 2.25 events/hours; low RERA was defined as ≤2.5; high RERA was defined as >2.5. Diurnal variation of TNF-α was significantly correlated to low arousal and low RERA index (r=0.92, p=0.0013; r=0.87, p=0.007, respectively). There was a positive correlation between diurnal variation of IL-6 and low arousal, but it did not reach a statistical significance (r=0.74, p=0.054). In addition, high RERA index was negatively correlated to diurnal variation of IL-8, but it failed to reach statistical significance (r=-0.73, p=0.062). Partial correlation analysis was conducted to identify the relationship between the indices of sleep fragmentation, including arousal index and RERA index and oxygen desaturation, including OSD 80%, TS 88%, average O₂, and O₂ nadir (Table 4.11).

**Table 4.11. Partial Correlation Matrix: Sleep Fragmentation and Oxygen Desaturation**

<table>
<thead>
<tr>
<th></th>
<th>Arousal Index</th>
<th>RERA Index</th>
<th>OSD80%</th>
<th>TS 88%</th>
<th>Aver O₂</th>
<th>O₂ Nadir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arousal Index</td>
<td>1</td>
<td>0.890***</td>
<td>-0.236</td>
<td>-0.433</td>
<td>0.304</td>
<td>0.567**</td>
</tr>
<tr>
<td>RERA Index</td>
<td>1</td>
<td>-0.243</td>
<td>-0.508*</td>
<td>0.162</td>
<td></td>
<td>0.704**</td>
</tr>
<tr>
<td>OSD 80%</td>
<td>1</td>
<td>0.868***</td>
<td>-0.783***</td>
<td>-0.468*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS 88%</td>
<td></td>
<td></td>
<td>-0.555*</td>
<td>-0.768***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>O₂ Nadir</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05; **p-value: ≤0.01; ***p-value: ≤0.01

**Notes.** Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

RERA Index, respiratory effort related arousal index; OSD 80%, total sleep time spent with O₂ saturations between 80% and 90%; TS 88%, total time spent with O₂ saturations less than or equal to 88%; Aver O₂, average oxygen saturation; O₂ nadir, lowest oxygen saturation
Sleep fragmentation, indicated by the arousal index variable (events/hour) and RERA index (events/hr), was negatively correlated with the total time with O₂ saturations less than or equal to 88% (Arousal Index*TS 88%: r=-0.433, p=0.072; RERA Index*TS 88%: r=-0.508, p=0.029), whereas both indices of sleep fragmentation were positively correlated with the lowest oxygen saturation (Arousal Index*O₂ Nadir: r=0.567, p=0.012; RERA Index*O₂ Nadir: r=0.704, p=0.0007).

**Research Question 2.** Is there an association between inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP and everyday symptoms, including excessive daytime sleepiness and fatigue, in adults with OSA?

Momentary symptoms were measured as current moment symptoms; lasting symptoms were measured at baseline and necessitated historical recall period, reflecting on the past week or longer. The relationship between concurrently-measured inflammatory biomarkers and symptoms at each time point is shown in Tables 4.12 and 4.13.

**Table 4.12. The Relationship Between Evening Inflammatory Biomarkers and Momentary Symptoms Including Outliers**

<table>
<thead>
<tr>
<th></th>
<th>IL-10E</th>
<th>IL-6E</th>
<th>IL-8E</th>
<th>TNF-α E</th>
<th>CRP E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepiness E</td>
<td>0.42</td>
<td>0.71***</td>
<td>0.11</td>
<td>0.15</td>
<td>-0.34</td>
</tr>
<tr>
<td>Fatigue E</td>
<td>0.08</td>
<td>0.30</td>
<td>-0.02</td>
<td>-0.01</td>
<td>-0.15</td>
</tr>
<tr>
<td>Energy E</td>
<td>-0.43</td>
<td>-0.68**</td>
<td>-0.03</td>
<td>-0.28</td>
<td>0.23</td>
</tr>
<tr>
<td>Sleepiness M</td>
<td>0.27</td>
<td>0.02</td>
<td>0.30</td>
<td>0.28</td>
<td>0.15</td>
</tr>
<tr>
<td>Fatigue M</td>
<td>0.38</td>
<td>0.05</td>
<td>0.21</td>
<td>0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>Energy M</td>
<td>-0.41</td>
<td>-0.12</td>
<td>-0.04</td>
<td>-0.31</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05; **p-value: ≤0.01; ***p-value: ≤0.001

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes
IL-10E, interleukin-10 measured in the evening; IL-6E, interleukin-6 measured in the evening; IL-8E, interleukin-8 measured in the evening; TNF-αE, tumor necrosis factor-α measured in the evening; CRPE, C-reactive protein measured in the evening; Sleepiness E, sleepiness measured in the evening; Fatigue E, fatigue measured in the evening; Energy E, energy measured in the evening; Sleepiness M, sleepiness measured in the morning; Fatigue M, fatigue measured in the morning; Energy M, energy measured in the morning

Table 4.13. The Relationship Between Morning Inflammatory Biomarkers and Momentary Symptoms Including Outliers

<table>
<thead>
<tr>
<th></th>
<th>IL-10M</th>
<th>IL-6M</th>
<th>IL-8M</th>
<th>TNF-α M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepiness E</td>
<td>0.21</td>
<td>0.37</td>
<td>-0.22</td>
<td>0.15</td>
</tr>
<tr>
<td>Fatigue E</td>
<td>0.20</td>
<td>0.14</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>Energy E</td>
<td>0.02</td>
<td>-0.33</td>
<td>0.18</td>
<td>-0.14</td>
</tr>
<tr>
<td>Sleepiness M</td>
<td>-0.09</td>
<td>0.18</td>
<td>-0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Fatigue M</td>
<td>0.40</td>
<td>0.36</td>
<td><strong>0.58</strong></td>
<td><strong>0.45</strong></td>
</tr>
<tr>
<td>Energy M</td>
<td>-0.16</td>
<td>-0.32</td>
<td>-0.42</td>
<td>-0.24</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

IL-10M, interleukin-10 measured in the morning; IL-6M, interleukin-6 measured in the morning; IL-8M, interleukin-8 measured in the morning; TNF-αM, tumor necrosis factor-α measured in the morning; Sleepiness E, sleepiness measured in the evening; Fatigue E, fatigue measured in the evening; Energy E, energy measured in the evening; Sleepiness M, sleepiness measured in the morning; Fatigue M, fatigue measured in the morning; Energy M, energy measured in the morning

There were no significant relationships between IL-10 and momentary symptoms. Notably, there were distinct trends between evening IL-6 and evening symptoms and between morning IL-8 and morning symptoms. Evening IL-6 was positively correlated with evening sleepiness (r=0.719, p<0.001), (i.e., increased evening IL-6 was correlated to increased evening
sleepiness) and negatively correlated to evening energy ($r=-0.685$, $p=0.001$) (i.e., increased evening IL-6 was correlated to decreased energy level); whereas, morning fatigue was positively correlated with morning IL-8 ($r=0.582$, $p=0.021$) and morning IL-8 was negatively correlated to morning energy ($r=-0.428$, $p=0.072$). Likewise, there was a positive correlation between morning TNF-α and morning fatigue ($r=0.453$, $p=0.05$).

To exclude the potential effect of outliers on the results, the Spearman partial rank correlation was repeated after removing outliers, which identified with evening IL-10, evening and morning IL-6, and morning IL-8 (Tables 4.14 and 4.15).

**Table 4.14. Relationship Between Evening Inflammatory Biomarkers and Momentary Symptoms Excluding Outliers**

<table>
<thead>
<tr>
<th></th>
<th>IL-10E (n=21)</th>
<th>IL-6E (n=20)</th>
<th>IL-8E (n=22)</th>
<th>TNF-α E (n=22)</th>
<th>CRPE (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepiness E</td>
<td>0.40</td>
<td>0.69**</td>
<td>0.11</td>
<td>0.15</td>
<td>-0.41</td>
</tr>
<tr>
<td>Fatigue E</td>
<td>0.11</td>
<td>0.25</td>
<td>-0.02</td>
<td>-0.01</td>
<td>-0.07</td>
</tr>
<tr>
<td>Energy E</td>
<td>-0.36</td>
<td>-0.57*</td>
<td>-0.03</td>
<td>-0.28</td>
<td>0.42</td>
</tr>
<tr>
<td>Sleepiness M</td>
<td>0.34</td>
<td>-0.05</td>
<td>0.30</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Fatigue M</td>
<td>0.31</td>
<td>-0.09</td>
<td>0.21</td>
<td>0.30</td>
<td>-0.01</td>
</tr>
<tr>
<td>Energy M</td>
<td>-0.36</td>
<td>0.21</td>
<td>-0.04</td>
<td>-0.31</td>
<td>-0.001</td>
</tr>
</tbody>
</table>

*p-value: $\leq$0.05; **p-value: $\leq$0.01

**Notes.** Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

*IL-10E, interleukin-10 measured in the evening; IL-6E, interleukin-6 measured in the evening; IL-8E, interleukin-8 measured in the evening; TNF-αE, tumor necrosis factor-α measured in the evening; CRPE, C-reactive protein measured in the evening; Sleepiness E, sleepiness measured in the evening; Fatigue E, fatigue measured in the evening; Energy E, energy measured in the evening; Sleepiness M, sleepiness measured in the morning; Fatigue M, fatigue measured in the morning; Energy M, energy measured in the morning*
Table 4.5. Relationship Between Morning Inflammatory Biomarkers and Momentary Symptoms

Excluding Outliers

<table>
<thead>
<tr>
<th></th>
<th>IL-10M (n=22)</th>
<th>IL-6M (n=19)</th>
<th>IL-8M (n=21)</th>
<th>TNF-α M (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepiness E</td>
<td>0.21</td>
<td>0.49</td>
<td>-0.30</td>
<td>0.15</td>
</tr>
<tr>
<td>Fatigue E</td>
<td>0.20</td>
<td>0.10</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Energy E</td>
<td>0.02</td>
<td>-0.24</td>
<td>0.36</td>
<td>-0.14</td>
</tr>
<tr>
<td>Sleepiness M</td>
<td>-0.09</td>
<td>-0.11</td>
<td>-0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Fatigue M</td>
<td>0.40</td>
<td>0.38</td>
<td><strong>0.51</strong></td>
<td><strong>0.45</strong></td>
</tr>
<tr>
<td>Energy M</td>
<td>-0.16</td>
<td>-0.44</td>
<td>-0.36</td>
<td>-0.24</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05

Notes. Spearman Partial Rank Correlation, adjusted for age, body mass index (BMI), cardiovascular diseases, and type II diabetes

IL-10M, interleukin-10 measured in the morning; IL-6M, interleukin-6 measured in the morning; IL-8M, interleukin-8 measured in the morning; TNF-α M, tumor necrosis factor-α measured in the morning; Sleepiness E, sleepiness measured in the evening; Fatigue E, fatigue measured in the evening; Energy E, energy measured in the evening; Sleepiness M, sleepiness measured in the morning; Fatigue M, fatigue measured in the morning; Energy M, energy measured in the morning

The results were similar with and without outliers. The significant relationships between evening IL-6 and evening symptoms and between morning IL-8 and morning symptoms remained.

Research Question 3: Is diurnal variation of inflammatory biomarkers, including IL-1β, IL-6, IL-8, IL-10, TNF-α, and CRP associated with symptoms in adults with OSA?

The relationship between diurnal variation of each inflammatory biomarker and symptoms was examined. With outliers included in the analysis, the relationship between diurnal variation of IL-10 and evening energy was the only significant relationship identified as shown in the Figure 4.6 (r=0.478, p=0.043).
**Figure 4.6. Scatterplot Illustrating the Relationship Between Diurnal Variation of IL-10 and Evening Energy**

![Scatterplot](image)

**Notes.** IL10D, diurnal variation of interleukin-10; ELEEE, energy measured in the evening

When outliers were removed from the analyses (i.e., the identified outliers with evening IL-10, evening and morning IL-6, and morning IL-8), other significant findings were identified for the association between diurnal variation of inflammatory biomarkers and symptoms (Table 4.16). Diurnal variation of IL-8 was positively correlated to morning fatigue ($r=0.54$, $p=0.03$) and negatively correlated to morning energy ($r=-0.45$, $p=0.08$). The relationship between diurnal variation of IL-10 and the evening energy remained significant after removing outliers ($r=0.49$, $p=0.04$). Diurnal variation of IL-6 and IL-8 and change in energy measured between evening and morning had a negative relationship ($r=-0.48$, $p=0.07$; $r=-0.53$, $p=0.03$, respectively), showing that the increased levels of IL-6 and IL-8 were associated with less energy, and vice versa.
Table 4.16. Relationship Between Diurnal Variation of Inflammatory Biomarkers and Symptoms

Excluding Outliers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>IL-10D (n=20)</th>
<th>IL-6D (n=18)</th>
<th>IL-8D (n=19)</th>
<th>TNF-αD (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleepiness E</td>
<td>-0.013</td>
<td>0.114</td>
<td>-0.379</td>
<td>-0.012</td>
</tr>
<tr>
<td>Sleepiness M</td>
<td>-0.045</td>
<td>-0.022</td>
<td>0.087</td>
<td>-0.287</td>
</tr>
<tr>
<td>Sleepiness D</td>
<td>-0.070</td>
<td>-0.193</td>
<td>0.376</td>
<td>-0.138</td>
</tr>
<tr>
<td>Fatigue E</td>
<td>-0.062</td>
<td>0.196</td>
<td>0.070</td>
<td>0.351</td>
</tr>
<tr>
<td>Fatigue M</td>
<td>-0.143</td>
<td>0.192</td>
<td><strong>0.548</strong></td>
<td>0.229</td>
</tr>
<tr>
<td>Fatigue D</td>
<td>0.014</td>
<td>-0.087</td>
<td>0.359</td>
<td>0.015</td>
</tr>
<tr>
<td>Energy E</td>
<td><strong>0.496</strong></td>
<td>0.383</td>
<td>0.320</td>
<td>0.329</td>
</tr>
<tr>
<td>Energy M</td>
<td>0.416</td>
<td>-0.309</td>
<td>-0.455</td>
<td>0.195</td>
</tr>
<tr>
<td>Energy D</td>
<td>0.091</td>
<td>-0.486</td>
<td><strong>-0.537</strong></td>
<td>-0.107</td>
</tr>
</tbody>
</table>

*p-value: ≤0.05

Notes. IL-10D, diurnal variation of interleukin-10; IL-6D, diurnal variation of interleukin-6; IL-8D, diurnal variation of interleukin-8; TNF-αD, diurnal variation of tumor necrosis factor-α; Sleepiness E, sleepiness measured in the evening; Sleepiness M, sleepiness measured in the morning; Sleepiness D, diurnal variation of sleepiness; Fatigue E, fatigue measured in the evening; Fatigue M, fatigue measured in the morning; Fatigue D, diurnal variation of fatigue; Energy E, energy measured in the evening; Energy M, energy measured in the morning; Energy D, diurnal variation of energy

Research question 4: What is symptom phenotype in adults with OSA?

Cosine similarity was calculated between the symptom vectors among 22 OSA participants. The resulting network, which represents natural clusters of the symptoms, is shown in Figure 4.5. The lightest solid line indicates the similarity of 0.90-0.94 between two vectors; the medium solid line shows the similarity of 0.95-0.96 between two vectors; and the heaviest solid line defines the similarity of greater than 0.97 between two vectors; a cosine cutoff of
greater than 0.9 was applied to indicate a similarity (Dumais et al., 1988). Two distinct symptom clusters were identified (Figure 4.7). Cluster 1 included the momentary symptoms of sleepiness (evening and morning) and fatigue (evening and morning) in adults with OSA; cluster 2 included sleep-related quality of life (all domains) and momentary symptom of energy levels (evening and morning).

In cluster 1, evening fatigue and evening sleepiness had the strongest connection (0.94), and perceived stress was connected to four different momentary symptoms, including evening sleepiness, morning sleepiness, morning fatigue, and diurnal symptoms, a sleep-related quality of life construct. Evening sleepiness had a connection with evening fatigue and morning sleepiness, indicating that OSA patients who have evening sleepiness are likely to have morning sleepiness and evening fatigue. Cluster 1 can be characterized as momentary symptoms in adults with OSA.

In cluster 2, symptom vectors shared at least three connections with each other, excepting evening energy. The nocturnal symptom, a sleep-related quality of life construct, was the strongest variable, showing six connections with other variables of which four symptoms had strong similarity. Nocturnal symptoms in adults with OSA were linked to daytime sleepiness, evening and morning energy, diurnal symptoms, social interactions, and emotions. Diurnal symptom was connected to all of the symptom variables in cluster 2 except evening energy. Cluster 2 can be characterized as sleep quality symptoms in adults with OSA. Sleepiness over the past month, mood disturbance, and depression were not grouped in any cluster and remained independent of other symptoms.
Figure 4.7. Symptom Phenotype in OSA

0.9 ≤ similarity ≤ 0.94
0.95 ≤ similarity ≤ 0.96
0.97 ≤ similarity
Notes. ELEEFSC, momentary evening fatigue; ESSS, momentary evening sleepiness; MSSS, momentary morning sleepiness; MALEEFS, momentary morning fatigue; STRESS, perceived stress; ELEEESC, momentary evening energy; QSQDSSx, lasting daytime sleepiness; QSQNOSx, lasting nocturnal symptoms; MALEEES, momentary morning energy; QSQDISx, lasting diurnal symptoms; QSQEMSx, lasting emotion status; QSQSCSx, lasting social interaction; EPWORTH, lasting daytimes sleepiness; POMS, lasting mood disturbance; BDI, lasting depressive symptoms
Chapter 5

Summary of Significant Findings

The purpose of this study was to identify whether inflammatory biomarkers are associated with everyday symptoms including excessive daytime sleepiness and fatigue after adjustment for confounding variables in terms of obesity, age, T2DM, and cardiovascular disease; additionally, the study sought to determine whether diurnal variation of inflammatory biomarkers influences everyday symptoms in adults with OSA. Significant relationships of (1) IL-6 diurnal variation, and (2) morning IL-6, were found with oxygen desaturation and sleep fragmentation indices. However, diurnal variation of IL-6 and morning IL-6 were not correlated with AHI, which is considered a gold standard metric for determining OSA severity. Additional significant relationships were found between evening IL-6 and evening symptoms, including sleepiness and energy level, and between morning IL-8 and morning symptoms, including fatigue and energy level. It is important to note that diurnal variation of IL-8 was correlated to morning symptoms, including fatigue and energy symptoms, and that diurnal variation of both IL-6 and IL-8 were correlated with diurnal variation of energy levels. Two distinct symptom clusters were identified including momentary symptoms and lasting symptoms in mild OSA.

Inflammatory Biomarkers in Mild OSA

No studies have specifically examined the prevalence of mild OSA, but several studies have shown the overall prevalence of OSA across AHI values. The estimated prevalence of OSA with AHI ≥5 ranges from 3 to 28%; whereas, when AHI ≥15 criterion was used, OSA prevalence estimates were lower with a range of 1 to 14% (Bearpark et al., 1993; Bixler et al., 2001; Durán, Esnaola, Rubio, & Iztueta, 2001; Gislason, Almqvist, Eriksson, Taube, & Boman, 1988; Kripke et al., 1997; Young et al., 1993). In spite of the apparent high prevalence of mild OSA and the
clinical importance of early detection, there are a limited number of studies that have focused on intervention, treatment, and prognosis of mild OSA.

In the current study, the majority of study participants had mild OSA (68.2%). Previous studies have suggested the relationship between mild OSA and adverse cardiovascular consequences; in detail, approximately 60% of mild OSA cases had hypertension even after adjustment for BMI (Nieto et al., 2000). Similarly, Peppard et al. (2000b) examined the incidence of developing hypertension and found the odds ratio for hypertension incidence in mild OSA was 2.03 (95% CI 1.29, 3.17) compared to non-OSA. These findings imply that mild OSA confers risks for the development of cardiovascular disease. Endothelial function (i.e., endothelium-dependent venodilation) has been shown to be decreased in mild OSA, suggesting an increased atherosclerotic load and potential for local or systemic inflammation (Duchna, Stoohs, Guilleminault, Christine-Anspach, Schultze-Werninghaus, & Orth, 2006). These findings suggest mild OSA, per se, is a risk factor for systemic inflammation and cardiovascular disease.

The results from a cross-sectional study demonstrate increased levels of pro-inflammatory cytokines (TNF-α and IL-1β) and anti-inflammatory cytokines (IL-10 and interleukin-1 receptor antagonist) in mild OSA compared to those of non-OSA (Sahlman et al., 2010). These results were significant even after controlling for the effects of confounding variables including age, BMI, and comorbidities (Sahlman et al., 2010). Previous studies of inflammatory biomarkers and OSA have primarily included moderate to severe OSA; therefore, inflammatory biomarkers in mild OSA are not well-understood. The current study included primarily adults with mild OSA, providing new insights to the field about the association between inflammatory mediators and mild OSA.
Diurnal Variation of Inflammatory Biomarkers

Since this study intended to only measure inflammatory biomarkers at two different time points (pre-sleep and post-sleep), the variability of the level of inflammatory biomarkers that occurs across a 24-hour period (i.e., circadian variability) was not addressed. Diurnal variability, however, was examined; the measurement approach therefore supported examining the impact of sleep apnea on the natural change of inflammatory biomarkers over sleep. Vgontzas et al. (1999) examined circadian rhythm of IL-6 and the association between the circadian rhythm of IL-6 and quantity/depth of sleep. IL-6 was measured every hour from an indwelling catheter. The results delineated the circadian rhythm of IL-6, which preliminarily indicates an increasing pattern of IL-6 over the sleep period and a sharp decrease of IL-6 in the morning. Consistent with Vgontzas et al study, the current study identified increased evening IL-6 and decreased morning IL-6, resulting in diurnal variability of IL-6.

In accordance with the study of Entzian and colleagues (1996), study participants in this study had lower levels of IL-6 in the morning (measured between 6am and 7am) and higher levels of IL-6 in the evening (measured between 8pm and 10pm). The current study’s IL-6 measurement interval was consistent with the Entzian et al. (1996) study wherein a peak level of IL-6 occurred at 8:47pm and a nadir level of IL-6 occurred at 8am (Entzian et al., 1996). In the Entzian et al. study (1996), variation of IL-6 was consistent during the daytime between healthy adults and adults with OSA; however, diurnal variation of IL-6 was different over the sleep period between the two groups. Healthy adults had a distinct increase over the sleep period between 12:00am and 4:00am, whereas adults with OSA had peak levels of IL-6 during sleep and a gradual decline to a minimum concentration at 8:00am (Entzian et al., 1996). The findings of the current study in an OSA cohort, which are consistent with prior studies, imply that
disturbed sleep caused by OSA contributes to chronic low-grade inflammation. IL-6 is also a mediator of sleep regulation, influenced by sleep, itself, and the continuity of sleep (Rohleder, Aringer, & Boentert, 2012). Therefore, alteration of the biological clock (i.e., circadian clock) by IL-6 may not only influence sleep regulation in OSA but may be further altered by intermittent hypoxia and therefore be a molecular basis of sleep-related symptoms in OSA.

To the author’s knowledge, there are no published studies that have addressed diurnal variation of IL-8 or IL-10 in adults with OSA of any severity. However, preliminary evidence suggests that hypoxia stimulates the expression of IL-8 (Wilson, Wallin, Della-Cioppa, Sandstrom, & Holgate, 2001). It has also been shown that the level of IL-8 in untreated adults with OSA was significantly greater than those in a control group (Ohga et al., 2003). The current study findings are consistent with prior preliminary evidence; participants in this study had significant oxygen desaturation over the sleep period and an increased level of IL-8 after the sleep bout. However, the increasing pattern of IL-8 during the sleep period in OSA may be a natural diurnal variation of IL-8 since in healthy adults, the diurnal variation of IL-8 showed a similar pattern (Hermann et al., 2006). It is therefore important to continue research focused on diurnal variation of IL-8, which necessitates comparison groups of OSA and healthy adults to more explicitly address the diurnal variation of IL-8.

There is no evidence regarding diurnal variation of anti-inflammatory cytokines (i.e., IL-10), as opposed to pro-inflammatory cytokines (IL-6 and IL-8), in adults with OSA. Little is known about IL-10. In the current study, there were no relationships identified between IL-10 levels and AHI or between IL-10 and hypoxic indices. However, diurnal variation of IL-10 was positively correlated to evening energy levels. These results must be considered relative to the scant evidence about IL-10, in OSA and non-OSA. IL-10 inhibits the production of somnogenic
cytokines and substances related to sleep regulation such as neurotrophins (Krueger, 2008). Adults with OSA experience a reduction in AHI in slow wave sleep (i.e., N3 stage of sleep) compared to N1 and N2 stages of sleep; the highest AHI is typically recorded during N1 stage of sleep (McCaskill, Ho, Auerbach, & Brass, 2013). Delineating diurnal variation of IL-10 both in healthy and OSA adults will suggest a biological mechanism whereby sleep may be altered in response to an activated inflammatory response due to intermittent hypoxia.

Published evidence on diurnal variation of IL-1β shows the peak levels of IL-1β during the sleep period and the nadir in the morning (Entzian et al., 1996). In this study, IL-1β was not detectable and therefore no analysis of this pro-inflammatory cytokine can be addressed. IL-1β has been evaluated as a potent proinflammatory cytokine, along with IL-6 and TNF-α, which contribute to the regulation of sleep in healthy adults (Kang et al., 2013). In the current study, sleep fragmentation identified by arousal index and RERA index is noteworthy and may be confounding the current study results for IL-1β. Previous evidence has argued that although inflammatory response is an important mechanism of the pathogenesis of OSA, systemic changes of inflammatory biomarkers in mild OSA may not be detectable (de Lima, Mazzotti, Tufik, S., & Bittencourt, 2016). These findings suggest that future studies will need to include larger samples with heterogeneity for OSA severity to determine if venous sampling of IL-1β is a marker with utility.

Proinflammatory cytokines including IL-1β, IL-6, and TNF-α are also known as endogenous somnogens, regulating the sleep-wake cycle (Krueger & Majde, 1995). Previous evidence has found a distinct diurnal variation of these cytokines, which shows the highest levels during the sleep period in healthy adults (Entzian et al., 1996). The distinct diurnal variation disappeared in adults with OSA, wherein TNF-α exhibited the peak level at noon and the
minimum concentration at the night (Entzian et al., 1996). In the current study, TNF-α was only measured pre- and post-sleep bout, which makes it difficult to draw any conclusions relative to prior studies that employed measurement of TNF-α at different times of day/night. Nonetheless, the significant difference between evening TNF-α and morning TNF-α (i.e., higher level of TNF-α in the morning compared to evening TNF-α) in the current study suggests that TNF-α may play a role in the pathophysiologic sequelae of OSA and contribute to daytime somnolence.

**Diurnal Variation of Inflammatory Biomarkers and AHI**

AHI (apnea-hypopnea index) is the most commonly employed measure of OSA severity and is employed clinically to guide treatment decisions, such as positive airway pressure (PAP) (American Academy of Sleep Medicine, 2012). However, more recent studies have cast doubt on the role of the AHI as the most appropriate metric to determine OSA severity (Asghari & Mohammadi, 2013; Muntarbhorn & Kunachak, 2016). The concerns about using this metric to guide decisions are two-fold: 1) AHI considers only the frequency of apneic and hypopneic events; and 2) AHI does not consider the degree of oxygen desaturation with apneic and hypopneic events (Asghari & Mohammadi, 2013; Muntarbhorn & Kunachak, 2016). As such, using only the AHI to guide treatment decisions is problematic. Patients with OSA experience different degrees of decreased airflow during sleep; for example, one can experience apneas of long duration but the number of episodic interruptions of breathing is not frequent; whereas others can experience the opposite (i.e., more frequent apneic episodes but of short duration). The long-lasting complete breathing pause, or apnea, can result in a larger reduction of arterial oxygen saturation than hypopneic events or short duration apneic events, and consequently likely contribute to worse outcomes/sequelae. Hypopneas, which are not complete airway closures but rather reductions in upper airway airflow, may be less specific indicators of OSA severity as
oxygen saturation criteria for hypopneas requires only 3-4% decrement and hypopneas tend to be of short duration. Therefore, AHI, based on the number of apneic/hypopneic events, may not be a specific metric for determining OSA severity if OSA sequelae are highly dependent on oxygenation.

With this controversy in mind, the current study identified no significant relationships between morning inflammatory biomarkers and AHI, diurnal variation of inflammatory biomarkers and AHI, or between diurnal variation of inflammatory biomarkers and apnea index (AI), or hypopnea index (HI), individually. When outliers were removed from the analysis and AI and HI were individually examined in relationship to inflammatory biomarkers, there was a moderate positive relationship between diurnal variation of IL-8 and the AI (r=0.51, p=0.049). Apnea index solely, rather than AHI, may be more informative regarding OSA severity as apneas are defined by ≥ 90% decrease from baseline airflow, lasting at least 10 seconds (American Academy of Sleep Medicine, 2012). In addition to apnea index (AI), oxygenation criteria such as OSD 80%, TS88%, average O₂, and O₂ nadir may be more deterministic of OSA severity. Recurrent nocturnal airway obstruction, followed by the reduction in oxygen saturation and normalization with ventilation, contributes to the development of reactive oxygen species (ROS) which affect the ischemic or hypoxic tissue damage (McCord, 2000). It is known that oxidative stress stimulates IL-8 secretion; therefore, the positive relationship between the apnea index and IL-8 shown in this study suggests that the recurrent hypoxia/re-oxygenation during sleep in OSA should be further explored as a causal mechanism (Vlahopoulos, Boldogh, Casola, & Brasier, 1999).

Notably, there were no significant relationships between the apneic/hypopneic events (i.e., AHI index, AI index, and HI index) and any morning inflammatory biomarker. As
previously reported, diurnal variation of IL-8 was significantly correlated to the AI; however, morning IL-8 did not show any significant relationships with AHI index, AI index, and HI index. This finding may be explained in terms of the natural trend of increasing IL-8 in OSA through the day. Therefore, diurnal variation of IL-8, rather than post-sleep bout IL-8, may indicate the true impact of the severity of apneic episodes on oxidative distress.

Diurnal Variation of Inflammatory Biomarkers and Hypoxia

The most significant feature of obstructive sleep apnea may be a disruption of normal gas exchange (i.e., recurrent hypoxia and hypercapnia). A number of studies have argued that the episodes of oxygenation/re-oxygenation in adults with OSA are likely to increase cardiovascular complications, which include endothelial dysfunction, sympathetic nerve activation, and inflammation (Ryan, Taylor, & McNicholas, 2005). Recurrent oxygenation/re-oxygenation during sleep, every night, may result in cell stress; more specifically, mitochondrial dysfunction (Li & Jackson, 2002) and consequently, the downstream effects of the activation of proinflammatory responses (Ross, 1999). Therefore, maintaining cell function is of major importance in establishing a positive relationship between recurrent oxygenation/re-oxygenation in OSA and inflammation. The present study examined the relationship between inflammatory biomarkers and diverse indicators of hypoxic episodes, considering not only the total sleep time spent with hypoxia but also the degree of hypoxia.

This study found a significant relationship between oxygen desaturation and diurnal variation of IL-6 and morning IL-6. IL-6 is released by T-cells and macrophages as well as produced by adipocytes; thereby, any significant relationship between oxygen desaturation and IL-6 is potentially affected by obesity and must be considered (Askevold et al., 2014). However, this study controlled for obesity as a confounding variable, so the potential effects of obesity on
the relationship were controlled. Diurnal variation of IL-6 was positively correlated to increased total time spent with low oxygen saturation and negatively correlated to low oxygen saturation. When psychological (depression, anxiety) or physiological stress (infection, inflammation, and hypoxic status) occurs, IL-6 induces local and systemic reactions by circulating cell activation of leukocytes, neutrophils, and endothelial cells (Jain, Gautam, & Naseem, 2011). IL-6 is produced by Kupffer cells in the liver. Stress enhances the production of adrenocorticotropic hormone (ACTH) and cortisol and subsequently, the activation of ACTH triggers IL-6 release in liver cells into the circulation (Moshage, 1997). To address the potential confounding effects of comorbid conditions on IL-6 release, the present study exclusion criteria ensured that no study participants had hepatic diseases or ACTH deficiency diseases.

Study participants had a mean 17.1% time of oxygen desaturation with O₂ saturation between 80% and 90% over the total sleep time (calculated by \[ \frac{\text{mean OSD}_80\%}{\text{total sleep time}} \times 100\% = \frac{63.05\text{min}}{368.8\text{min}} = 17.1\% \]) and a mean O₂ nadir of 82.5%. The results of the present study are in keeping with those reported in healthy humans exposed to hypoxemia (Klausen, Olsen, Poulsen, Richalet, & Pedersen, 1997). Klausen et al. (1997) found that serum IL-6 was negatively correlated to SaO₂; there were no significant correlations with other proinflammatory cytokines including IL1-β, IL-1 receptor antagonist (IL-1ra), and TNF-α. Beyond serum IL-6, IL-6 induced by sputum was also significantly correlated to oxygen desaturation indices (the lowest SpO₂) in OSA (Aihara et al., 2013). Even though participants in this study had mild OSA, there were consistent relationships identified between IL-6 and oxygen desaturation indices (OSD 80%, TS88%, average O₂, and O₂ Nadir). Therefore, further studies are needed to confirm the findings by including more variation in OSA severity (i.e., moderate OSA and severe OSA) and ensure heterogeneity for severity classification that incorporate oxygenation indices.
Diurnal Variation of Inflammatory Biomarkers and Total Sleep Time

This study found opposite relationships for diurnal variation of IL-6 and total sleep time (negative correlation) and diurnal variation of IL-8 and total sleep time (positive correlation). IL-6 has a nadir level in the morning and peak level at night in both healthy and OSA adults (Entzian et al., 1996). The negative correlation between diurnal variation of IL-6 and total sleep time was therefore expected and can be understood in the circadian rhythm of IL-6. In accordance with this study, the study of Hong et al. (2004) demonstrated the negative correlation between morning IL-6 and total sleep time in healthy adults with a respiratory disturbance index of less than 15 (RDI; calculated as [the number of apnea events per hour + the number of hypopnea events per hour + respiratory effort related arousals per hour] / total sleep time [in minutes]). In addition, total sleep time was negatively correlated to morning IL-6 levels in patients with disorders with excessive daytime sleepiness (Vgontzas et al., 1997) and in healthy adults with sleep deprivation (Vgontzas et al., 1999). Recognizing IL-6 peak levels are at night and nadir levels are in morning, the findings of this study are consistent with what is currently known about IL-6 rhythmicity.

IL-8 influences the regulation of sleep by promoting non-rapid eye movement sleep (Krueger, 2008). However, the evidence which explores the relationship between IL-8 and total sleep time in OSA is scarce. Total sleep time is defined as the number of minutes in stages N1-3 and REM and is one of the markers of sleep quality (American Academy of Sleep Medicine, 2012). In the present study, total sleep time was positively associated with diurnal variation of IL-8 \( r=0.66, p=0.002 \). In OSA, total sleep time is typically lower than in non-OSA due to sleep fragmentation associated with respiratory-related arousals. As total sleep time is reduced in OSA, IL-8 diurnal variation is similarly reduced; as total sleep time is increased in OSA, IL-8 diurnal
variation is similarly increased. To explore whether this relationship is specific to continuity of sleep relative to cortical arousals (i.e., sleep fragmentation) or respiratory-event related arousals, preliminary exploration of arousal indices and respiratory-event related arousals with inflammatory biomarker diurnal variation was conducted.

**Diurnal Variation of Inflammatory Biomarkers and Arousal**

The physiological determinants of airway patency predominantly include ventilatory control stability; more specifically, loop gain and arousal threshold. Both loop gain and arousal threshold are important physiologic determinants for maintaining stable respiratory conditions, but both are abnormal in OSA and consequently contribute to the degree of airway collapse in OSA (Jordan, McSharry, & Malhotra, 2014). Pharyngeal critical closing pressure (Pcrit) is defined as the pressure inside pharyngeal airway where the airway obstruction does occur (i.e., collapsibility of the upper airway) (Kuna & Sant'Ambrogio, 1991). Patients with OSA have higher Pcrit compared to those without OSA because of narrow upper airway caliber (Kuna & Sant'Ambrogio, 1991). The pharyngeal airway dilator muscles are response dependent on both the negative intra-pharyngeal pressure and PCO₂ levels, which serve as respiratory stimuli. The repetitive collapse of the upper airway in OSA accelerates the failure of activating the upper airway dilator muscles against the collapsing pressure (Horner, Hughes, & Malhotra, 2014; Jordan et al., 2014).

During sleep, patients with OSA experience frequent arousals as the result of ventilatory stimulation in response to upper airway resistance. As arousals occur at a threshold level of ventilatory drive, it is crucial to determine whether apnea or hypopnea will occur (Deacon & Catcheside, 2015; Wellman et al., 2011). The arousal threshold can be divided into a high arousal threshold (hard to wake up) and a low arousal threshold (wake up easily). A high arousal
threshold permits time for the activation of pharyngeal dilator muscles to maintain airway stability; whereas, a low arousal threshold does not allow enough time for respiratory stimuli to trigger pharyngeal dilator muscles, and consequently leads to unstable ventilatory control (Wellman et al., 2011). Workload to increase ventilation to a sustainable level contributes to oxidative stress and resultant increased inflammatory biomarkers (Deacon & Catcheside, 2015).

Patients with OSA show an elevation in arousal threshold; it is thought to be an adaptive phenomenon (i.e., permits time for pharyngeal dilator muscle activation). In contrast to the adaptive high arousal threshold, gradual increases in arousal threshold are thought to be an accumulation of respiratory stimuli to maintain upper airway patency (Eckert et al., 2011; Wellman et al., 2011). In the present study, morning IL-6 had a negative relationship with arousal index and RERA index, indicating that fewer arousals, cortical and/or respiratory-event related arousals, result in higher morning IL-6 levels and vice versa. As study participants had OSA of unknown duration prior to study enrollment, this observation may suggest adaptation to the frequent arousals. In spite of oxygen desaturation, responsivity to ventilator demand may be dulled/blunted. In support of these findings, O₂ nadir was positively correlated to both arousal index and RERA index. The findings suggest an adaptive increase in arousal threshold in adults with even mild OSA.

**IL-6 and Everyday Symptoms**

The proinflammatory cytokine, IL-6, is multifunctional cytokine that plays a crucial role in not only immune response, but also biological processes such as inflammation, stress, and sleep (Rohleder, Aringer, & Boentert, 2012). IL-6 is secreted by both immune cells including T-cells and macrophages and nonimmune cells including endothelial cells and epithelial cells to stimulate immune response during acute and chronic inflammation. Effects of which are
apparent at peripheral tissues as well as in the central nervous system (CNS). IL-6 is capable of altering the metabolism and activity of neurotransmitters and decreasing neurotrophic factors since it crosses the blood-brain barrier (BBB) (Chen, Castro, Chow, & Reichlin, 1997; Romero, Kakucska, Lechan, & Reichlin, 1996). It is therefore hypothesized that during the inflammatory process, IL-6 mediates everyday symptoms such as excessive daytime sleepiness and fatigue (Dantzer, Heijnen, Kavelaars, Laye, & Capuron, 2014; Rohleder et al., 2012).

Several studies examined the relationship between IL-6 and OSA symptoms, including sleepiness and fatigue in OSA, and have found significant relationships between IL-6 and OSA symptoms (Vgontzas et al., 1997; Yokoe et al., 2003). However, the findings should be interpreted with caution due to methodological concerns. Vgontzas et al. (1997) suggested that both IL-6 and TNF-α may mediate excessive daytime sleepiness and fatigue in adults with OSA. However, the study did not include a symptom measurement (i.e., subjective symptom) but instead used mean nap sleep latency as an objective measure of excessive daytime sleepiness intensity; the protocol period for OSA symptom measurement based on nap latency was also therefore not concurrent with cytokine sampling. Even though blood samples for proinflammatory cytokines were reportedly obtained in the morning, after PSG removal, variability for time of collection was not reported in the study. Therefore, any conclusions from this prior work must be considered preliminary as OSA symptom expression cannot be reliably determined with the afore-mentioned methodological limitations.

The present study, which measured both OSA symptoms and IL-6 at nearly the same time, showed a significant correlation between evening IL-6 and evening sleepiness and between evening IL-6 and evening energy levels. These findings, therefore, imply that the IL-6 expression potentially underlies the biological mechanism of OSA symptoms. Similarly, Li et al. (2016)
examined the relationship not only between IL-6 and subjective sleepiness measured by Epworth Sleepiness Scale and Stanford Sleepiness Scale, but also between IL-6 and objective sleepiness measured by the Multiple Sleep Latency Test (MSLT) in adults with AHI ≥10. IL-6 was collected every hour through an indwelling catheter. Notably, evening or morning subjective sleepiness was not significantly associated with evening or morning IL-6; however, objective sleepiness (by MSLT) was significantly related to elevated 24-hour IL-6 (β = −0.34, p = 0.01), morning IL-6 (β = −0.30, p = 0.02) and evening IL-6 (β = −0.38, p < 0.01) levels. This study identified that asymptomatic OSA patients (i.e., normal levels of subjective sleepiness) may instead demonstrate OSA-related symptoms by objective testing and objectively-measured symptoms correlate with proinflammatory cytokines. The findings of no relationships between IL-6 and daytime sleepiness (measured by ESS) or between IL-6 and daytime sleepiness (measured by SSS) should be interpreted with caution. The protocol included 4-24hr laboratory days for specimen collection and polysomnography. Concurrently-assessed daytime sleepiness was measured on the last protocol day. The laboratory environment and protocol activities may affect everyday symptom levels; this may result in a lack of external validity. A second methodological concern is the timing of measuring momentary symptoms by SSS. Measurements were assessed at 09:00 am, 12:00 pm, 3:00 pm, and 5:00 pm. In adults with OSA, the peak levels of IL-6 occur at pre-sleep bout (about 9:00pm) and the nadir levels of IL-6 occurred at post-sleep bout (between 7:00-8:00) (Entzian et al., 1996). Therefore, measurement of momentary symptoms would ideally include periods relative to the known rhythmicity of IL-6 in OSA. Future studies with rigorous methodology, carefully designed relative to the current state of science addressing IL-6 in OSA, are needed to determine the underlying pathophysiologic mechanisms between subjective sleepiness and IL-6. To improve external
validity of any such studies, consideration of home PSG, continuous or high-frequency blood sampling, and technology-delivered measures of momentary symptoms are recommended.

**IL-8 and Everyday Symptoms**

To the best of the author’s knowledge, the current study is the first study to examine the relationship between the levels of IL-8 and symptoms including fatigue, energy levels, and sleepiness and between diurnal variation of IL-8 and symptoms in OSA. This study found that increased levels of IL-8 in the morning were correlated with increased morning fatigue as well as low morning energy level. Previous evidence has suggested that hypoxia activates expression of IL-8 (Hirani et al., 2001; Shi et al., 1999). Study participants in this study showed decreased hemoglobin oxygen levels, indicating the increased possibility of upregulation of IL-8 expression; however, there was no significant relationship between IL-8 and hypoxia indices. Due to the relatively small sample size in the current study, further studies with larger sample sizes are needed to confirm the relationship between IL-8, everyday symptoms, and hypoxia associated with OSA.

Beyond OSA, there are several studies, which identified the relationship between IL-8 and fatigue. A significant relationship between fatigue and IL-8 has been observed among patients with lung cancer and chronic fatigue syndrome (CFS) (Natelson, Weaver, Tseng, & Ottenweller, 2005; Reyes-Gibby et al., 2013). Interestingly, IL-8 levels were significantly higher in adults with CFS than in fatigued, non-CFS adults. CFS is diagnosed based on three criteria. First, simultaneous presence of 4 or more of the following symptoms: 1) unrefreshing sleep; 2) headaches; 3) frequent sore throat 4) impairment of short-term memory or concentration; 5) lasting post-exertion malaise; 6) muscle pain; 7) multi-joint pain; and 8) tender cervical or axillary lymph nodes. Second, severe chronic fatigue exists for six or more months. Third, severe
chronic fatigue significantly interrupts daily activities and work (Centers for Disease Control and Prevention, CDC). The chronicity of fatigue symptom and underlying inflammation may be an important differentiating factor relative to IL-8 as compared to non-CFS fatigue, though studies to date have not prospectively and longitudinally examined such relationships.

A plausible mechanism of fatigue and IL-8 takes into consideration the HPA axis. The normal function of the HPA axis is altered in OSA (Buckley & Schatzberg, 2005). Similarly, in CFS, supporting evidence showed a significant relationship between fatigue severity and abnormal patterns of cortisol, indicating dysregulation of the HPA axis in adults with CFS (Torres-Harding et al., 2008). There is no published evidence regarding diurnal variation of IL-8 in OSA; however, the current study found the decreased level of IL-8 during pre-sleep bout and increased level of IL-8 during post-sleep bout. The IL-8 diurnal variability in OSA showed the opposite pattern with the circadian rhythm of cortisol in OSA (i.e., the highest levels of cortisol occur after sleep and the lowest levels occur after wakefulness) (Ghiciuc, et al., 2015; Raff et al., 2011). IL-8 expression is regulated by the inflammatory transcriptional factor, nuclear factor-kB (NF-kB). When inflammatory transcription by NF-Kb is initiated, the levels of glucocorticoids (i.e., cortisol) are reduced. Over the sleep period, patients with OSA experience hypoxia and resultant inflammatory response; increased inflammatory transcription leads to HPA axis changes (i.e., reduced levels of cortisol in the morning). Previous studies have found that low circulating cortisol was associated with increased fatigue (Cleare, Blair, Chambers, & Wessely, 2001). Therefore, the abnormal pattern of cortisol may explain increased levels of IL-8, contributing to inflammatory processes shown in OSA and symptom expression of fatigue.

Carneiro et al. (2008) examined HPA axis and the relationship between HPA axis and sympathetic nervous system in adults with OSA. The study found that 24-hour heart rate in
adults with OSA was greater than those without OSA due to the stress responses including recurrent episodes of upper airway obstruction, hypoxia, and sleep fragmentation; furthermore, the cortisol levels and heart rate in adults with OSA were significantly decreased after CPAP treatment. Previous studies have found that fatigue was correlated to irritable hemodynamic states such as heart rate irritability (Freeman, & Komaroff, 1997). In this study, the diurnal variation of IL-8 showed the similar pattern with cortisol (i.e., decreased evening level, but increased morning level). Therefore, the abnormal pattern of cortisol may explain increased levels of IL-8, contributing to inflammatory processes in OSA.

**Symptom Cluster in OSA**

Although excessive daytime sleepiness is prevalent in OSA and it is considered a clinically significant symptom, few efforts exist to identify groups of symptoms and the underlying mechanism of symptom expression in the OSA population (Seneviatne & Puvanendran, 2004; Ye et al., 2014). OSA is perceived as a disorder that disrupts life since the obstructive episodes that occur during sleep cause impaired daytime functions (Feng et al., 2012; Gooneratne et al., 2011; Kapur et al., 2008). Frequent arousals and hypoxia occur during sleep, every night; therefore, patients with OSA experience accumulated symptoms (i.e., chronic, or lasting) such as sleepiness, mood disturbance, stress, and depression, all of which are often perceived as “a way of life.” On the other hand, patients with OSA also experience immediate symptoms from the obstructive, hypoxic events and sleep fragmentation (i.e. lack of energy, fatigue, excessive sleepiness, and headache after awakening) (American Academy of Sleep Medicine, 2009).

Several studies failed to identify the relationship between daytime sleepiness (measured by Epworth Sleepiness Scale) and OSA and have argued that excessive sleepiness may not be a
distinct symptom of OSA (Barbé et al., 2001; Young et al., 1993). However, it is plausible that adults with OSA may not express OSA-related symptoms when they are adapted to the accumulated symptoms (i.e., high threshold of symptoms). Likewise, the accumulated symptoms can co-occur with other lasting symptoms such as perceived stress and depression (Lenz et al., 1997). In this study, two distinct symptom clusters were identified; that is, momentary symptoms and lasting symptoms. Symptom components in each of the clusters were importantly connected with each other and the probabilities that symptoms in the same cluster are expressed simultaneously was identified. For instance, if adults with OSA express excessive sleepiness in the evening, simultaneous fatigue in the evening, excessive sleepiness in the morning, and stress, are likely to co-occur, but not lasting symptoms.

Another study that applied cluster analysis identified three subgroups of moderate to severe OSA patients who have combinations of symptoms and comorbidities. The identified subgroups were disturbed sleep group, minimally symptomatic group, and excessive daytime sleepiness group (Ye et al., 2014). The study evaluated sleep-related symptoms by using Basic Nordic Sleep Questionnaire, Epworth Sleepiness Scale, and Short Form Health Survey; of importance, these instruments are designed to measure lasting symptoms. Questions from the instruments were restricted to excessive daytime sleepiness, snoring, and stop breathing at night, but the questions did not include any other relevant symptoms such as depression, anxiety, stress, and fatigue. Interestingly, the minimal symptomatic group did not express distinct symptoms and had a higher probability of developing comorbidities such as hypertension and obstructive lung disease.

Untreated mild OSA is highly likely to progress to more severe OSA (i.e., moderate to severe OSA) (Peppard et al., 2000a) and lead to long-term serious conditions such as
cardiovascular disease (Motamedi, McClary, & Amedee, 2009). Momentary symptoms identified in adults with mild OSA are therefore likely to progress to be increasingly “symptomatic” moderate to severe OSA; lasting symptoms in mild OSA may also progress with progressively worsening OSA. Perception of such symptoms may be “accommodated” over time in OSA. Larger future symptom phenotyping studies are needed to address this hypothesis. For now, the current study suggests it is important to diagnose and treat the early signs and symptoms of OSA, which includes mild OSA.

An Adapted Model of Symptom Phenotypes in Mild OSA

There is continuing discussion of the mechanisms that contribute to increased levels of inflammatory biomarkers and everyday symptoms in OSA (Slater & Steier, 2012). OSA symptoms cannot be simply explained since several contributing symptom factors coexist, such as intermittent nocturnal hypoxia (physiological factor), sleep fragmentation (physiological factor), sleep disturbance (situational factor), increased age (confounding factor), and obesity (confounding factor) (Vgontzas et al., 1997; Vgontzas et al., 1999). The prevalence of OSA is higher in obese individuals than non-obese adults; however, higher prevalence of OSA in obese individuals is not necessarily accompanied by increased rates of excessive daytime sleepiness in obese individuals (Slater & Steier, 2012). To identify the underlying mechanism(s) of OSA symptoms, it is important to gain understanding of the biological mechanisms of upregulation in the symptoms as well as other factors that may affect the symptoms. The current study employed an adapted model that combines the pathophysiological framework in OSA and the Theory of Unpleasant Symptoms.

In this study, hypoxia and frequent apnea episodes were objectively measured to identify not only the underlying oxygenation/re-oxygenation condition in OSA, but also the relationship
with inflammation responses in OSA. The significant relationships between IL-6 and oxygenation indices and between IL-8 and apnea index (AI) were identified after controlling the confounding effects (i.e., the upregulated mechanism of OSA symptoms). Influencing factors in terms of arousals (total arousal index, respiratory efforts-related arousals) and sleep duration (total sleep time) showed an interaction with OSA symptom expression. Through the adapted model, the protein expression related to specific symptoms was identified. It may be difficult to shed light on the OSA symptom expression without an advanced pathophysiological understanding and a comprehensive conceptual framework that permits consideration of the complexity of factors that affect symptoms and inflammation. Though this line of inquiry is yet emergent, the employ of a conceptual model that is comprehensive will potentially support future clinical practice applications relative to symptom management approaches.
Figure 5.1. An Adapted Model of Symptom Phenotypes in Mild OSA

**Diseases**
- Decreased pharyngeal muscle activity
- Sleep onset
- Airway obstruction

**Confounding factors**
- Age
- Obesity
- Type 2 diabetes mellitus
- Cardiovascular disease

**Influencing factors**
- Physiologic factors: Inflammation
  - IL-10M
  - IL-1βM
  - IL-10E
  - IL-1βE
  - IL-10D
  - IL-1βD
  - IL-6M
  - IL-6E
  - IL-6D
  - IL-8M
  - IL-8E
  - IL-8D
  - TNF-αM
  - TNF-αE
  - TNF-αD

**Psychological factor**
- Mood disturbance

**Situational factor**
- Short sleep duration

**Symptoms**
- Sleepiness M
- Sleepiness E
- Sleepiness D
- Fatigue M
- Fatigue E
- Fatigue D
- Energy M
- Energy E
- Energy D

**Consequences/outcomes**
- Poor quality of life

**Notes:** ←→ Relationship; Bold, significant relationship; Red textbox, denotes future research opportunities; Abbreviations: M, morning; E, evening; D, diurnal variation.
Study Strengths

To the best of the author’s knowledge, this is the first study to identify a relationship between inflammatory biomarkers and symptom expression and between diurnal variation of inflammatory biomarkers and symptom expression in adults with mild OSA, inclusive of a balanced sample for sex. It was believed that OSA is more prevalent in men than in women whereby the prevalence of OSA in males is almost twice that in females (Young et al., 1993). However, the prevalence rate in women is likely to increase if the undiagnosed rate of OSA in women is considered. More than 90% of women are undiagnosed with OSA (Young et al., 1997). The majority of studies related to OSA have focused on males and therefore, understanding of OSA in women is limited.

Mild OSA is highly prevalent, affecting approximately 30% of the general population; however, there is limited evidence that has examined prognosis, intervention, and treatment of mild OSA per se (Young et al., 1993). Furthermore, prior studies of mild OSA did not consistently adjust for relevant confounding factors, including obesity, cardiovascular diseases, and diabetes (Chowdhuri et al. 2016). There are urgent needs for further research focused on the impact of mild OSA on cardiovascular events, neurocognitive diseases as well as the risk for developing such co-morbidities. Causal mechanism studies are of critical importance to discerning risks and potentially mitigating such risks.

Lastly, this study simultaneously measured both inflammatory biomarkers and symptom expression to identify molecular signatures of OSA based on the levels of inflammatory biomarkers and symptom-related molecular signature in adults with OSA. The careful planning of the measurement in this study allows for hypothesis generation to address symptom expression and underlying mechanism of symptoms expression in OSA.
Study Limitations

There are several study limitations; consequently, the study findings must be interpreted with caution. This study had no comparison/control group, making it difficult to conclude that any relationships observed in adults with OSA is due to the true relationship, rather than to other factors. The natural course of diurnal variation of inflammatory biomarkers both in untreated OSA and healthy adults is not clear or consistent based on the extant literature. To clarify the relationship between inflammatory biomarkers and symptom expression in adults with OSA, comparison or control groups are particularly important. This study was a cross-sectional cohort study, which limits any causal inferences. A larger, longitudinal cohort study, or case-control study, inclusive of a carefully planned comparison group, may provide more definitive evidence for causal mechanisms of the symptom expression and inflammation in OSA and permit a priori planned analyses to discern the influence of hypoxia and fragmented sleep in OSA on the relationship.

A second limitation of the current study is related to the study sample. Study participants were referred to the sleep clinic, rather than a population-based sample. Owing to the nature of this study, selection bias, which affects internal validity for study findings, cannot be avoided. There remains a possibility that the observed relationship between inflammatory biomarkers and symptom expression in adults with OSA may have arisen from such a bias. In addition to the selection bias, this study included a relatively small sample size, making difficult to confidently identify significant relationships from the data. The risk of Type II error also cannot be overlooked as the sample size was small, multiple tests were conducted, and no statistical correction was employed in this pilot study for multiplicities.
Third, this study included non-diverse study participants; that is, 86.4% of the study participants were non-Hispanic Whites. Previous population-based studies reported that African-Americans have a higher prevalence of OSA compared to Caucasians and the severity of OSA in African-Americans was more severe than counterparts (Ancoli-Israel et al., 1995). Another population-based study, which examined the prevalence of OSA among different racial groups found that the prevalence of OSA measured by the oxygen desaturation index 4% (ODI4) ≥20 was higher among Asians than non-Hispanic Whites, Hispanics, and Blacks (Kripke et al., 1997). The observed racial differences in the severity of OSA highlight the need for further studies that reflect the diversity of the population and examine if inflammatory biomarkers and symptom expression is similar among heterogeneous adults with mild OSA and explore differences of genetic etiology.

Next, this study did not exclude study participants who took anti-inflammatory drugs such as Ibuprofen, Aspirin, and Tylenol 24 hours prior to the study. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibits the activity of the cyclooxygenase (COX) enzymes, which contribute to produce prostanoids, including thromboxane and prostaglandins. NSAIDs, including ibuprofen and aspirin, inhibit COX, and consequently result in reduction of symptoms such as inflammation and pain (Kurumbail, Stevens, Gierse, & McDonald, 1996). Therefore, the concern regarding the influence of NSAIDs on the level of inflammatory biomarkers and the relationship with symptom expression cannot be avoided. This study could potentially underestimate the levels of inflammatory biomarkers and the relationship with symptom expression.

Finally, patients with relatively mild degrees of OSA were included in this study. Homogeneity of the study sample with regard to the severity of OSA can be seen as a strength of
this study; however, it is also a limitation with regard to generalizability. This was a relatively small study that was not designed for generalizability, but rather for hypothesis generation. Last but not least, the current study identified correlations between inflammatory biomarkers and symptom expressions and between diurnal variation of inflammatory biomarkers and symptom expressions in adults with mild OSA but causality cannot be evaluated based on correlations.

Implications and Recommendations for Future Research and Practice

This study was exploratory in nature and therefore confirmatory evidence is necessary with regard to the levels of and diurnal variation of inflammatory biomarkers and the relationship with symptoms expression in adults with mild OSA. However, the study provides further insights for the underlying biological mechanism of symptom expression in OSA as well as research directions for the future.

Although clinical practice implications are not immediate as a result of this study, there are potential clinical implications of this line of inquiry with continued research. Examining the diurnal variation of inflammatory biomarkers and the relationship with symptom expression in OSA, particularly in mild OSA, is absent in the sleep literature. The identified two symptom clusters, momentary symptoms and lasting symptoms, suggest reference points for baseline evaluation in mild OSA and potential treatment response evaluation opportunities in OSA symptom phenotypes. From such insights, symptom management strategies can be developed, evaluated and translated in the future. Health care providers do not necessarily follow up if the symptoms resolve after treatment but rather evaluate treatment response based only on efficacy of sleep disordered breathing treatment. Using a symptom management perspective will bring attention in the disciplines of both nursing and medicine to the importance of not only evaluating efficacy but also evaluating effectiveness of treatment for symptom resolution.
Apnea-hypopnea index (AHI), the total number of apneic or hypopneic events per hour during sleep is used to diagnose OSA and/or assess the severity of OSA, is the basis upon which treatment recommendation for OSA is determined. However, this study implies that AHI should not be assumed to directly relate to biological dysregulation in OSA. AHI may be an appropriate descriptor for epidemiologic prevalence (Macey, Woo, Kumar, Cross, & Harper, 2010), but AHI may not be an accurate index for determining OSA severity and making the decision on which sleep apnea treatment options should be applied. Rather, hypoxic indices, indicating the degree of deoxygenation during sleep are possibly more representative and specific for obstructive sleep apnea morbidity than AHI. To better predict the severity of OSA, health care providers should consider not only AHI, but also other desaturation indices, indicating the degree and frequency of hypoxic events.

There has been controversy whether mild OSA affects adverse health outcomes and should be treated. Untreated OSA causes increased risk of fatal cardiovascular events (Gami, 2005; Marin, 2005); inflammatory responses are considered the underlying mechanisms of the association between OSA and adverse cardiovascular outcomes (Nadem et al., 2013). This study clearly shows that treatment of mild OSA is important to pursue. With rigorous, well-controlled treatment response evaluations, inclusive of inflammatory biomarkers and immediate/lasting symptoms, additional insights will be gained for the potential beneficial impact of treatment on the reduction of cardiovascular disease risk as well as management of accompanying symptoms.

For future studies, a case-control study with a larger number of study participants and variation in OSA severity is necessary in order to determine whether certain subgroups are more susceptible to inflammatory responses and whether the levels of inflammatory biomarkers affect symptom expression. As for sampling, 26% of study participants initially enrolled in this study
had split-night sleep studies (diagnostic plus treatment trial). If recruiting/enrolling in a clinical sleep laboratory is planned in subsequent studies, it is important to incorporate an inflation rate of at least 20% or higher to account for split-night studies. Yet, this sampling plan will likely lead to little variation in OSA severity by AHI, as polysomnography protocols transition those with >20 events/hr AHI to split-night studies. It is therefore likely necessary to conduct research polysomnography for the purposes of future studies addressing inflammatory biomarkers assessed pre- and post-sleep, absent treatment exposure. Funding is an essential consideration for conducting future studies.

**Conclusion**

Pre-sleep bout IL-6 was positively associated with momentary symptoms of sleepiness and negatively associated with energy; post-sleep bout IL-8 and TNF-α positively associated with the momentary symptom of fatigue. Diurnal variation of IL-8 were associated with the morning momentary symptom of fatigue and diurnal variation of the momentary symptom of energy. Based on the findings, IL-8 may be specific to OSA for fatigue and energy symptoms; IL-6 was may be specific to OSA for sleepiness. This research will serve as a foundation to support future development of tailored symptom management or future research to address inflammatory biomarkers and symptom expression in adults with OSA.
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Karaca, Z., Ismailogullari, S., Korkmaz, S., Cakir, I., Aksu, M., Baydemir, R., ... & Bayram, F. (2013). Obstructive sleep apnoea syndrome is associated with relative hypocortisolemia and decreased hypothalmo–pituitary–adrenal axis response to 1 and 250μg ACTH and glucagon stimulation tests. *Sleep Medicine, 14*(2), 160-164.


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antioxidant sensitive activating pathway distinct from nuclear translocation. *Blood, 94*(6), 1878-1889.


Appendix A

Human Research Approval

APPROVAL OF SUBMISSION

Date: August 5, 2015

From: Heidi Watts, IRB Analyst

To: Hyunjoo Yang

<table>
<thead>
<tr>
<th>Type of Submission:</th>
<th>Modification</th>
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<tbody>
<tr>
<td>Title of Study:</td>
<td>Inflammation, excessive daytime sleepiness, and fatigue in patients with obstructive sleep apnea</td>
</tr>
<tr>
<td>Principal Investigator:</td>
<td>Hyunjoo Yang</td>
</tr>
<tr>
<td>Study ID:</td>
<td>STUDY00002746</td>
</tr>
<tr>
<td>Submission ID:</td>
<td>MDD00007721</td>
</tr>
<tr>
<td>Funding:</td>
<td>Nursing (UNIVERSITY PARK)</td>
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Documents Approved:
- Beck's Depression Inventory_v.7.18.2016 (v.7.18.15), Category: Data Collection Instrument
- Health Questionnaire v.7.18.16 (v.7.18.16), Category: Data Collection Instrument
- CONSENT FOR RESEARCH (v.7.18.16), Category: Consent Form
- Demographic Questionnaire clean copy v.7.18.16 (0.01), Category: Data Collection Instrument
- Epworth Sleepiness Scale v.7.18.16 (v.7.18.16), Category: Data Collection Instrument
- HRP-591 - Protocol for Human Subject Research (v.7.18.16), Category: IRB Protocol
- HRP-593 - Research Data Plan Review Form Application Supplement (0.01), Category: IRB Protocol
- Lee Fatigue Scale v.7.18.16 (v.7.18.16), Category: Data Collection Instrument
- Perceived Stress Scale_v.7.18.2016 (v.7.18.16), Category: Data Collection Instrument
- POMS-SF_v.7.18.2016 (v.7.18.16), Category: Data Collection Instrument
- Quebec Sleep Questionnaire_v.7.18.2016 (v.7.18.16), Category: Data Collection Instrument
- Stanford Sleepiness Scale_v.7.18.2016 (v.7.18.16), Category: Data Collection Instrument
- Study Recruitment Template for PSG Techs v.7.18.16 Clean Copy (0.01), Category: Recruitment Materials

Review Level: Expedited
IRB Board Meeting Date:  

On 8/5/2016, the IRB approved the above-referenced Modification. This approval is effective through 7/7/2017 inclusive. You must submit a continuing review form with all required explanations for this study at least 45 days before the study’s approval end date. You can submit a continuing review by navigating to the active study and clicking ‘Create Modification / CR’.

If continuing review approval is not granted before 7/7/2017, approval of this study expires on that date. To document consent, use the consent documents that were approved and stamped by the IRB. Go to the Documents tab to download them.

In conducting this study, you are required to follow the requirements listed in the Investigator Manual (HRP-103), which can be found by navigating to the IRB Library within CATS IRB (http://irb.psu.edu). These requirements include, but are not limited to:

- Documenting consent
- Requesting modification(s)
- Requesting continuing review
- Closing a study
- Reporting new information about a study
- Registering an applicable clinical trial
- Maintaining research records

This correspondence should be maintained with your records.
Title of Project: Inflammation, Excessive Daytime Sleepiness, and Fatigue in Patients with Obstructive Sleep Apnea

Principal Investigator: Hyunju Yang, MSN, RN, PhD Candidate, Penn State University College of Nursing

Address: 307 Nursing Sciences Building, University Park, PA 16802

Telephone Numbers: Weekdays: 8:00 a.m. to 5:00 p.m. (717) 531-8520, Sleep Center, or 814-380-7731, mobile number, or 814-863-7546, office number. After hours call (814) 380-7731.

We are asking you to be in a research study.

Whether or not you take part is up to you. You can choose not to take part. You can agree to take part and later change your mind. Your decision will not be held against you.

This form gives you information about the research. Please ask questions about anything that is unclear to you and take your time to make your choice.

1. Why is this research study being done?

We are asking you to be in this research because your sleep healthcare provider suspects that you are likely to have obstructive sleep apnea (OSA). Sleep apnea causes frequent arousals from sleep (or awakenings) and disrupts sleep during the night; this results in daily symptoms such as sleepiness, fatigue, and poor quality of life. Sleep apnea is also known to increase risks for heart disease. Inflammation is one of the potential causes of heart disease in sleep apnea. If you are diagnosed with OSA, it is likely that you will have an increased level of inflammation and impaired daily symptoms. This research is being done to identify if inflammation and daily symptoms in sleep apnea are related to each other. We are seeking persons to be in this study who are suspected to have OSA.
Approximately 72 people will take part in this research study at Hershey Medical Center.

2. What will happen in this research study?

The day of the sleep study, After signing the consent form to participate in this research study, you will complete (8) questionnaires and a sample of your blood will be drawn to measure inflammation. One questionnaire is about your demographics (i.e. age, race, sex), two questionnaires are about your daytime sleepiness, one questionnaire is about your fatigue, one questionnaire is about your quality of life, and questionnaire is about your mood disturbance, one questionnaire is about your depression, and one questionnaire is about your stress. You may skip any questions that make you uncomfortable. You will have a total of 10 ml of blood (about 4 teaspoons) drawn for this study. This will be done at the sleep center by one researcher, who is trained to draw blood and a registered nurse. Blood samples will be stored and tested for inflammation levels. Questionnaire completion and blood draw will take approximately 45 minutes. After completing questionnaires and the blood draw, you will have your scheduled overnight sleep study (polysomnography) at the sleep center.

In the morning following the sleep study, you will meet with a researcher who will draw another 10 ml of blood (about 4 teaspoons) and you will complete three (3) of the same questionnaires regarding daytime sleepiness and fatigue immediately upon awakening.

The blood will be drawn from a different vessel from the previous night’s blood draw site. After a 1-hr wake period, you will complete two of the same questionnaires again. Questionnaire completion and blood draw will take approximately 45 minutes.

What are my responsibilities if I take part in this research?

If you take part in this research, your major responsibilities will include:

- Provide answers to the eight (8) questionnaires in the evening and three (3) questionnaires in the morning at six in the a.m. and eight in the a.m. (i.e., 1-hr after awakening).
- Provide two (2) blood samples; one in the evening and one in the morning
- Complete the sleep study.

3. What are the risks and possible discomforts from being in this research study?

This study involves drawing blood at two times (evening and morning). The risks of the blood draw are discomfort during the blood draw, bleeding/bruising at the blood draw site, and infection at the blood draw site. To prevent against these risks, a trained registered nurse will closely monitor you during and after the blood draw. Pressure will be applied to the blood draw site to reduce bleeding and bruising. The blood draw will be performed using standard procedure to minimize risk of infection
at the blood draw site. This includes cleaning the blood draw site with antiseptic prior to and after blood draw, the researcher will wear the gloves during the procedure, and a sterile pressure bandage will be applied after blood draw.

There is also a risk of loss of confidentiality, which means that information collected about you or your participation in this research could become known by others outside of the research. To prevent this, your information will be assigned a code number (i.e., de-identified). A master list of participant’s names linking them to their identification number will be stored in a locked filing cabinet in Ms. Yang’s office.

4. What are the possible benefits from being in this research study?

4a. What are the possible benefits to me?
You will not directly benefit from this research study. However, results of your diagnostic polysomnography, which is being done at the request of your sleep provider, will be shared with you by your clinical sleep provider.

4b. What are the possible benefits to others?
The results of this research may guide the future treatment of OSA.

5. What other options are available instead of being in this research study?
You may choose not to be in this research study.

6. How long will I take part in this research study?
Your sleep studies (polysomnography) to diagnose OSA will require that you spend one-night (approximately 8 hours) in the sleep center. All study procedures are timed to occur immediately before your sleep study and immediately after your sleep study. This study will occur over a one-night period of time. Your sleep study will be completed whether you participate in this study or not.

7. How will you protect my privacy and confidentiality if I decide to take part in this research study?
7a. What happens to the information collected for the research?

Efforts will be made to limit the use and sharing of your personal research information. In our research files at The Milton S. Hershey Medical Center (HMC) and Penn State College of Medicine (PSU) we will include these identifiers (your name, address, phone number, date of birth, medical record number, and a research code number).

- A list that matches your name with your code number will be kept in a locked file in Ms. Yang’s locked office.
- Your blood samples will be labeled in a de-identified manner (by subject number) and will be stored in the laboratory of Dr. Engeland (co-investigator; 147 HHD Building, Penn State University at University Park) until analyzed. Only Ms. Yang, Dr. Engeland, Dr. Sawyer (co-investigator; 307 Nursing Sciences Building, Penn State University at University Park, and their immediate laboratory staff will have access to these samples. The codes for the subject numbers will be stored in a locked filing cabinet and only the investigators will have access to the code. All computer files will be password protected. Any personal identifiers will be removed from your data in the final database to be analyzed.
- A copy of this signed consent form will be included in your HMC medical record. This means that other HMC healthcare providers will know you are in this study. You will also receive a copy of the signed consent form for your own records.
- Results of some of the research-related tests, including only your sleep study results, will be kept in your HMC medical record.

In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

7b. How will my identifiable health information be used?

If you give your consent, health information that can be traced to you will be collected for this research study. In general, under federal law, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use, and share your health information for research and why they may need to do so.

The research team may use the following health information:

- Past, present, and future medical records
- New health information from tests, procedures, visits, interviews, or forms filled out as part of this research study.
The following people/groups may see, use, and share your identifiable health information:

- HMC/PSU research staff involved in this study
- The HMC/PSU Institutional Review Board (IRB), a group of people who review the research study to protect subjects’ rights and welfare
- The HMC/PSU Human Subjects Protection Office
- The HMC/PSU Research Quality Assurance Office
- Non-research staff within HMC/PSU who need this information to do their jobs (such as for treatment, payment (billing), or health care operations)
- Federal and state agencies (such as the U.S. Food and Drug Administration, the Office for Human Research Protections, the Department of Health and Human Services, the National Institutes of Health, and other U.S. or foreign government bodies that oversee or review research)

- Organizations that provide independent accreditation and oversight of hospitals and research

These groups may also review and/or copy your original PSU/HMC records while looking at the results of the research study. It is possible that some of the other people/groups who receive your health information may not be required by Federal privacy laws to protect your information. We share your information only when we must, and we ask anyone who receives it from us to protect your privacy.

Because research is an ongoing process, your permission for the use, storage and sharing of your health information will continue indefinitely.

Your privacy rights:

- You have the right to refuse to sign this form that allows us to use and share your health information for research; however, if you don’t sign it, you will not be able to take part in this research study.
- You have the right to withdraw your permission for us to use or share your health information for this research study. If you want to withdraw your permission, you must notify the person in charge of this research study in writing using the address on the front of this form. Once permission is withdrawn, you cannot continue to take part in the study.
- If you withdraw your permission, we will stop collecting health information about you for this study; we may continue to use and share your health information that we already have if it is necessary for safety and scientific soundness of the research study; and we will not be able to take back information that has already been used or shared with others.
You have the right to see and get a copy of your health information that is used or shared for treatment or for payment. However, you may not be allowed to see or copy certain health information that is a part of this research study. This is only for the period of the study. You will be allowed to see that information when the entire research study is complete.

8. What are the costs of taking part in this research study?

8a. What will I have to pay for if I take part in this research study?

There are no costs to you for participating in the study.

8b. What happens if I am injured as a result of taking part in this research study?

It is possible that you could develop complications or injuries as a result of being in this research study. If you experience a side effect or injury and emergency medical treatment is required, seek treatment immediately at any medical facility. If you experience a side effect or injury and you believe that emergency treatment is not necessary, you should contact the principal investigator listed on the first page of this consent form as soon as possible and he/she will arrange for medical treatment.

HMC/PSU compensation for injury

- There are no plans for HMC/PSU to provide financial compensation or free medical treatment for research-related injury.
- If an injury occurs, medical treatment is available at the usual charge.
- Costs will be charged to your insurance carrier or to you.
- Some insurance companies may not cover costs associated with research injuries.
- If these costs are not covered by your insurance, they will be your responsibility.

When you sign this form you are not giving up any legal right to seek compensation for injury.

9. Will I be paid to take part in this research study?

You will receive $20.00 for your participation in this research study. If you do not complete the study for any reason, you will be paid for the research activities that you have completed.
• $10 for the two (2) blood draws;
• $10 for the time required to complete questionnaires

If you decide to withdraw from the study before the study is over, your compensation will be consistent with the research-related activities that you have completed at the time of withdraw from the study

10. Who is paying for this research study?

This study is supported by Ms. Yang’s College-supported research funds and the American Nurses Foundation (ANF) funds.

11. What are my rights if I take part in this research study?

Taking part in this research study is voluntary.

▪ You do not have to be in this research.
▪ If you choose to be in this research, you have the right to stop at any time.
▪ If you decide not to be in this research or if you decide to stop at a later date, there will be no penalty or loss of benefits to which you are entitled.

Your research doctor may take you out of the research study without your permission.

▪ Some possible reasons for this are: continuing the research would be harmful, your condition has become worse, you become pregnant, you did not follow the instructions of the study doctor, you experience serious side effects.
▪ Also, the sponsor of the research may end the research study early.
▪ If your participation ends early, you may be asked to visit the research doctor for a final visit.

During the course of the research you will be provided with any new information that may affect your health, welfare or your decision to continue participating in this research.

12. If I have questions or concerns about this research study, whom should I call?

Please call the head of the research study, Ms. Hyunju Yang at 814-380-7731, mobile number (anytime) or 814-863-7546, if you:

▪ Have questions, complaints or concerns about the research.
Believe you may have been harmed by being in the research study.

You may also contact the research protection advocate in the HMC Human Subjects Protection Office (HSPO) at 717-531-5687 if you:
- Have questions regarding your rights as a person in a research study.
- Have concerns or general questions about the research.
- Have questions about your privacy and the use of your personal health information.
- You may also call this number if you cannot reach the research team or wish to talk to someone else about any concerns related to the research.

You may visit the HSPO’s web site at http://pennstatehershey.org/irb under research subject information for:
- Information about your rights when you are in a research study;
- Information about the Institutional Review Board (IRB), a group of people who review the research to protect your rights; and
- Links to the federal regulations and information about the protection of people who are in research studies. If you do not have access to the internet, copies of these federal regulations are available by calling the HSPO at (717) 531-5687.

INFORMED CONSENT AND AUTHORIZATION TO TAKE PART IN RESEARCH

**Signature of Person Obtaining Informed Consent**

Your signature below means that you have explained the research to the subject or subject representative and have answered any questions he/she has about the research.

______________________________

Signature of Person Giving Informed Consent and Authorization

Before making the decision about being in this research you should have:
- Discussed this research study with an investigator,
- Read the information in this form, and
- Had the opportunity to ask any questions you may have.
Your signature below means that you have received this information, have asked the questions you currently have about the research and those questions have been answered. You will receive a copy of the signed and dated form to keep for future reference.

**Signature of Subject**

By signing this consent form, you indicate that you voluntarily choose to be in this research and agree to allow your information to be used and shared as described above.

__________________________________  ____________  ____________  ______________________
Signature of Subject                Date                  Time                  Printed Name
Appendix B

Study Instruments

Berlin questionnaire

SLEEP EVALUATION

1. Complete the following:
   height _______ age _______
   weight _______ male/female ___

2. Do you snore?
   yes
   no
   don't know

If you snore:
3. Your snoring is?
   slightly louder than breathing
   as loud as talking
   louder than talking
   very loud. Can be heard
   in adjacent rooms.

4. How often do you snore?
   nearly every day
   3-4 times a week
   1-2 times a week
   1-2 times a month
   never or nearly never

5. Has your snoring ever bothered other
   people?
   yes
   no

6. Has anyone noticed that you quit
   breathing during your sleep?
   nearly every day
   3-4 times a week
   1-2 times a week
   1-2 times a month
   never or nearly never

Scoring Questions: Any answer within box outline is a positive response.
Scoring Categories: Category 1 is positive with 2 or more positive responses to questions 2-6
Category 2 is positive with 2 or more positive responses to questions 7-9
Category 3 is positive with 1 or more positive responses and/or a BMI > 30
Final Results: 2 or more positive categories indicates a high likelihood of sleep disordered breathing.
DEMOGRAPHIC INFORMATION

For Office use only

Protocol ID: ______________________

Data Entry Date: ________________ Visit: ______________________

DE Initials: ________________ Study ID: ______________________

1. Sex:
   1) Male
   2) Female

2. Date of birth (mm/dd/yy): ________________

3. Ethnicity:
   1) Hispanic or Latino
   2) Not Hispanic or Latino

4. Race:
   1) White
   2) Black or African American
   3) Asian
   4) Native Hawaiian or Other Pacific Islander
   5) American Indian/Alaskan Native

5. Marital Status:
   1) Married (or Common Law)
   2) Single
   3) Separated/Divorced
   4) Widow(er)

6. Last year, what was your total family income from all sources, before taxes?
   1) $0-25,999
   2) $26,000-$51,999
   3) $52,000-$74,999
   4) more than $75,000
   5) don’t know/decline to say
7. Problems you are seeking help for from the Sleep Clinic (Check All that apply):
   1) Snoring
   2) My breathing stops
   3) Sleepiness during the day
   4) High Blood Pressure
   5) Restless sleep
   6) Can't fall asleep

8. Who referred you here? (Check One)
   1) My family doctor
   2) A lung specialist
   3) A heart specialist
   4) A neurologist
   5) A psychiatrist
   6) A bariatric program
   7) Myself

9. Highest level of schooling completed: (Check One)
   1) Elementary School
   2) Middle School
   3) High School
   4) 2 Years College/Trade School
   5) 4 Years College
   6) Graduate School

10. Are you? (Check One)
    1) Working Full Time
    2) Working Part Time
    3) Home Keeper
    4) Unemployed
    5) A Student
    6) Retired
    7) Unable to work/disabled

11. Do you work rotating night shift work?
    1) YES    2) NO

12. Do you work steady night shift work?
    1) YES    2) NO
13. Please mark if you have taken any of the following medicines or supplements for your inflammation or infection

<table>
<thead>
<tr>
<th>Medicine/Supplement</th>
<th>Last 24hrs</th>
<th>2 weeks ago</th>
<th>2-4 weeks ago</th>
<th>1-3 months ago</th>
<th>3+ months ago</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anti-Inflammatory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Ibuprofen (Motrin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Aspirin e.g., Bayer, Acetylsalicylic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Other Anti-Inflammatory:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Herbal Remedy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Nutritional Supplement:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Injection:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Tylenol (Acetaminophen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Other Medication for inflammation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Epworth Sleepiness Scale

Study ID: ________________________ Evening or Morning

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times.

Even if you haven’t done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:

<table>
<thead>
<tr>
<th>0 = would never doze</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = slight chance of dozing</td>
</tr>
<tr>
<td>2 = moderate chance of dozing</td>
</tr>
<tr>
<td>3 = high chance of dozing</td>
</tr>
</tbody>
</table>

It is important that you answer each question as best you can.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of Dozing (0-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting, inactive in a public place (e.g. a theatre or a meeting)</td>
<td></td>
</tr>
<tr>
<td>As a passenger in a car for an hour without a break</td>
<td></td>
</tr>
<tr>
<td>Lying down to rest in the afternoon when circumstances permit</td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after a lunch without alcohol</td>
<td></td>
</tr>
<tr>
<td>In a car, while stopped for a few minutes in the traffic</td>
<td></td>
</tr>
</tbody>
</table>

THANK YOU FOR YOUR COOPERATION

M.W. Johns 1990-97
Stanford Sleepiness Scale

This is a quick way to assess how alert you are feeling. If it is during the day when you go about your business, ideally you would want a rating of a one. Take into account that most people have two peak times of alertness daily, at about 9 a.m. and 9 p.m. Alertness wanes to its lowest point at around 3 p.m.; after that it begins to build again. Rate your alertness at different times during the day. If you go below a three when you should be feeling alert, this is an indication that you have a serious sleep debt and you need more sleep.

An Introspective Measure of Sleepiness
The Stanford Sleepiness Scale (SSS)

<table>
<thead>
<tr>
<th>Degree of Sleepiness</th>
<th>Scale Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeling active, vital, alert, or wide awake</td>
<td>1</td>
</tr>
<tr>
<td>Functioning at high levels, but not at peak; able to concentrate</td>
<td>2</td>
</tr>
<tr>
<td>Awake, but relaxed; responsive but not fully alert</td>
<td>3</td>
</tr>
<tr>
<td>Somewhat foggy, let down</td>
<td>4</td>
</tr>
<tr>
<td>Foggy; losing interest in remaining awake; slowed down</td>
<td>5</td>
</tr>
<tr>
<td>Sleepy, woozy, fighting sleep; prefer to lie down</td>
<td>6</td>
</tr>
<tr>
<td>No longer fighting sleep, sleep onset soon; having dream-like thoughts</td>
<td>7</td>
</tr>
<tr>
<td>Asleep</td>
<td>X</td>
</tr>
</tbody>
</table>
Inflammatory Biomarkers in OSA

Lee Fatigue Scale

ID # ________ Date ________ Time ________ a.m. ________ p.m.

We are trying to find out about your level of energy before and after your night of sleep. There are 18 items we would like you to respond to. This should take less than 1 minute of your time. Thank you!

DIRECTIONS: You are asked to circle a number on each of the following lines to indicate how you are feeling RIGHT NOW.

For example, suppose you have not eaten since yesterday. What number would you circle below?

not at all hungry 0 1 2 3 4 5 6 7 8 9 10 extremely hungry

You would probably circle a number closer to the “extremely hungry” end of the line. This is where I put it:

not at all hungry 0 1 2 3 4 5 6 7 8 9 10 extremely hungry

NOW PLEASE COMPLETE THE FOLLOWING ITEMS:

1. not at all tired 0 1 2 3 4 5 6 7 8 9 10 extremely tired
2. not at all sleepy 0 1 2 3 4 5 6 7 8 9 10 extremely sleepy
3. not at all drowsy 0 1 2 3 4 5 6 7 8 9 10 extremely drowsy
4. not at all fatigued 0 1 2 3 4 5 6 7 8 9 10 extremely fatigued
5. not at all worn out 0 1 2 3 4 5 6 7 8 9 10 extremely worn out
6. not at all energetic 0 1 2 3 4 5 6 7 8 9 10 extremely energetic
7. not at all active 0 1 2 3 4 5 6 7 8 9 10 extremely active
8. not at all vigorous 0 1 2 3 4 5 6 7 8 9 10 extremely vigorous
<table>
<thead>
<tr>
<th>Item</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>9. not at all efficient</td>
<td>0 1 2 3 4 5 6 7 8 9 10 extremely efficient</td>
</tr>
<tr>
<td>10. not at all lively</td>
<td>0 1 2 3 4 5 6 7 8 9 10 extremely lively</td>
</tr>
<tr>
<td>11. not at all bushed</td>
<td>0 1 2 3 4 5 6 7 8 9 10 totally bushed</td>
</tr>
<tr>
<td>12. not at all exhausted</td>
<td>0 1 2 3 4 5 6 7 8 9 10 totally exhausted</td>
</tr>
<tr>
<td>13. keeping my eyes open is no effort at all</td>
<td>0 1 2 3 4 5 6 7 8 9 10 keeping my eyes open is a tremendous chore</td>
</tr>
<tr>
<td>14. moving my body is no effort at all</td>
<td>0 1 2 3 4 5 6 7 8 9 10 moving my body is a tremendous chore</td>
</tr>
<tr>
<td>15. concentrating is no effort at all</td>
<td>0 1 2 3 4 5 6 7 8 9 10 concentrating is a tremendous chore</td>
</tr>
<tr>
<td>16. carrying on a conversation is no effort at all</td>
<td>0 1 2 3 4 5 6 7 8 9 10 carrying on a conversation is a tremendous chore</td>
</tr>
<tr>
<td>17. I have absolutely no desire to close my eyes</td>
<td>0 1 2 3 4 5 6 7 8 9 10 I have a tremendous desire to close my eyes</td>
</tr>
<tr>
<td>18. I have absolutely no desire to lie down</td>
<td>0 1 2 3 4 5 6 7 8 9 10 I have a tremendous desire to lie down</td>
</tr>
</tbody>
</table>
The Profile of Mood States-Short Form (POMS-SF)

Reprinting of full instrument violates copyright agreement, and therefore sample questions are described.

Mood State

- Friendly
- Tense
- Angry
- Worn out
- Lively
- Confused
### QUEBEC SLEEP QUESTIONNAIRE

This questionnaire has been designed to find out how you have been doing and feeling over the last 4 weeks. You will be questioned about the impact that sleep apnea may have had on your daily activities, your emotional functioning, and your social interactions, and about any symptoms it might have caused.

<table>
<thead>
<tr>
<th>During the last 4 weeks :</th>
<th>All the time</th>
<th>A large amount of the time</th>
<th>A moderate to large amount of the time</th>
<th>A moderate amount of the time</th>
<th>A small to moderate amount of the time</th>
<th>A small amount of the time</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you had to force yourself to do your activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>2. Have you disturbed everyone at night while staying with friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>3. Have you felt like not wanting to do things together with your partner, children or friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4. Have you woken up more than once per night to urinate?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>5. Have you been feeling depressed?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>6. Have you been feeling anxious or fearful about what was wrong?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>7. Have you needed to nap during the day?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
### Inflammatory Biomarkers in OSA

**Quebec Sleep Questionnaire**

**Protocol #0002746**  
**Version 7.18.2016**

<table>
<thead>
<tr>
<th>During the last 4 weeks :</th>
<th>All the time</th>
<th>A large amount of the time</th>
<th>A moderate to large amount of the time</th>
<th>A moderate amount of the time</th>
<th>A small to moderate amount of the time</th>
<th>A small amount of the time</th>
<th>Not at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. Have you been feeling impatient?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>9. Have you woken up often (more than twice) during the night?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>During the last 4 weeks :</th>
<th>A very large amount</th>
<th>A large amount</th>
<th>A moderate to large amount</th>
<th>A moderate amount</th>
<th>A small to moderate amount</th>
<th>A small amount</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. Have you had difficulty with trying to remember things?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>11. Have you had difficulty with trying to concentrate?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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<tr>
<td>12. Have you been upset about being told that your snoring was bothersome or irritating?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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<tr>
<td>13. Have you felt guilty about your relationship with family members or close personal friends?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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<tr>
<td>14. Have you noticed a decrease in your performance at work?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>15. Have you been concerned about heart problems or premature death?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>During the last 4 weeks, how much of a problem have you had with:</td>
<td>A very large problem</td>
<td>A large problem</td>
<td>A moderate to large problem</td>
<td>A moderate problem</td>
<td>A small to moderate problem</td>
<td>A small problem</td>
<td>No problem</td>
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<tr>
<td>16. Having to fight to stay awake during the day?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>17. Feeling decreased energy?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>18. Feeling excessive fatigue?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>19. Feeling that ordinary activities require an extra effort to perform or complete?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>20. Falling asleep if not stimulated or active?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>21. Difficulty with a dry or sore mouth/throat upon awakening?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>22. Difficulty returning to sleep if you wake up in the night?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>23. Feeling that you lack energy?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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</table>
## Inflammatory Biomarkers in OSA

### Quebec Sleep Questionnaire

**Protocol #0002745**  
**Version 7.18.2016**

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<th>During the last 4 weeks, how much of a problem have you had with:</th>
<th>A very large problem</th>
<th>A large problem</th>
<th>A moderate to large problem</th>
<th>A moderate problem</th>
<th>A small to moderate problem</th>
<th>A small problem</th>
<th>No problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>24. Concern about the times you stop breathing at night?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>25. Loud snoring?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>26. Difficulties with attention?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>27. Falling asleep suddenly?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>28. Waking up at night feeling like you were chasing?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>29. Waking up in the morning feeling unrefreshed and/or tired?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>30. A feeling that your sleep is restless?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>31. Difficulty staying awake while reading?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>32. Fighting the urge to fall asleep while driving?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

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Perceived Stress Scale (PSS)

Patient ID: _________________  Date: _________________

The questions in this scale ask you about your feelings and thoughts **during the past week**. In each case, please indicate how often you felt or thought a certain way by circling the number in the column. The best approach is to answer each question fairly quickly. That is, don’t try to count up the number of times you felt a particular way, but rather indicate the answer that seems like a reasonable estimate.

[0=Never/1=Almost never/2=Sometimes/3=Fairly often/4=Very often]

<table>
<thead>
<tr>
<th>In the last week.....</th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Fairly Often</th>
<th>Very Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. How often have you been upset because of something that happened unexpectedly?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. How often have you felt unable to control the important things in your life?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. How often have you felt nervous or stressed?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. How often have you felt confident about your ability to handle personal problems?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. How often have you felt that things were going your way?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6. How often have you found that you could not cope with all the things you had to do?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. How often have you been able to control irritations in your life?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. How often have you felt that you were on top of things?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. How often have you been angry because of things that happened that were outside of your control?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. How often have you felt that difficulties were piling up so high that you could not overcome them?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Beck's Depression Inventory
This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1. 0 I do not feel sad.
    1 I feel sad
    2 I am sad all the time and I can't snap out of it.
    3 I am so sad and unhappy that I can't stand it.

2. 0 I am not particularly discouraged about the future.
    1 I feel discouraged about the future.
    2 I feel I have nothing to look forward to.
    3 I feel the future is hopeless and that things cannot improve.

3. 0 I do not feel like a failure.
    1 I feel I have failed more than the average person.
    2 As I look back on my life, all I can see is a lot of failures.
    3 I feel I am a complete failure as a person.

4. 0 I get as much satisfaction out of things as I used to.
    1 I don't enjoy things the way I used to.
    2 I don't get real satisfaction out of anything anymore.
    3 I am dissatisfied or bored with everything.

5. 0 I don't feel particularly guilty.
    1 I feel guilty a good part of the time.
    2 I feel quite guilty most of the time.
    3 I feel guilty all of the time.

6. 0 I don't feel I am being punished.
    1 I feel I may be punished.
    2 I expect to be punished.
    3 I feel I am being punished.

7. 0 I don't feel disappointed in myself.
    1 I am disappointed in myself.
    2 I am disgusted with myself.
    3 I hate myself.

8. 0 I don't feel I am any worse than anybody else.
    1 I am critical of myself for my weaknesses or mistakes.
    2 I blame myself all the time for my faults.
    3 I blame myself for everything bad that happens.
Inflammatory Biomarkers in OSA

Beck's Depression Inventory

9.
0  I don't have any thoughts of killing myself.
1  I have thoughts of killing myself, but I would not carry them out.
2  I would like to kill myself.
3  I would kill myself if I had the chance.

10.
0  I don't cry any more than usual.
1  I cry more now than I used to.
2  I cry all the time now.
3  I used to be able to cry, but now I can't cry even though I want to.

11.
0  I am no more irritated by things than I ever was.
1  I am slightly more irritated now than usual.
2  I am quite annoyed or irritated a good deal of the time.
3  I feel irritated all the time.

12.
0  I have not lost interest in other people.
1  I am less interested in other people than I used to be.
2  I have lost most of my interest in other people.
3  I have lost all of my interest in other people.

13.
0  I make decisions about as well as I ever could.
1  I put off making decisions more than I used to.
2  I have greater difficulty in making decisions more than I used to.
3  I can't make decisions at all anymore.

14.
0  I don't feel that I look any worse than I used to.
1  I am worried that I am looking old or unattractive.
2  I feel there are permanent changes in my appearance that make me look unattractive.
3  I believe that I look ugly.

15.
0  I can work about as well as before.
1  It takes an extra effort to get started at doing something.
2  I have to push myself very hard to do anything.
3  I can't do any work at all.

16.
0  I can sleep as well as usual.
1  I don't sleep as well as I used to.
2  I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3  I wake up several hours earlier than I used to and cannot get back to sleep.
Inflammatory Biomarkers in OSA

Beck’s Depression Inventory

17. I don’t get more tired than usual.
    1. I get tired more easily than I used to.
    2. I get tired from doing almost anything.
    3. I am too tired to do anything.

18. My appetite is no worse than usual.
    1. My appetite is not as good as it used to be.
    2. My appetite is much worse now.
    3. I have no appetite at all anymore.

19. I haven’t lost much weight, if any, lately.
    1. I have lost more than five pounds.
    2. I have lost more than ten pounds.
    3. I have lost more than fifteen pounds.

20. I am no more worried about my health than usual.
    1. I am worried about physical problems like aches, pains, upset stomach, or constipation.
    2. I am very worried about physical problems and it’s hard to think of much else.
    3. I am so worried about my physical problems that I cannot think of anything else.

21. I have not noticed any recent change in my interest in sex.
    1. I am less interested in sex than I used to be.
    2. I have almost no interest in sex.
    3. I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

<table>
<thead>
<tr>
<th>Total Score</th>
<th>Levels of Depression</th>
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<tbody>
<tr>
<td>1-10</td>
<td>These ups and downs are considered normal</td>
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<tr>
<td>11-16</td>
<td>Mild mood disturbance</td>
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<tr>
<td>17-20</td>
<td>Borderline clinical depression</td>
</tr>
<tr>
<td>21-30</td>
<td>Moderate depression</td>
</tr>
<tr>
<td>31-40</td>
<td>Severe depression</td>
</tr>
<tr>
<td>over 40</td>
<td>Extreme depression</td>
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</table>
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<td>Figures 4-1, 4-2</td>
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