INFLAMMATION IN OBSTRUCTIVE SLEEP APNEA:
ITS MEDIATING ROLE AND POTENTIAL AS A MARKER OF SEVERITY

A Dissertation in
Neuroscience
by
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ABSTRACT

Obstructive sleep apnea (OSA) is a prevalent disorder characterized by upper airway obstruction during sleep. This airway obstruction creates irregular breathing pauses, resulting in intermittent hypoxia and fragmented sleep. Obesity – in particular, visceral (central) obesity – is the strongest risk factor for developing OSA. Primarily driven by inflammatory mechanisms, prolonged visceral obesity is also associated with development of the metabolic syndrome, a cluster of symptoms that increases the risk for cardiovascular disease and type 2 diabetes. Independent of obesity, however, OSA has also been linked to elevations in the proinflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and the acute phase reactant C-reactive protein (CRP) due to stresses induced by intermittent hypoxia. In the interminable quest to identify novel modifiable risk factors for cardiovascular disease and type 2 diabetes, the notion that OSA causes the metabolic syndrome is attractive; however, given the significant overlap in phenotypes, subclinical biomarkers, and health outcomes in obese non-apneics and patients with OSA, the model cannot be so simple. No study to date has attempted to disentangle the relative contribution of obesity-associated inflammation toward the development of OSA, nor how these biomarkers may reflect the cardiometabolic or neurocognitive severity of the disorder.

This dissertation explores these questions in a representative population sample of 421 adolescents, the Penn State Child Cohort, who were followed up in the sleep laboratory eight years after their initial study visit. Examining OSA in adolescents presents a unique advantage; given that the majority of childhood OSA cases do not persist into adolescence, this age group captures a unique perspective into the early pathophysiology of the disorder. Specifically, based on consistent evidence demonstrating that adipose tissue is a major source of systemic inflammation, we explored five overarching hypotheses. In Chapter 3, we demonstrate how,
similar to adults, OSA in adolescents is associated with elevated visceral fat area, particularly in boys. In Chapter 4, we show that inflammation is associated with obesity in adolescents, and is also elevated in those with OSA independent of obesity. In Chapter 5, we illustrate how a significant portion of the relationship between visceral obesity and OSA is mediated by systemic inflammation. Chapter 6 demonstrates how, using longitudinal data from the childhood baseline study, inflammation during childhood predicts apnea and blood pressure reactivity in adolescence. Finally, in Chapter 7, we explore the potential of inflammation (namely, CRP) to serve as a marker of the cardiometabolic and neurocognitive severity of OSA in adolescents.

Taken together, these findings do not support a simple linear model of OSA pathophysiology, but rather a more complex vicious cycle in which visceral fat – through primarily inflammatory mechanisms – results in OSA; in turn, OSA is not an endpoint, but contributes to a progressive worsening of related sequelae. The findings in this dissertation may provide a unique perspective and future directions for studying the pathophysiology, prognosis, and potential treatment for OSA in children, adolescents, and adults.
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<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>AHI</td>
<td>apnea/hypopnea index</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AT</td>
<td>adenotonsillectomy</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>cMetS</td>
<td>continuous metabolic syndrome score</td>
</tr>
<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
</tr>
<tr>
<td>CRC</td>
<td>Clinical Research Center</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DXA</td>
<td>dual(-energy) X-ray absorptiometry</td>
</tr>
<tr>
<td>EDS</td>
<td>excessive daytime sleepiness</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram/electroencephalography</td>
</tr>
<tr>
<td>EOG</td>
<td>electrooculogram/electrooculography</td>
</tr>
<tr>
<td>EKG</td>
<td>electrocardiogram/electrocardiography</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EMG</td>
<td>electromyogram/electromyography</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein [cholesterol]</td>
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<tr>
<td>HOMA</td>
<td>homeostatic model assessment</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
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<tr>
<td>ICC</td>
<td>intra-class correlation</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>IL-6 sR</td>
<td>interleukin-6 soluble receptor</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OSA</td>
<td>obstructive sleep apnea</td>
</tr>
<tr>
<td>PSCC</td>
<td>Penn State Child Cohort</td>
</tr>
<tr>
<td>PSG</td>
<td>polysomnography</td>
</tr>
<tr>
<td>SDB</td>
<td>sleep-disordered breathing</td>
</tr>
<tr>
<td>SE</td>
<td>sleep efficiency</td>
</tr>
<tr>
<td>SOL</td>
<td>sleep onset latency</td>
</tr>
<tr>
<td>SpO₂</td>
<td>hemoglobin oxygen (O₂) saturation</td>
</tr>
<tr>
<td>SWS</td>
<td>slow-wave sleep</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movement</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TNFR1</td>
<td>tumor necrosis factor receptor 1</td>
</tr>
<tr>
<td>TST</td>
<td>total sleep time</td>
</tr>
<tr>
<td>WASO</td>
<td>wake (time) after sleep onset</td>
</tr>
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</table>
ACKNOWLEDGMENTS

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Graduate school was, on a personal level, the hardest five years of my life thus far. At times, it was a roller coaster of the highest highs, the lowest lows, self-doubt, and learning to find resiliency in the face of adversity. But a lot of amazing things also happened, and I’ve learned more about myself in the past five years than all the years before that. Here I acknowledge
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And now, without further ado: **LET’S DO SCIENCE!**
Chapter 1

INTRODUCTION
1.1. OBSTRUCTIVE SLEEP APNEA

1.1.1. Clinical Definition, Prevalence, and Scope of the Problem

Obstructive sleep apnea (OSA) is a prevalent sleep disorder characterized by upper airway obstruction during sleep. This obstruction results in intermittent breathing pauses despite breathing effort, leading to acute reductions in hemoglobin oxygen saturation (SpO$_2$) and fragmented sleep. Importantly, OSA is associated with a number of chronic medical conditions which contribute to poor quality of life, including obesity, excessive daytime sleepiness (EDS), hypertension, diabetes, coronary artery disease, and diminished cognitive ability, among others.

General population cohort studies over the last two decades have estimated the prevalence of OSA at 17-24% of men and 5-9% of women (Young et al., 1993; Bixler et al., 1998; Bixler et al., 2001), though more recent work highlights how rates of OSA have increased by an additional 14% to 55%, depending on the subpopulation studied, in conjunction with the ongoing obesity epidemic (Peppard et al., 2013). The prevalence of OSA peaks around age 55 years for men and 65 years for women (Bixler et al., 1998; Bixler et al., 2001). Particularly in women, OSA is more frequent following hormonal changes such as menopause, which may explain the delay in peak prevalence compared to men (Bixler et al., 2001; Young et al., 2003). Among premenopausal women, OSA is also significantly more prevalent in those with polycystic ovarian syndrome (PCOS), an endocrine disorder characterized by insulin resistance and hyperandrogenism (Vgontzas et al., 2001; Fogel et al., 2001).
Patients with untreated OSA have been shown to contribute to significantly increased healthcare costs and use of resources, while treatment reduces healthcare use to that of the general population (Leger et al., 2012).

1.1.2. Measuring Obstructive Sleep Apnea

The gold standard for an objective, diagnostic measure of sleep is polysomnography (PSG). The term, derived from the Greek *polus* (“many”), Latin *somnus* (“sleep”), and Greek *graphein* (“to write”), represents the multi-parametric nature of the tool.

In general, the polysomnogram provides measures of brain electrical activity via electroencephalography (EEG); chin muscle tone; leg movements via electromyography (EMG); eye movements via electrooculography (EOG); and heart rate and rhythm via electrocardiography (EKG).

Each measure is critical for differentiating the hallmarks associated with each of the four sleep stages (non-REM [NREM] stages 1, 2, and 3 [slow-wave sleep] and REM sleep; Rechtschaffen & Kales, 1968; Iber et al., 2007), which repeat in roughly 90-minute cycles four or five times per night (Dement & Kleitman, 1957). EEG electrodes are applied according to the standard 10-20 electrode placement system to ensure reproducibility, so named because adjacent electrodes are either 10% or 20% of the total front-to-back or left-to-right distance of the skull (American Electroencephalographic Society, 1991; Figure 1.1).

![Figure 1.1. International 10-20 EEG electrode placement system. Frontal (F3/F4), central (C3/C4), occipital (O1/O2), and mastoid (A1/A2) regions are commonly measured during a sleep study. Image source: talk/Wikimedia Commons (public domain).](image-url)
In the early 1960s, Kuhl and colleagues described breathing cessations during sleep that were not captured by polysomnography (Kuhl, 1997); soon thereafter, measures of airflow and thoracic/upper abdominal wall movement (via belts) were added to standard PSG (Gastaut et al., 1965). Monitors for snoring (snore microphone) and oxygen saturation (pulse oximetry) are also now included in standard PSG as additional indicators of breathing problems during sleep (Figure 1.2).

When reviewing a PSG record, OSA is characterized by cessations in nasal airflow despite breathing effort in the thoracic and abdominal belts, suggesting obstruction of the upper airway. Specifically, apneas (total obstructions) are defined as breathing pauses for at least 10 seconds with associated decreases in SpO2 (Figure 1.3), while hypopneas (partial obstructions) are reductions of airflow by at least 50%.

**Figure 1.2.** Polysomnography sensors specifically designed to capture OSA and other sleep-related breathing disorders. Note nasal cannula (air flow), thoracic and upper abdominal wall belts (breathing effort), and pulse oximeter on finger (blood oxygen saturation). *Image source: NIH/NHLBI (public domain).*

**Figure 1.3.** Sample polysomnography record showing an obstructive apnea. While airflow has ceased for > 10 seconds (red box), there is still breathing effort in the thoracic and abdominal belts (sinusoidal blue lines). *Image source: NascarEd/Wikimedia Commons (public domain).*
Table 1.1. Classification of OSA severity according to AHI.

<table>
<thead>
<tr>
<th>OSA Severity</th>
<th>Classification</th>
</tr>
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<tbody>
<tr>
<td>AHI &lt; 5</td>
<td>No OSA</td>
</tr>
<tr>
<td>5 ≤ AHI &lt; 15</td>
<td>Mild OSA; treat if accompanied by cardiometabolic sequelae or EDS</td>
</tr>
<tr>
<td>15 ≤ AHI &lt; 30</td>
<td>Moderate OSA</td>
</tr>
<tr>
<td>AHI ≥ 30</td>
<td>Severe OSA</td>
</tr>
</tbody>
</table>

AHI = apnea/hypopnea index; OSA = obstructive sleep apnea; EDS = excessive daytime sleepiness.

OSA severity is defined by the apnea/hypopnea index (AHI), calculated as the number of apneas and hypopneas summed per hour of sleep. The International Classification of Sleep Disorders defines OSA as an AHI ≥ 15 events per hour; or AHI ≥ 5 if accompanied by cardiometabolic sequelae, such as hypertension or type 2 diabetes; complaints of EDS or insomnia; and/or complaints by the patient or bed partner of gasping, choking, or habitual snoring (American Academy of Sleep Medicine, 2014). OSA is considered severe if AHI ≥ 30 (Table 1.1).

Figure 1.4. Representative hypnograms of a night’s sleep in healthy non-apneics (top) and patients with OSA (bottom). Note frequent periods of wake and stage 1 sleep, with less stage 2, SWS (stages 3 & 4), and REM sleep in those with OSA. Image source: Wikimedia Commons (public domain).
As a result of arousals from sleep that contribute to sleep fragmentation, OSA is associated with greater wake time after sleep onset (WASO) and total wake time (TWT), lower sleep efficiency (SE), more stage 1 (“light”) sleep, and less stage 2 sleep, slow-wave sleep (SWS), and rapid eye movement (REM) sleep (Figure 1.4) compared to age-, gender-, and BMI-matched controls (Vgontzas et al., 2000).

**Definition of Sleep-Disordered Breathing.** OSA is just one sleep-related breathing disorder on a spectrum of several. The umbrella term sleep-disordered breathing (SDB) refers to any chronic condition in which partial or complete cessation of breathing occurs throughout the night. In addition to obstructive apneas and hypopneas, there are (Greenberg et al., 2016):

- **Central apneas:** complete cessations of breathing not accompanied by effort; typically due to neurological dysfunction.

- **Mixed apneas:** combination of obstructive and central apneas.

- **Snoring:** vibration of upper respiratory structures, often resulting in an unpleasant sound.

- **Respiratory effort related arousals (RERAs):** increasing respiratory efforts that lead to sleep arousals, but do not fulfill the criteria for apnea or hypopnea.

- **Upper airway resistance syndrome (UARS):** increased upper airway resistance during sleep with arousals, but not characterized by apneas, hypopneas, or significant hypoxia.

- **Hypoventilation:** insufficient gas exchange resulting in increased carbon dioxide in the blood; often naturally adjusted for by taking deeper or longer breaths as needed.
Cheyne-Stokes breathing: an abnormal breathing pattern – repeating in cycles of 30 seconds to two minutes – characterized by progressively deeper or faster breathing followed by a gradual decrease that ends in an apnea.

While SDB conditions vary in their causes and manifestations, common unifying complaints in patients include EDS and/or fatigue and morning headaches (Labanowski et al., 1996). In many cases, the individual is not aware of their SDB unless informed by a bed partner. Research has demonstrated that, on average, bed partners of those with SDB rate their quality of life as equal to or even lower than the individuals themselves with SDB (Breugelmans et al., 2004). In the case of SDB, then, the impact of the condition on public health goes beyond the patient.

Defining Sleep-Disordered Breathing and Obstructive Sleep Apnea in Children. OSA is increasingly recognized in children and adolescents. Depending on the definition used (Lumeng and Chervin, 2008), it is estimated that 1-4% of the general pediatric population have OSA (Bixler et al., 2008). A significantly greater number of young people (27-40%) have some form of SDB (Bixler et al., 2009; Dayyat et al., 2007), the most common of which is snoring.

For children and adolescents, there is no consensus definition of OSA. While it’s recognized that clinical complications of OSA occur at a much lower AHI threshold in children than in adults, definitions have ranged from $\text{AHI} \geq 1$, $\text{AHI} \geq 2$, $\text{AHI} \geq 3$, $\text{AHI} \geq 5$, or any of the previous three cut-offs in combination with a specific criteria for oxygen desaturation in various studies (Gileles-Hillel et al., 2014). Many sleep centers will treat children in the $2 \leq \text{AHI} < 5$ range (Beck & Marcus, 2009), often referring them for adenotonsillectomy, though this practice is controversial (see Section 1.1.4. Current Treatment).
1.1.3. Risk Factors for Obstructive Sleep Apnea

*Obesity.* The most widely-recognized risk factor for OSA is obesity. Early research seeking to characterize the natural course of OSA cited obesity and snoring as the “first phase[s] of developing the syndrome” (Lugaresi, 1975). As far back as the nineteenth century, physicians lumped a number of related symptoms into the catch-all term “Pickwickian syndrome,” inspired by the maladies of a Charles Dickens character in *The Pickwick Papers* (Bickelmann et al., 1956). Joe, a “fat and red-faced boy in a state of somnolency,” was described as having heavy, labored breathing and would constantly fall asleep, snoring, in the middle of everyday tasks.

Later re-named obesity hypoventilation syndrome, obesity has long been associated with inadequate ventilation and daytime hypercapnia, resulting in abnormally low blood oxygen and high carbon dioxide levels (Olson & Zwillich, 2005). The mechanism is not fully understood, but is likely the result of interplay between various processes. For one, excess fat tissue, particularly in the abdominal region, contributes to inefficient diaphragm and chest wall movement, causing respiratory muscles to become fatigued more easily (Rochester & Enson, 1974). Over time, chronic low oxygen levels can lead to hypoxic pulmonary vasoconstriction, or the tightening of small blood vessels in the lung. In turn, this vasoconstriction results in pulmonary hypertension, making the right ventricle of the heart less efficient at moving blood from the veins. Increased hydrostatic pressure due to inefficient blood flow can lead to edema in the skin or abdominal cavity, further contributing to the vicious cycle of obesity hypoventilation (Mokhlesi & Tulaimat, 2007).
Also associated with central obesity, specifically, is the accumulation of excess fat in the neck (Lubrano et al., 2012), which contributes to narrowing of the upper airway. In addition to less efficient gas exchange, the pattern of fat accumulation around the oropharynx alters the geometry of the upper airway; although a healthy upper airway is largest when measured laterally, its anterior-posterior dimension is significantly higher in patients with OSA, decreasing the efficiency of genioglossus muscle contraction and other dilator muscles (Deegan & McNicholas, 1995; Figure 1.5). Obesity in general is associated with sarcopenia (loss of muscle tissue) and skeletal muscle dysfunction (Atlantis et al., 2009) through lipid accumulation (Gueugneau et al., 2015), increased inflammatory gene expression in muscle tissue (Poelkens et al., 2013), and other mechanisms. Interestingly, a consistent finding across multiple studies of patients with OSA is a relative increase in type 2 fast-twitch muscle fibers in the genioglossus compared to type 1 fibers, the latter of which is less likely to fatigue (Carrera et al., 1999; Series et al., 2000); it’s not clear, however, whether these differences reflect a cause or effect of OSA (Carrera et al., 1999). Upper airway neuromuscular responses are also blunted by central obesity, and recent work in animals suggests that leptin may play a role (Pho et al., 2016). Leptin, a hormone produced by adipose cells, is elevated in obesity, and further elevated in SDB as a result of intermittent hypoxia; leptin levels have been shown to be

\[ \text{Figure 1.5. Anatomy of the upper airway. Image adapted from BruceBlaus/Wikimedia Commons (public domain).} \]
strong predictors of reduced respiratory drive and hypercapnia (Campo et al., 2007), though the exact mechanism is not well understood (Pho et al., 2016).

Another significant result of obesity, particularly central obesity, is systemic inflammation. Increases in visceral fat pad size are associated with low levels of tumor necrosis factor alpha (TNFα) secretion by adipocytes themselves. In turn, macrophages are recruited, which release the cytokines interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and TNFα, among others. Levels of the acute phase reactant C-reactive protein (CRP) synthesized by the liver, also rise in response to IL-6 secretion by macrophages and T-cells (Chrousos, 1995). (These mechanisms are explained in greater detail in Section 1.2.3. Adipocytes and Inflammation.) In addition to upper airway tissue damage and denervation by inflammatory cytokines, such chronic activation of the innate immune system may also contribute to upper airway narrowing due to swelling of the many lymph nodes located in the head and neck (Figure 1.6).

Recent prospective findings in the Wisconsin Sleep Cohort, a large general population-based cohort, indicate that the increased prevalence of OSA has mirrored the surge in obesity over the last two decades (Young et al., 2009). A four-year follow-up study in the same cohort reported that modest (10%) weight gain predicted a 32% increase in AHI and 6-fold odds of developing moderate-to-severe OSA. On the other hand, 10% weight loss predicted a 26% decrease in AHI (Peppard et al., 2000a).
Anatomic risk factors. In addition to anatomical changes in upper airway shape, musculature, and fat distribution resulting from obesity, overall increases in soft tissue volume predispose the upper airway to collapse during sleep. Magnetic resonance imaging (MRI) studies have demonstrated that some patients with OSA have greater pharyngeal wall, soft palate, and/or tongue volumes compared to non-apneics (Greenberg et al., 2016). Other craniofacial features that can narrow the upper airway include abnormal maxilla and mandible placement, such as mandibular retrognathia (overbite; Dempsey et al., 2002), an inferiorly placed hyoid bone (Guillemainault et al., 1984), and narrowed posterior airspace (Cistulli, 1996).

In children and young adolescents, adenotonsillar hypertrophy is considered the major determinant of OSA, with removal of the tonsils and adenoids regarded as the most effective first-line therapy (Shine et al., 2005). These two structures (Figure 1.7), part of the lymphatic system, continue growing until the time of puberty before atrophying thereafter; during young childhood (ages 3-6 years), these structures are the largest they’ll ever be relative to the diameter of the throat (Greenfield et al., 2003). Recent work, however, suggests that the association between pediatric tonsil size and objective OSA severity is relatively weak (Nolan & Brietzke, 2011). Furthermore, many children spontaneously remit from SDB after several months without surgical intervention (Marcus et al., 2013), with the vast majority remitting by adolescence (Spilsbury et al., 2015; Bixler et al., 2016). (Adenotonsillectomy in children with SDB is described in greater detail in Section 1.1.4. Current Treatment.)
**Inflammatory disease.** An emerging body of literature reports an association between sleep apnea in autoimmune and rheumatic disease populations, particularly rheumatoid arthritis (Reading et al., 2009; Taylor-Gjevre et al., 2013). Common to both conditions are increased levels of circulating proinflammatory cytokines; however, the potential direction of this association has not been explicitly examined. Interestingly, a recent study reported a significantly lower prevalence of OSA in patients with spondyloarthritis who take TNF-inhibitors compared to those who do not (Walsh, 2012), suggesting that systemic inflammation precedes the development – or at least the worsening – of OSA.

**Genetic risk factors.** A family history of OSA is a risk factor for snoring, daytime sleepiness, and apnea. Most genetic studies have focused on AHI as the outcome measure, though other metrics – such as duration of respiratory disturbance – have been shown to have heritability as high as 0.60, meaning that 60% of the variance is explained by familial factors (Cade et al., 2014).

A major confounding factor in studying the genetics and epigenetics of OSA is the significant overlap with obesity-related genes. Obesity-associated phenotypes, including BMI, body fat distribution, and leptin levels, have heritability estimates ranging from 40-70% (Farooqi & O’Rahilly, 2006), though a recent study reported that only about 35% of the genetic variance in OSA severity is shared with BMI (Patel et al., 2008). Several case-control studies of OSA have identified a functional polymorphism in the TNFα genes (Riha et al., 2005; Almpanidou et al., 2012), as well as variants in IL-6 and CRP genes (Larkin et al., 2010a; Larkin et al., 2010b). The genetic investigation of OSA has only emerged within the last decade; future studies
employing new, developing technologies in the coming years will provide a greater understanding of the potential genetic basis of OSA.

**1.1.4. Current Treatment**

Aside from weight loss, which has been shown to improve AHI, oxygen desaturation, sleep architecture, and daytime performance (Strobel & Rosen, 1996), a number of mechanical, surgical, and medication options are available or currently being developed for patients with OSA, each with their benefits and drawbacks.

*Continuous positive airway pressure.* Thirty-five years ago, Colin Sullivan and colleagues of the University of Sydney published their seminal paper demonstrating that continuous positive airway pressure (CPAP) prevented upper airway occlusion and improved sleep quality in five patients with OSA, describing the device as a “pneumatic splint for the nasopharyngeal airway” (Sullivan et al., 1981). The following year, David Rapoport and colleagues of New York University published a case report in a middle-aged, obese man, describing how long-term nocturnal CPAP use (13 months) “reversed” daytime sleepiness and nighttime hypoxia (Rapoport et al., 1982).

As the gold standard treatment for OSA, continuous positive airway pressure devices (Figure 1.8) apply continuous mild air pressure to keep airways open, allowing patients with OSA or other conditions (such as respiratory distress system in preterm infants) to breathe spontaneously on their own. Nasal masks are most common, though oral and naso-oral masks are also used. Depending on the air flow rate, nasal cannulae deliver between one to six liters of
oxygen per minute (Waugh & Granger, 2004),
and most devices include features such as heated
humidifiers. CPAP use has been consistently
associated with higher sleep efficiency, fewer
arousals, lower AHI, improved oxygen
saturation, and decreased sleepiness during the
daytime (Ha et al., 2014). There is evidence that
long-term CPAP use (at least three months) is
associated with a modest reduction in blood
pressure (Dimsdale et al., 2000; Vgontzas et al., 2008).

Despite a beneficial effect on sympathetic activity, however, a recent meta-analysis
concluded that CPAP does not alter lipid levels, insulin resistance, inflammatory markers, or the
proportion of patients with the metabolic syndrome (Jullian-Desayes et al., 2015). Specifically,
CPAP does not significantly reduce IL-6, TNFα, TNFR1 (Vgontzas et al., 2008; Arias et al.,
2008), CRP (Kohler et al., 2009), leptin (Hoyos et al., 2012; Kritikou et al., 2014), nor fasting
insulin and glucose (Vgontzas et al., 2008), even after comparing groups with low versus high
CPAP compliance (Kritikou et al., 2014). A sham-controlled study also demonstrated no reversal
of metabolic syndrome after 12 weeks of CPAP use (Hoyos et al., 2013). In a recent randomized
trial, improvements in inflammation, insulin resistance, and serum triglycerides were only
observed in apneics who combined their CPAP use with weight loss during a 24-week period
(Chirinos et al., 2014).

CPAP compliance is also problematic, particularly for patients in the mild-to-moderate
OSA range. In a recent long-term study of CPAP use, 31% of patients with OSA who were

Figure 1.8. Continuous positive airway pressure (CPAP)
device, showing airflow generator, hose, and full face
mask. Image source: PruebasBMA/Wikimedia Commons
(public domain).
prescribed CPAP never commenced therapy after diagnosis and titration, and an additional 15% abandoned their CPAP after an average of 10 months of use (Wolkove et al., 2008). Many patients cite an uncomfortable mask, the noise generated by the machine, and difficulty falling asleep as the major reasons for discontinuing CPAP (Wozniak et al., 2014).

**Upper airway surgeries.** A number of upper airway surgeries have been developed for treating OSA, particularly in patients who find it difficult to adhere to CPAP therapy or have severe anatomical abnormalities. Uvulopalatopharyngoplasty (UPPP) removes excess tissue in the throat – tonsils, posterior soft palate, and uvula (Fujita et al., 1981). If an enlarged tongue contributes to obstruction, a surgeon may also remove part of the tongue in what is called a uvulopalatopharyngoglossoplasty. A similar procedure, laser assisted uvulopalatoplasty (LAUP), uses a series of laser incisions to shorten the uvula and tighten the soft palate. Although UPPP and LAUP acutely reduce AHI, long-term success of these surgeries is low (Walker-Engström et al., 2002; Caples et al., 2010). For OSA patients with retrognathia, or severe overbite, maxillomandibular advancement (MMA) expands the skeletal framework without directly manipulating pharyngeal tissues, stabilizing the bone placement with screws, plates, or bone grafts (Caples et al., 2010).

Soft palate implants offer a somewhat less invasive procedure for patients with mild-to-moderate OSA. Under local anesthesia, Dacron rods are inserted into the soft palate to “stiffen” and provide structure to the soft palate to prevent collapse (Friedman et al., 2008). Recent work has been testing the feasibility, efficacy, and safety of implantable hypoglossal nerve stimulators that function to activate the genioglossus and other upper airway dilator muscles during sleep (Kezirian et al., 2014).
Adenotonsillectomy. Surgical removal of the tonsils and/or adenoids represents the most common major operation in children; in the United States, it’s estimated that over half a million adenotonsillectomy (AT) procedures are performed annually (Bhattacharyya & Lin, 2010), often due to recurrent infections or SDB. The procedure involves removing both the tonsils and adenoids (Figure 1.7) from the surrounding fascia. Potential complications include hemorrhage, infection, and nasopharyngeal stenosis (Kavanagh & Beckford, 1988), and the risk of complication is increased in children with obesity (Stratham & Myer, 2010).

Between the ages of 3 to 6 years, the tonsils and adenoids are the largest they’ll ever be relative to the diameter of the throat, atrophying after the onset of puberty (Greenfield et al., 2003). As reported in the recent Childhood Adenotonsillectomy Trial (CHAT) study, AT normalizes PSG findings in 79% of children (5-9 years) with OSA seven months after surgery; interestingly, however, 46% of children in the “watchful waiting” group remitted without intervention (Marcus et al., 2013). Furthermore, two large longitudinal cohorts recently reported OSA remission rates of 91.3% (Spilsbury et al., 2015) and 100% (Bixler et al., 2016) from childhood to adolescence. In both of these studies, a history of AT in childhood actually predicted incident SDB in adolescence, suggesting that the surgical intervention did not address the root cause of OSA. Although adenotonsillar hypertrophy is considered the major cause of OSA in young children, these findings suggest that, like in adults, most cases of OSA tend to be systemic, rather than a result of a local anatomical or inflammatory process. Furthermore, OSA that manifests in adolescence does not appear to be on a continuum with childhood OSA.

Anti-inflammatory therapy. In line with the hypothesis that OSA stems from a systemic cause, several pilot treatment studies have modestly reduced OSA severity in both children and
adults without CPAP treatment or surgical intervention. In a placebo-controlled, double-blind study of eight obese men with severe OSA (AHI = 52.8 ± 9.1 events/h), a three-week trial of the TNFα antagonist etanercept significantly reduced AHI (to 44.3 ± 10.3 events/h), IL-6 levels, and objective daytime sleepiness (as assessed by increased sleep latency during daytime nap opportunities in the Multiple Sleep Latency Test [MSLT]; Vgontzas et al., 2004).

In adults with OSA, inflammation has been detected in nasal and oropharyngeal mucosa (Rubinstein, 1995; Sekosan et al., 1996; Olopade et al., 1997). Similarly, more recent research has reported mediators of inflammation, including leukotrienes and prostaglandins, present in the exhaled breath condensate of children with OSA (Goldbart et al., 2006). Based on these findings, several randomized controlled trials in children, spanning across several weeks to months, report reduced AHI and relative normalization of sleep parameters with intranasal corticosteroid treatment in combination with oral anti-inflammatory medication, despite no change in adenotonsillar size (Brouillette et al., 2001; Goldbart et al., 2005; Kheirandish-Gozal & Gozal, 2008; Kuhle & Urschitz, 2011; Kheirandish-Gozal et al., 2014).

For children and adolescents, such treatment may provide a less invasive, disruptive alternative to surgery or CPAP, especially for those with milder forms of SDB. Furthermore, the preliminary success of these treatment studies suggests that anti-inflammatory therapy may be better addressing the systemic root cause of OSA for many patients.
1.2. OBESITY

1.2.1. Definition, Prevalence, and Worldwide Impact

In 1990, less than 15% of the U.S. adult population was obese. Two decades later, this figure jumped to 25% in 36 states, with 12 states at 30% or higher (Ogden et al., 2014). Currently, 17% of U.S. children under the age of 20 years are considered obese (Ogden et al., 2014), up from 10% in 2007 (Ogden et al., 2012).

In 1997, the World Health Organization (WHO) formally recognized obesity as a global health epidemic (Caballero, 2007). Once considered a “first-world” problem, obesity rates have steadily risen worldwide, with the greatest impact in urban locations. In 2013, the number of overweight or obese adults in the world was estimated at 2.1 billion, compared to 857 million in 1980 (Ng et al., 2014). That same year, the American Medical Association publicly recognized obesity as a “disease.” The cost of obesity-related illnesses is estimated at $190.2 billion annually, or 21% of all medical spending in the U.S. (Cawley & Meyerhoefer, 2012). Regression modeling forecasts an obesity prevalence of 42% and “severe obesity” prevalence of 11% by 2030; if the prevalence were to remain at current levels, the U.S. would save $549.5 billion in medical expenditures across this time period (Finkelstein et al., 2012). In addition to direct medical costs, obesity has also been associated with many indirect economic costs, including absenteeism, disability, and even transportation due to excess fuel usage (Hammond & Levine, 2010).

The technical definition of obesity is based on the body mass index (BMI), defined as one’s mass (in kilograms) divided by the square of one’s height (in meters). According to the
WHO, a BMI of 25 kg/m$^2$ is considered “overweight,” while any BMI $\geq 30$ kg/m$^2$ is considered “obese.” These findings have been validated by a number of large studies demonstrating significant morbidity and mortality beginning at the cut-off of BMI $\geq 30$ (Berrington de Gonzalez et al., 2010); however, Asian populations tend to develop negative health consequences at a lower BMI than Caucasians. As such, China has redefined obesity as BMI $\geq 28$ (Zhou et al., 2002), while Japan uses a definition of BMI $\geq 25$ (Kanazawa et al., 2005). Because the BMI measure only captures measures of height and weight and not body composition (namely, the proportion and distribution of fat to muscle, bone, and other tissue), it is considered a rather crude marker of overall health.

Since body composition changes so rapidly with age in children and adolescents (< 20 years), it is customary to use age- and gender-specific charts to calculate a BMI percentile (Figure 1.9). BMI percentile allows for comparison of a child’s BMI relative to other children of his or her age and gender in the U.S. A BMI percentile $\geq 85$ is considered “overweight,” while BMI percentile $\geq 95$ is considered “obese.”

Figure 1.9. BMI-for-age growth charts in boys (left) and girls (right), ages 2-20 years. Image source: Centers for Disease Control, 2000 (public domain).
1.2.2. Visceral vs. Subcutaneous Fat

Adipose (fat) tissue is a type of loose connective tissue composed primarily of adipocytes (fat cells), as well as preadipocytes, various fibroblasts, vascular endothelial cells, and adipose tissue macrophages. Brown adipose tissue functions to generate body heat, while white adipose tissue – the majority of overall body fat – stores energy in the form of lipids. White adipocytes contain a large lipid droplet composed primarily of triglycerides and cholesteryl ester. Once considered to be relatively fixed in number throughout the lifetime, these cells can increase in size roughly fourfold before needing to divide and multiply (Lefterova & Lazar, 2009). With weight loss, lipolysis breaks down these lipids into glycerol and free fatty acids, decreasing the size of adipocytes (Verhoef et al., 2013).

White adipose tissue is divided into two major types of body fat: subcutaneous and visceral. In vertebrates, subcutaneous fat tissue is located in the subcutis (hypodermis), which composes the bottommost layer of the integumentary system (Figure 1.10). Surrounded also by blood vessels, lymphatic vessels, nerves, hair follicles, and other cell types, subcutaneous adipocytes are grouped in lobules separated by connective tissue. Subcutaneous fat is thickest in the buttocks, palms, and soles of the feet in humans (Janigan et al., 1993).

Figure 1.10. Subcutaneous fat (yellow) is located in the subcutis layer of the integumentary system. Image source: US-Gov/Wikimedia Commons (public domain).
Visceral fat, on the other hand – also known as intra-abdominal fat – is localized in the peritoneal cavity, packed between the stomach, liver, kidneys, intestines and other internal organs (Figure 1.11). Excess visceral fat is referred to as central obesity, as the waistline is greatly expanded relative to other body regions. Compared to subcutaneous adipose tissue, visceral fat contains proportionally more adipocytes that are capable of growing quite large before dividing (Ibrahim, 2009).

![Figure 1.11. Visceral fat is packed between the organs of the peritoneal cavity. Image source: Wikimedia Commons (public domain).](image)

There are several major cellular, molecular, metabolic, and prognostic differences between these two types of fat. For one, while visceral fat accounts for 5-8% of total body fat in women, up to 10-20% of body fat is located in the viscera of men (Wajchenberg, 2000). In women, estrogen promotes the storage of subcutaneous fat preferentially in the buttocks, thighs,
and hips; at the time of menopause, when estrogen declines, fat then begins to accumulate more rapidly in the belly (Krotkiewski et al., 1983; Poehlman et al., 1995).

Furthermore, adipocytes capable of accommodating larger lipid droplets become dysfunctional as they grow, showing a higher rate of glucose uptake and developing resistance to the anti-lipolytic effect of insulin (Márin et al., 1992). Because these large adipocytes are more common in visceral fat tissue, visceral adipose tissue has been increasingly regarded as a metabolically-active organ in recent years. Compounding the health risk is the fact that visceral fat tissue is rich in vasculature and innervation compared to subcutaneous fat, making the adipocytes more vulnerable to circulating hormones and cytokines. Visceral adipose tissue has a relatively higher concentration of glucocorticoid, androgen, and adrenergic receptors than subcutaneous fat, making the adipocytes more susceptible to stress hormone, catecholamine, and testosterone signaling (Rebuffé-Scrive et al., 1985; Arner et al., 1990; Björntorp, 1995). Relatedly, visceral fat also readily contributes adipokines into the bloodstream and nervous system (Ibrahim, 2009). While both subcutaneous and visceral adipose tissues synthesize peptides and proteins, including leptin, adiponectin, and pro-inflammatory cytokines, the proximity of visceral fat to vasculature, central nerves, and critical organs such as the liver make this tissue type relatively more dangerous to overall health (Ibrahim, 2009).

Indeed, compared to subcutaneous fat, elevations in visceral fat have been strongly linked to development of the metabolic syndrome (see Section 1.2.4. Metabolic Syndrome), cardiovascular events (Dobbelsteyn et al., 2001), and cardiovascular and all-cause mortality, particularly in men (Kuk et al., 2006).
1.2.3. Adipocytes and Inflammation

Obesity is recognized as a chronic, low-grade inflammatory state. In addition to adipocytes, fibroblasts, and vascular endothelial cells, adipose tissue contains immune cells including mast cells, eosinophils, B cells, T cells, and macrophages (Schipper et al., 2012). Many studies in animals and humans have linked obesity to increased levels of the proinflammatory proteins TNFα, IL-6, IL-1β, CRP, inducible nitric oxide synthase (iNOS), transforming growth factor beta 1 (TGF-β1), soluble intercellular adhesion molecule (ICAM), and monocyte chemoattractant protein-1 (MCP-1) compared to lean individuals (Hotamisligil et al., 1993; Vgontzas et al., 1997; Samad et al., 1997; Fried et al., 1998; Perreault & Marette, 2001; Sartipy et al., 2003). The increased production of these so-called “adipokines” (adipocyte cytokines) is implicated in the metabolic sequelae associated with obesity; TNFα has been shown to directly decrease insulin sensitivity and increase lipolysis (Hotamisligil et al., 1994; Zhang et al., 2002), while IL-6 stimulates lipolysis and triglyceride secretion (Nonogaki et al., 1995). Increased volume and number of adipocytes is correlated with increased leptin production (a hormone involved in energy intake and storage) and decreased adiponectin (a hormone associated with insulin sensitivity and free fatty acid oxidation), metabolic rate, and insulin sensitivity (Maffei et al., 1995; Rosenbaum & Leibel, 1999).

Macrophages are a type of phagocyte, a component of the innate immune system responsible for ingesting harmful foreign bodies and dead cells, secreting antimicrobial peptides, and releasing molecules that attract other immune cells to sites of infection (Gordon, 1998). Macrophages adopt tissue-specific morphologies, residing in nearly all bodily tissues in distinct populations. For example, in the central nervous system, macrophages, called microglia, are
interspersed throughout neurons and account for 10-15% of all cells in the brain (Lawson et al., 1992); in the skeletal system, macrophages form osteoclasts in the connective tissue surrounding bone (the periosteum), absorbing bone tissue during growth and healing (Teitelbaum, 2000). The specific function of macrophages in adipose tissue is not fully understood, but it’s been demonstrated that the concentration of macrophages in adipose tissue is positively correlated with adipocyte size and BMI (Weisberg et al., 2003). In lean mice, the percentage of macrophages relative to total adipose tissue is estimated at < 10%, while ≥ 50% in obese, leptin-deficient mice; in obese humans, this figure is estimated to be around 40% (Weisberg et al., 2003).

In obesity, there are several mechanisms of macrophage recruitment that, in turn, lead to elevations in proinflammatory cytokines. As the size of lipid droplets within adipocytes increases, adipocytes themselves begin to secrete low levels of TNFα. Elevations in TNFα stimulate both preadipocytes and surrounding endothelial cells to produce MCP-1, a signal that attracts macrophages to adipose tissue (Xu et al., 2003). With growing fat pad size, increased production of leptin and decreased adiponectin are also shown to recruit macrophages to adipose tissue (Sierra-Honigmann et al., 1998) as well as promote macrophage adhesion to endothelial cells (Maeda et al., 2002). As adipocytes grow and divide, endothelial damage due to oxidative stress or crowding may also recruit additional macrophages (Wellen & Hotamisligil, 2003). It’s not clear which of these stimuli are responsible for initial macrophage recruitment; regardless, these macrophages begin secreting TNFα, IL-6, IL-1β, and other proinflammatory cytokines that perpetuate a vicious cycle of macrophage recruitment, impaired adipocyte functioning, altered leptin and adiponectin expression, and activation of transcription factors, (such as NF-κB) for genes encoding inflammatory proteins (Wellen & Hotamisligil, 2003).
Taken together, obesity brings about a chronic activation of the innate immune system with long-term release of proinflammatory cytokines, termed chronic systemic inflammation. As a result, plasma levels of a number of other proteins, called acute-phase reactants, also become elevated. One such acute-phase reactant is C-reactive protein (CRP), a protein synthesized in the liver when stimulated by elevated IL-6 levels (Pepys & Hirschfield, 2003). In addition to adipocyte growth, IL-6 and other cytokines trigger CRP synthesis due to infection, chronic inflammatory disease, tissue injury, and malignancy; CRP levels rise within two hours of acute infection and peak at 48 hours. Because its half-life (18 hours) is constant, it does not exhibit diurnal rhythmicity like other cytokines, and its levels are dependent upon the severity of overall inflammation in the body, CRP is considered to be a good nonspecific marker of inflammation and a rough proxy for cardiovascular disease risk (Seo, 2012); a plasma concentration of ≥ 3.0 mg/L is considered to put one at high risk (Ridker, 2005).

1.2.4. Metabolic Syndrome

The constellation of adverse health outcomes associated with prolonged obesity – namely, central obesity, elevated blood pressure, insulin resistance, high triglycerides, and reduced high-density lipoprotein (HDL) cholesterol levels – is termed the metabolic syndrome. Collectively, the metabolic syndrome raises the risk of cardiovascular morbidity and mortality, type 2 diabetes, some cancers, all-cause mortality, and even neurological disorders (Ford, 2005a). Depending on the definition used, 35-40% of U.S. adults have the metabolic syndrome (Ford, 2005b). Risk factors for metabolic syndrome include older age, sedentary behavior, poor diet, and genetics (Pollex & Hegele, 2006; Malik et al., 2010; Edwardson et al., 2012). Other
names for the disorder over the years include dysmetabolic syndrome, insulin resistance syndrome, obesity syndrome, and syndrome X (Eckel et al., 2005).

A number of definitions for the metabolic syndrome in adults have been put forward by various organizations and health federations over the last two decades (Table 1.2). While the definitions provide considerable insight into the pathophysiology of the syndrome, in some ways they have also complicated what was designed to be a simple screening tool. Today, the two most recent definitions – by the National Cholesterol Education Program Adult Treatment Program III (NCEP ATP III; 2005) and International Diabetes Federation (IDF; 2006) – are very similar and the most commonly used criteria (Parikh & Mohan, 2012).

Table 1.2. Definitions of the metabolic syndrome in adults.

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<td>Obesity</td>
<td>Waist/hip ratio &gt; 0.90 (men), &gt; 0.85 (women), or BMI ≥ 30</td>
<td>Waist circumference ≥ 94 cm (men), ≥ 80 cm (women)</td>
<td>Waist circumference &gt; 40 in (men), &gt; 35 in (women)</td>
<td>Waist circumference ≥ 94 cm (men), ≥ 80 cm (women)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>≥ 140/90 mmHg</td>
<td>≥ 140/90 mmHg or medication</td>
<td>&gt; 130 mmHg systolic or &gt; 85 mmHg diastolic or medication</td>
<td>&gt; 130 mmHg systolic or &gt; 85 mmHg diastolic or medication</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Triglycerides</td>
<td>TG ≥ 150 mg/dL or HDL-C criteria</td>
<td>TG ≥ 177 mg/dL or HDL-C criteria</td>
<td>≥ 150 mg/dL or medication</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>HDL-C &lt; 35 mg/dL (men), &lt; 39 mg/dL (women) or TG criteria</td>
<td>HDL-C &lt; 39 mg/dL or TG criteria</td>
<td>&lt; 40 mg/dL (men) or &lt; 50 mg/dL (women) or medication</td>
<td>&lt; 40 mg/dL (men) or &lt; 50 mg/dL (women) or medication</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>Any evidence of IR (impaired glucose tolerance or fasting glucose, type 2 diabetes, etc.)</td>
<td>Plasma insulin &gt; 75th percentile</td>
<td>Fasting glucose ≥ 100 mg/dL or medication</td>
<td>Fasting glucose ≥ 100 mg/dL or medication</td>
</tr>
<tr>
<td>Criteria</td>
<td>IR or type 2 diabetes, plus any 2 of the other 4 criteria</td>
<td>Hyperinsulinemia, plus any 2 of the other 4 criteria</td>
<td>Any 3 of the 5 criteria</td>
<td>Central obesity, plus any 2 of the other 4 criteria</td>
</tr>
</tbody>
</table>

WHO = World Health Organization; EGIR = European Group for the Study of Insulin Resistance; NCEP ATP III = National Cholesterol Education Program Adult Treatment Program III; IDF = International Diabetes Federation; TG = triglycerides; HDL-C = HDL cholesterol; IR = insulin resistance. Data from Alberti & Zimmet, 1998; NCEP, 2002; Alberti et al., 2006.
A consensus for defining and estimating the prevalence of metabolic syndrome in children and adolescents (< 18 years) remains challenging. Over 40 definitions adapted from adult criteria have been proposed (Ford & Li, 2008), most of which include some form of each of the five components of the adult criteria (Table 1.2). Two commonly-used definitions in children and adolescents are detailed below (Table 1.3). According to findings from the third National Health and Nutrition Examination Survey (NHANES III), the prevalence of metabolic syndrome in adolescents aged 12-19 years was 6.1% in boys and 2.1% in girls. Furthermore, metabolic syndrome was present in 28.7% of obese adolescents (BMI ≥ 95th percentile), 6.8% of overweight adolescents, and 0.1% of normal-weight adolescents (Cook et al., 2003).

Table 1.3. Definitions of the metabolic syndrome in children.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obesity</strong></td>
<td>Waist circumference ≥ 90th percentile, according to age- and gender-specific values from NHANES III</td>
<td>Waist circumference ≥ 90th percentile</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>Systolic or diastolic &gt; 90th percentile, according to age-, gender-, and height-specific values from NHANES III</td>
<td>Systolic &gt; 130 mmHg or diastolic &gt; 85 mmHg</td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt; 1.24 mmol/L</td>
<td>&gt; 1.7 mmol/L</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&lt; 1.03 mmol/L</td>
<td>&lt; 1.03 mmol/L</td>
</tr>
<tr>
<td><strong>Hyperglycemia</strong></td>
<td>Glucose ≥ 6.1 mmol/L</td>
<td>Glucose ≥ 5.6 mmol/L or type 2 diabetes</td>
</tr>
<tr>
<td><strong>Age criteria</strong></td>
<td>12-19 years</td>
<td>10-16 years</td>
</tr>
</tbody>
</table>

NHANES = National Health and Nutrition Examination Survey.

Khoury and colleagues (1980) of the University of Cincinnati are first credited with clustering risk factors for cardiovascular disease in children aged 6-19 years. Citing the need to quantify metabolic syndrome risk without dichotomizing each variable, literature over the last two decades have put forth several definitions of a continuous metabolic syndrome score.
(cMetS), employing everything from Z-scores (Brage et al., 2004) to percentile rankings (Bao et al., 1994) and principal component analyses (Katzmarzyk et al., 2001) in their equations. In 2008, Eisenmann first proposed a cMetS calculation for children and adolescents, validated in 2010, that included the individual components of the metabolic syndrome (waist circumference, mean arterial pressure [MAP; average blood pressure], homeostatic model assessment [HOMA; insulin resistance], HDL cholesterol [multiplied by -1], and triglycerides) standardized by regressing them onto age, gender, and race to account for any confounder-related differences in these variables. (See Methods, Section 2.4. Measures of Inflammation and Metabolic Health, for more details about MAP and HOMA calculations). Once regressed, the standardized residuals (Z-scores) for these values were summed to generate the cMetS (Eisenmann et al., 2010). Indeed, the cMetS score correlates strongly with the cumulative number of risk factors (dichotomized) in children, according to NCEP ATP III criteria (Table 1.3).

A number of studies have since adopted this definition (Okosun et al., 2010; Pandit et al., 2011; Heshmat et al., 2015); currently, given the challenges and controversies associated with defining specific cut-off points, the cMetS appears to be the best tool for assessing metabolic risk in this heterogeneous age group.
1.3. OBESITY, METABOLIC SYNDROME, AND OBSTRUCTIVE SLEEP APNEA

Epidemiological studies have reported that metabolic syndrome is 6 to 9 times more likely to be present in individuals with OSA compared to the general population (Coughlin et al., 2004; Gruber et al., 2006). While the causative association between obesity and metabolic syndrome is relatively straightforward, findings on the link between OSA and the metabolic syndrome have been inconsistent.

1.3.1. Obesity Precedes the Development of Obstructive Sleep Apnea

Physicians as far back as the nineteenth century recognized the cluster of symptoms (obesity, snoring, daytime sleepiness, labored breathing) related to obesity hypoventilation, or “Pickwickian,” syndrome. In adults, longitudinal findings in the large general population Wisconsin Sleep Cohort indicate that the increased prevalence of OSA has mirrored the growing prevalence of obesity over the last 20 years (Young et al. 2009). More recently, rates of SDB have increased 14% to 55%, depending on the subpopulation studied, in parallel with the ongoing obesity epidemic (Peppard et al. 2013). Another prospective study in the same cohort reported how a 10% weight gain predicts a 32% increase in AHI and 6-fold odds of developing moderate-to-severe OSA, while a 10% weight loss results in a 26% reduction in AHI (Peppard et al. 2000a). Together, these findings suggest that obesity precedes OSA, and that OSA, conversely, can be attenuated by weight loss.

Specifically, compared to age- and BMI-matched controls, visceral fat area in obese (Vgontzas et al. 2000) and even non-obese (Kritikou et al. 2013) men as measured by CT scan is
elevated in adults with OSA. Many studies have since reported a strong cross-sectional association between OSA and visceral, as opposed to subcutaneous, adiposity in both adults (Shinohara et al., 1997; Harada et al., 2014) and children/adolescents (Hannon et al., 2011). A recent longitudinal general population study examining incident SDB in adolescents reported that childhood waist circumference, a proxy measure of visceral adiposity, was a significant predictor of adolescent SDB; visceral fat area, measured cross-sectionally via dual X-ray absorptiometry (DXA), was the strongest predictor of all variables examined (Bixler et al. 2016).

As previously mentioned (Section 1.1.3. Risk Factors for Obstructive Sleep Apnea), visceral obesity in particular has been associated with increased neck fat (Lubrano et al., 2012), altered upper airway anatomy (Deegan & McNicholas, 1995), airway dilator muscle dysfunction (Atlantis et al., 2009), and systemic inflammation, which may all contribute to the development and worsening of OSA.

1.3.2. Inflammation and Obstructive Sleep Apnea: A Two-Way Street

While there is little doubt that central obesity and obesity-induced inflammation are strong causative factors in the majority of OSA cases, the link between inflammation and OSA, specifically, appears to be more strongly bidirectional. There is considerable overlap in many biomarkers and health outcomes in obese individuals with and without apnea, which makes it difficult to disentangle cause, effect, and whether certain treatments, like CPAP, can improve these markers.

Proinflammatory cytokine elevations have been reported independent of obesity in both adults and children with OSA. Compared to age- and BMI-matched controls, patients with OSA
demonstrate significantly elevated plasma IL-6, TNFα (Vgontzas et al., 2000; Yokoe et al., 2003), CRP (Shamsuzzaman et al., 2002; Chien et al., 2012), and leptin (Vgontzas et al., 2000; Phillips et al., 2000; Tsaoussoglou et al., 2010), suggesting that the presence of OSA – on top of pre-existing obesity – exacerbates systemic inflammation. In children and adolescents with SDB, levels of CRP have been positively correlated with AHI, arousal index, and oxygen desaturation (Tauman et al., 2004; Larkin et al., 2005).

Inflammation independent of obesity in OSA is thought to originate from two major sources. For one, mechanical damage due to snoring, breathing effort, and upper airway obstruction has been associated with elevated immune cells in nasal and oropharyngeal mucosa, breath condensate, and sputum in adults (Rubinstein, 1995; Sekosan et al., 1996; Olopade et al., 1997; Salerno et al., 2004) and children (Goldbart et al., 2006), suggesting a local inflammatory process.

The second source appears to be more systemic. In addition to sleep fragmentation, OSA patients experience a unique pattern of oxygen deficiency – termed intermittent hypoxia – in which short, repetitive cycles of oxygen desaturation are followed by rapid reoxygentation of tissue. In an in vitro model, Ryan and colleagues (2005) described how intermittent hypoxia preferentially activates inflammatory pathways mediated by the transcription factor nuclear factor kappa B (NF-κB) which, when activated, increase the transcription of genes coding for TNFα, interleukins, and other immune proteins. On the other hand, more sustained, long-term hypoxic conditions (like living in high altitudes, for example) are associated with activation of the adaptive hypoxia-inducible factor-1 (HIF-1)-dependent pathway (Ryan et al., 2005), which helps the body adapt by promoting oxygen delivery to hypoxic regions. Intermittent hypoxia has also been uniquely associated with activation of other inflammatory transcription factors,
including activator protein complex-1 (AP-1), which is formed by c-Fos and c-Jun proteins induced by inflammatory cytokines (Shaulian & Karin, 2002); interestingly, however, the fact that CPAP therapy corrects intermittent hypoxia, but not does significantly reduce inflammation (Jullian-Desayes et al., 2015), suggests that OSA may affect inflammation through a mechanism(s) other than intermittent hypoxia alone.

In sum, both obesity- and OSA-induced inflammation launch a vicious cycle of worsening OSA and cardiometabolic health outcomes. In line with this evidence, it is not surprising that modest improvement in both AHI and sleep quality have been demonstrated with anti-inflammatory therapies in adults (Vgontzas et al., 2004) and children (Brouillette et al., 2001; Goldbart et al., 2005; Kheirandish-Gozal & Gozal, 2008; Kuhle & Urschitz, 2011; Kheirandish-Gozal et al., 2014) independent of weight loss, CPAP therapy, or upper airway surgery.

1.3.3. Obstructive Sleep Apnea and the Metabolic Syndrome: Chicken or Egg?

In the Penn State Adult Cohort, a general population sample of 1,741 adults, the prevalence of OSA peaks around age 55 years for men and 65 years for women (Bixler et al., 1998; Bixler et al., 2001). Interestingly, this quadratic relationship mirrors the prevalence of the metabolic syndrome in U.S. adults according to NHANES III data (1988-1994) collected during the same time frame (Ford et al., 2002; Vgontzas et al., 2005; Figure 1.12).

Particularly in women, OSA is more prevalent following hormonal changes such as menopause, which may explain the delay in peak prevalence compared to men (Bixler et al., 2001). Among premenopausal women, OSA is also 30 times more common in those with PCOS,
an endocrine disorder characterized by insulin resistance, oligoanovulation, hyperandogenism, and central obesity. Together, these findings suggest that the metabolic syndrome plays a causative role in the development of OSA.

![Graph showing prevalence of OSA and metabolic syndrome by age and gender](image)

**Figure 1.12.** The prevalence of OSA mirrors that of the metabolic syndrome in the U.S. general population. OSA peaks around age 55 years for men and 65 years for women. *Data from Bixler et al., 1998; Bixler et al., 2001; Ford et al., 2002.*

Interestingly, just as the prevalence of metabolic syndrome declines with older age, the cardiometabolic effects associated with OSA also diminish with age. A cross-sectional study of
the Penn State Adult Cohort reported that although AHI was independently associated with blood pressure, the relationship was strongest in the youngest, pointing to the hypothesis that OSA is not an independent risk factor for hypertension in the elderly (Bixler et al., 2000). Similarly, a prospective investigation of the Sleep Heart Health Study found that the risk of coronary heart disease and heart failure were not significantly increased for older men or women, even at the level of “severe” OSA (AHI ≥ 30; Gottlieb et al., 2010). Mortality was also not increased in an elderly cohort (≥ 65 years), even in those with a respiratory disturbance index (AHI plus RERAs) of > 40 events/hour (Lavie & Lavie, 2009). Together, these findings suggest that (a) the declining cardiometabolic morbidities in older age occur independently from – and are not a result of – OSA, and (b) OSA in old age is not as strongly associated with the metabolic syndrome as in younger age.

A key component in the pathophysiology of the metabolic syndrome is chronic systemic inflammation. In adipose tissue, TNFα concentrations have been associated with insulin resistance in individuals both with and without type 2 diabetes (Hotamisligil & Spiegelman, 1994; Kern et al., 2001). TNFα messenger RNA expression in fat tissue is positively correlated with fasting plasma glucose, insulin, and triacylglycerol levels (Hotamisligil et al., 1993); TNFα increases systemic insulin resistance by releasing adipose tissue fatty acids into the bloodstream, acting then on other organ systems (Greenberg & Obin, 2006). IL-6 is expressed 10-fold higher in the adipose tissue of obese versus lean individuals, particularly in visceral fat (Fried et al., 1998), and adipose IL-6 accounts for 30% of the body’s total production (Mohamed-Ali et al., 1997). Like TNFα, IL-6 causes fat oxidation and lipolysis (van Hall et al., 2003); plasma IL-6 concentrations correlate with insulin resistance (Kern et al., 2001), and predict type 2 diabetes and myocardial infarction (Ridker et al., 2000; Pradhan et al., 2001).
In addition to obesity, however, growing evidence suggests that hypoxia-induced inflammation in OSA also contributes independently to the development of metabolic syndrome. Several rat and dog models of intermittent hypoxia have demonstrated increased blood pressure, which remains even after animals are returned to normal conditions (Fletcher et al., 1992; Brooks et al., 1997); it’s thought that chronic sympathetic activation is the major pathophysiological mechanism (Bao et al., 1997). Furthermore, activation of the transcription factor NF-κB has been demonstrated in a mouse model of intermittent hypoxia (Greenberg et al., 2006), as well as in cultured monocytes of human patients with OSA compared to non-apneics (Yamauchi et al., 2006). NF-κB activates genes that code for proinflammatory cytokines, and has been shown to contribute to insulin resistance through a variety of proinflammatory signaling pathways (Baker et al., 2011).

Intermittent hypoxia during the sleep phase in rats is also associated with apoptosis in the CA1 region of the hippocampus and subsequent poor performance in the Morris water maze, a spatial learning task (Goldbart et al., 2003). Interesting, in a 2007 study, Gozal and colleagues demonstrated that CRP is a marker of cognitive dysfunction in non-obese children with OSA. Participants (ages 5-7 years) were divided into OSA (AHI ≥ 2 plus nadir SpO₂ < 92%) and no-OSA (AHI < 1) groups. The OSA group was further subdivided into those with and without cognitive deficits, defined as a score ≥ 1 standard deviation below the mean on at least two subtests of the Differential Ability Scales (DAS) or the NeuroPsychological Assessment Battery (NEPSY). While CRP levels were relatively similar between children with OSA/normal cognitive scores and controls without OSA (0.21 ± 0.08 mg/dL vs. 0.19 ± 0.07 mg/dL), non-obese children with OSA and cognitive deficits had more than twice the concentration of CRP (0.48 ± 0.12 mg/dL), as measured by a fasting morning blood draw (Gozal et al., 2007). It is
unclear, however, whether specific cognitive domains were more strongly associated with elevated CRP than others. Furthermore, while the association between cardiometabolic aberrations and inflammation has been demonstrated in many studies of patients with OSA, none have reported whether elevated inflammation may serve as a sensitive biomarker of the medical severity of the disorder in either adults or children, as this study of cognition in OSA suggests.

In sum, individuals with obesity and those with OSA share nearly identical phenotypes, as well as overlapping inflammatory mechanisms in promoting cardiometabolic and neurocognitive aberrations. Research focused on disentangling the “chicken or egg” relationship between OSA and the metabolic syndrome will have strong implications in OSA prognosis and treatment.
1.4. GAP IN THE LITERATURE

In the interminable quest to identify novel modifiable risk factors for cardiovascular disease and type 2 diabetes – which contribute to or account for nearly half of all deaths in the U.S. (Mensah & Brown, 2007) – the notion that OSA causes the metabolic syndrome is attractive. Indeed, most textbooks, original research papers, literature reviews, and research proposals on the topic currently detail a simplistic linear model in which hypoxia-induced inflammation results in cardiovascular and metabolic sequelae. Given the significant overlap in phenotypes, subclinical biomarkers, and health outcomes in obese non-apneics and apneics (who are often also obese), however, the model cannot be so simple. No study to date has attempted to tease apart the relative contribution of obesity and obesity-associated biomarkers in the development of OSA, nor how these biomarkers may reflect the cardiometabolic or neurocognitive severity of the disorder.

Studying OSA in adolescents presents a unique advantage. For one, because the vast majority of childhood OSA cases do not persist into adolescence (Spilsbury et al., 2015; Bixler et al., 2016), adolescent OSA is often new-onset and more likely to persist young adulthood, thus capturing a unique perspective into the early pathophysiology of the disorder. Furthermore, no longitudinal general population cohorts with objective sleep data in this age group also have also attained precise measures of body fat composition via DXA scan, nor longitudinal inflammation data from childhood. Thus, the work of the Penn State Child Cohort presented in this dissertation may provide a new viewpoint and future directions for studying the pathophysiology, prognosis, and potential treatment for OSA in children, adolescents, and perhaps even adults.
1.4.1. Vicious Cycle

In 2005, Vgontzas et al. put forth a feed-forward model (Figure 1.13) in which visceral obesity and insulin resistance contribute to increased inflammation as well as impaired diaphragm and airway motility. Subsequently, OSA and resulting sleep fragmentation contribute to sympathetic activation and further inflammation, which function to exacerbate central obesity. In short, obesity sets off a vicious cycle in which OSA is not an endpoint, but rather contributes to a progressive worsening of related sequelae.

**Figure 1.13.** Heuristic model of the feed-forward association between visceral fat, insulin resistance, inflammatory cytokines, sleep apnea, and other associated components. *Source: Vgontzas et al., 2005.*
1.4.2. Study Aim and Hypothesis

The overall aim of the studies presented in this dissertation is to explore the relative contributions of obesity and inflammation in the development of OSA. Specifically, based on consistent evidence demonstrating that adipose tissue – particularly visceral fat – is a major source of inflammation in the body, we explored the following five hypotheses in a large general population sample of adolescents, the Penn State Child Cohort:

1. Similar to adults, OSA in adolescents is associated with elevated visceral fat (Chapter 3).

2. Inflammation is associated with obesity in adolescents, and is elevated in OSA independent of obesity (Chapter 4).

3. Inflammation mediates (explains) a significant proportion of the relationship between visceral obesity and OSA in adolescents (Chapter 5).

4. Elevated inflammation during childhood predicts OSA and cardiometabolic aberrations in adolescence (Chapter 6).

5. Inflammation is a marker of the cardiometabolic and cognitive severity of OSA in adolescents (Chapter 7).
Chapter 2

METHODS
2.1. PENN STATE CHILD COHORT

2.1.1. Baseline Recruitment

The Penn State Child Cohort (PSCC) is a representative cohort of 700 children originally established in the early 2000s. The baseline recruitment was designed as a two-phase study. In the Phase I, elementary schools (kindergarten through grade 5) were selected so that ~1,500 students were enrolled per year. A questionnaire and consent form completed by parents was sent home with every child based on a survey (Ali et al., 1993) validated to identify children at high risk for SDB, with added questions assessing height, weight, age, gender, race, and ethnicity. Over five years, all 18 elementary schools within three districts of Dauphin County, Pennsylvania were assessed, with 5,740 of 7,312 total questionnaires returned (78.5% response rate).

During Phase II, 200 children selected from the questionnaires returned each year. Using stratification for grade level, gender, and risk for SDB (according to the parents’ responses), children were randomly selected from each stratum to maintain the representative nature of the original sample. After five years, the final sample included 700 children with a response rate of 70%. Each child spent one night in our Clinical Research Center (CRC, Penn State University College of Medicine, Hershey, PA, USA) with a parent. Each child completed detailed physical and psychometric evaluations. The study protocol was approved by the Penn State University College of Medicine Institutional Review Board. Written informed consents were obtained from participants’ parents or legal guardians.
Basic demographic, physical, and sleep characteristics of the baseline sample are detailed in Table 2.1. Participants identified their race/ethnicity from one of six options; “ethnic minority status” was re-defined as “Caucasian/non-white” for statistical purposes.

Table 2.1. Demographic characteristics of children who participated in the Penn State Child Cohort baseline study.

<table>
<thead>
<tr>
<th>Penn State Child Cohort (baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Females (%)</td>
</tr>
<tr>
<td>Ethnic minority (%)</td>
</tr>
<tr>
<td>BMI percentile</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
</tr>
<tr>
<td>Apnea-hypopnea index (events/h)</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM).

2.1.2. Follow-up Recruitment

The 700 participants were invited to return for another visit to the CRC as adolescents after an average of 8.4 years. Between 2010 and 2014, 421 of the original 700 participants completed a follow-up examination, yielding a 60.1% response rate. The loss to follow-up was mainly due to subjects moving out of the central Pennsylvania area. Basic demographic, physical, and sleep characteristics of the follow-up sample are detailed in Table 2.2. No differences in the baseline demographic characteristics were observed between subjects who participated in the follow-up examination and those who did not (Table 2.3). Written informed consents were obtained from participants, as well as their parents or legal guardians if younger than 18 years.
Table 2.2. Demographic characteristics of adolescents who participated in the Penn State Child Cohort follow-up study.

<table>
<thead>
<tr>
<th></th>
<th>Penn State Child Cohort (follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>421</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17.0 (0.1)</td>
</tr>
<tr>
<td>Females (%)</td>
<td>46.1</td>
</tr>
<tr>
<td>Ethnic minority (%)</td>
<td>21.9</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>65.3 (1.4)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.4 (0.7)</td>
</tr>
<tr>
<td>Apnea-hypopnea index (events/h)</td>
<td>2.7 (0.3)</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM).

Table 2.3. Baseline characteristics of children who did and did not participate in the Penn State Child Cohort follow-up study as adolescents.

<table>
<thead>
<tr>
<th></th>
<th>Participated in follow-up</th>
<th>Did not participate in follow-up</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>421</td>
<td>279</td>
<td>0.53</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.2 (0.8)</td>
<td>9.1 (0.1)</td>
<td>0.29</td>
</tr>
<tr>
<td>Females (%)</td>
<td>46.1</td>
<td>50.2</td>
<td>0.16</td>
</tr>
<tr>
<td>Ethnic minority (%)</td>
<td>21.9</td>
<td>26.5</td>
<td>0.89</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>63.3 (1.4)</td>
<td>63.0 (1.8)</td>
<td>0.40</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>28.9 (0.2)</td>
<td>28.9 (0.3)</td>
<td>0.92</td>
</tr>
<tr>
<td>Apnea-hypopnea index (events/h)</td>
<td>0.7 (0.1)</td>
<td>0.9 (0.1)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM).

2.2. HEALTH HISTORY AND PHYSICAL ASSESSMENT

At both baseline and follow-up, physical examinations were completed in the afternoon before each participant’s night in the sleep laboratory. Height was measured in centimeters using a stadiometer (SECA Corp., Hanover, MD, USA) and weight assessed in kilograms (Cardinal Scale Manufacturing, Webb City, MO, USA). Age- and gender-adjusted body mass index (BMI) percentile was calculated based on the formula and data from the 2000 U.S. Centers for Disease Control and Prevention growth charts (Kuczmarski et al., 2002). A demography was also
administered by a trained technologist or medical student, and a parent filled out a family health history questionnaire.

Waist circumference was measured in centimeters at the top of the iliac crest, and neck circumference was measured at the cricothyroid membrane.

Pubertal development (Tanner staging) was determined via a self-administered rating scale (Carskadon & Acebo, 1993).

Blood pressure was measured using an automated system (Vital Signs Monitor; Welch Allyn, Skaneateles Fall, NY, USA). The averages of three measures each of systolic and diastolic blood pressure were recorded in the seated, supine, and standing position. Systolic and diastolic blood pressure reactivity was calculated as the difference in blood pressure in the standing minus the supine position; these measures capture the rapid response of blood pressure to gravity changes, which has been associated with higher risk of hypertension, cardiovascular disease, and diabetes in adults (Ewing et al., 1985; Nardo et al., 1999). Mean arterial pressure (MAP), defined as arterial pressure during a single cardiac cycle, was defined as \( \frac{(\text{systolic blood pressure} - \text{diastolic blood pressure})}{3} + \text{diastolic blood pressure} \).

During the follow-up examination only, participants underwent whole-body dual-energy X-ray absorptiometry (DXA) scans to measure the distribution of adipose tissue in the abdominal region (Hologic Discovery W; Hologic Inc., Waltham, MA, USA). In this method, two beams of low-energy X-ray pass are attenuated through body tissue, then collected by detectors. Soft tissue is resolved using mass attenuation coefficients derived from tissue equivalent standards for fat-free versus fat tissues. Android (waist), gynoid (hips), visceral adipose tissue, subcutaneous adipose tissue, and total adipose tissue (sum of visceral and subcutaneous adipose tissue) areas were selected as regions of interest and identified by Hologic APEX 4.0 software (Hologic Inc.,
Bedford, MA, USA) then verified by an experienced investigator. Methods for defining each region of interest are detailed elsewhere (Kelly et al., 2010). Quality control and calibration were performed daily on the DXA scanner to ensure data validity.

2.3. SLEEP LABORATORY PROTOCOL

At both baseline and follow-up examinations, all participants underwent a single 9-hour polysomnography (PSG) recording in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was continuously monitored from 22:00 h until 7:00 h using 7-channel recordings of electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG). Respiration was monitored via nasal pressure (Pro-Tech PTAF Lite; Mukilteo, WA), thermocouple (Salter Labs; Lake Forest, IL), and thoracic/abdominal strain gauges (Model 1312, Sleepmate Technologies; Midlothian, VA). Hemoglobin oxygen saturation (SpO2) was assessed using a pulse oximeter placed on the index finger (Model 3011 Xpod, Nonin Medical, Inc.; Plymouth, MN). Snoring sounds were monitored via a sensor attached to the throat. All data were recorded using Twin Recording & Analysis software (Grass-Telefactor; West Warwick, RI). Visual sleep stage scoring was conducted by a single registered polysomnography technologist according to standardized criteria (Rechtshaffen & Kales, 1968).
2.3.1. Measuring Obstructive Apneas and Hypopneas

Apnea/hypopnea index (AHI; number of apneas and hypopneas summed per hour) was ascertained. An obstructive apnea was defined as a cessation of airflow with a minimum duration of 5 seconds (for those aged < 16 years) or 10 seconds (for those ≥ 16 years) and an associated out-of-phase strain gauge movement; a hypopnea was characterized by a reduction of airflow by approximately 50% with an associated decrease in SpO₂ of at least 3% or an associated EEG arousal (Iber et al., 2007).

2.4. MEASURES OF INFLAMMATION AND METABOLIC HEALTH

Of 421 participants at follow-up, 392 (93.1%) provided a fasting blood sample upon awakening at 7:00; 56 of the 421 (13.3%) had also provided a blood sample at baseline.

Blood samples were collected in an EDTA-containing tube and spun for 10 minutes at 3000 RPM. Plasma was aliquoted into cryotubes and stored at -80°C until assayed. Plasma interleukin-6 (IL-6), interleukin-6 soluble receptor (IL-6 sR), tumor necrosis factor alpha (TNFα), tumor necrosis factor receptor superfamily member 1A (TNFR1), high-sensitivity C-reactive protein (CRP), leptin, and adiponectin were measured via enzyme-linked immunosorbent assay (ELISA; R&D Systems; Minneapolis, MN). Briefly, diluted specimens and controls solutions were incubated on a 96-well plate, followed by separate incubation times with diluted conjugate solution, substrate solution to produce a colorimetric change, and stop solution. Immediately after application of the stop solution, the color change was detected using a Multiskan FC microplate reader (Thermo Scientific; Walthan, MA, USA) with filters set at
450nm (IL-6 sR, TNFR1, CRP, leptin, adiponectin) or 490nm (IL-6, TNFα). Cytokine concentrations were quantified for each microwell based on a standard curve of known calibrator concentrations. The intra- and interassay coefficients of variation were 4.7% and 5.1%, respectively (IL-6), 5.4% and 6.2% (IL-6 sR), 4.6% and 4.9% (TNFα), 5.1% and 6.4% (TNFR1), 5.8% and 5.3% (CRP), 6.5% and 7.0% (leptin), and 5.6% and 5.6% (adiponectin). The lower detection limits were 0.039 pg/mL (IL-6), 6.5 pg/mL (IL-6 sR), 0.106 pg/mL (TNFα), 0.77 pg/mL (TNFR1), 0.010 ng/mL (CRP), 7.2 pg/mL (leptin), and 0.25 ng/mL (adiponectin). All samples and standards were run in duplicate.

Additional fasting blood samples were sent to Quest Diagnostics™ for a complete health profile, including measures of glucose, insulin, HDL cholesterol, and triglycerides. Insulin resistance, as measured by homeostatic model assessment (HOMA), was calculated as (glucose x insulin) / 405. To be consistent with adult metabolic syndrome criteria, the continuous metabolic syndrome score (cMetS) was defined as the sum of Z-transformed, age- and gender-adjusted waist circumference, MAP, HOMA, HDL cholesterol (multiplied by -1), and triglycerides, after each of the five components was adjusted for age and gender (Eisenmann et al., 2010). A higher cMetS score is indicative of a less favorable cardiometabolic profile and greater metabolic syndrome burden.

2.5. NEUROCOGNITIVE TESTING

At both baseline and follow-up, all participants underwent a 2.5-hour neurocognitive and neuropsychological evaluation prior to their overnight stay. The tests were administered individually to each child by a trained psychometrist over a single session, and scores were
converted to age-standardized scores based on published normative data. In general, the tests measured intelligence, and neurocognitive functioning including attention, memory, processing speed, verbal fluency, mental flexibility, visual-motor skills, and academic achievement.

_Wechsler Abbreviated Scales of Intelligence_. The WASI consists of four subtests (block design, matrix reasoning, vocabulary, similarities) that correspond to the subtests on the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV).

_Gordon Diagnostic System_. The Vigilance and Distractibility subtests of the Gordon Diagnostic System (Gordon, 1983) were administered. The Vigilance Task yields data regarding an individual's ability to focus and maintain attention over time and in the absence of feedback. In this subtest, a series of digits flash, one at a time, on the electronic display of a portable microprocessor. The Distractibility subtest flashes irrelevant digits on either side of the column that displays the target stimuli. The subject is told to press a button every time a "1" is followed by a "9," and the number of correct responses, incorrect responses, and failures to respond to the "1/9" combination are recorded. There is significant agreement between subtest scores on the Gordon Diagnostic subtests with behavior rating scales, standardized observations, attentional performance tests, and diagnoses of ADHD (Mariani & Barkley, 1997).

_Digit Span_. The WISC-IV/WAIS-III Digit Span subtest requires the participant to repeat number sequences after the evaluator in forward and reverse order, with an increasing number of digits each round. Digit Span is a measure of attention, working memory, and concentration.

_Coding – Digit Symbol_. The WISC-IV/WAIS-III Coding subtest presents symbols paired with numbers. The participant has 120 seconds to go through a grid of numbers and draw the correct symbol below each one. The task assesses visual-motor speed and coordination.
Symbol Search. The WISC-IV/WAIS-III Symbol Search presents several rows of symbols. To the right of each row, two additional symbols are presented. The participant has 120 seconds to go through and determine whether or not at least one of the two symbols are the right are also depicted in the series of symbols to the left. The task assesses visual-motor speed and accuracy.

Wide Range Achievement Test. The Wide Range Achievement Test 3 (WRAT3) measures an individual’s ability to read words and compute solutions to math problems (Wilkinson & Robertson, 2006). The test is designed to assess basic academic skills necessary for effective learning, thinking, and communication.

Developmental Test of Visual-Motor Integration. The Developmental Test of Visual-Motor Integration, 5th edition (VMI-5) is an untimed test in which the participant copies geometric forms of increasing difficulty with a pencil without erasing. The VMI has validity as a measure of dysgraphia or handwriting difficulty (Maeland, 1992), and children with neurological disorders (autism, ADHD) earn relatively lower scores on the test (Mayes & Calhoun, 2003).

California Verbal Learning Test. The California Verbal Learning Test (CVLT) is a test of episodic verbal learning and memory that includes immediate and delayed recall. Participants are asked to recall as many words as possible from a list of 15 items read by the evaluator five times, the final time being after a 20-minute break.

Stroop Color and Word Test. The Stroop Color and Word Test is a measure of executive functioning – the ability to shift cognitive ability to inhibit a dominant response in favor of an unusual one (Golden et al., 2003). There are three sections to the test. In the first section, the participant reads a list of colors written in black ink. In the second section, the participant reads the color of “XXX”es written in colored ink. In the final section, the participant is asked to list
the color of ink for each word, though the word itself does not match the ink color. The participant is timed during each section, and an interference score is calculated. The Stroop effect has been replicated over 700 times due to its sensitivity to individual differences (McLeod, 1991).

2.6. ADDITIONAL MEASURES

Actigraphy and Daily Logs

After their night in the laboratory, each participant was sent home with an ActiGraph (GT3X; ActiGraph Corp., Pensacola, FL, USA) that was worn on the wrist (nighttime) or belt (daytime) for the next seven days. The ActiGraph contains X-Y-Z acceleration sensors to detect movement and yields a “typical” assessment of sleep and physical activity away from the laboratory. Activity data was downloaded onto a PC and total sleep time, sleep onset latency, and sleep efficiency were determined based on self-reported daily sleep and activity logs.

Ambulatory Holter Electrocardiography

A high-fidelity (Hz sampling frequency) 12-lead HScribe Holter system (Mortara Instrument, Inc.; Milwaukee, WI, USA) collected 39-hour beat-to-beat electrocardiogram (EKG) data. All Holter recordings started around 17:00 during the evening of the in-laboratory sleep study and were collected from the participants’ homes two days later during the morning. A trained investigator retrieved and archived beat-to-beat data for offline processing, including
verifying Holter-identified EKG waves and labeling additional artifacts and arrhythmias. Heart rate variability (HRV) analysis was performed using beat-to-beat (RR) interval data.

**Salivary Cortisol**

At 19:00 the night of and 7:00 the morning after the in-laboratory sleep recording, participants chewed a piece of cotton for 30 seconds (or until soaked) and deposited it into a Salivette vial (Sarstedt; Nümbrecht, Germany) to obtain two saliva samples. Samples were immediately transferred to -20°C for storage until analysis. The 12-hour difference in morning/evening collection was designed to capture the typical circadian secretion pattern of cortisol, which is lowest in the evening and highest upon awakening (Rose et al., 1972). Salivettes were centrifuged at 1000 RPM for two minutes then assayed via ELISA (ALPCO Diagnostics; Salem, NH, USA). Immediately after application of the stop solution, the color change was detected using a Multiskan FC microplate reader (Thermo Scientific; Walthan, MA, USA) with a filter set at 450nm. Cortisol concentrations were quantified for each microwell based on a standard curve of known calibrator concentrations. All samples and standards were run in duplicate.

**Questionnaires**

After their night in the sleep laboratory, participants were sent home with a packet of surveys that were returned a week later with their ActiGraph.
**Behavior Rating Scales.** The 113-item Child Behavior Checklist (CBCL; filled out by parents for participants younger than 18 years) or the 126-item Adult Behavior Checklist (ABCL; filled out by parents for participants if at least 18 years or older) was given. Responses are recorded on a Likert scale (0 = Not True, 1 = Somewhat or Sometimes True, 2 = Very Often or Very True) and refer to the child’s behavior (e.g. “Acts too younger for his or her age.”) The scales are used as a diagnostic tool for a variety of behavioral, social, and emotional problems, including conduct disorder, separation anxiety, and phobias.

The 165-item Pediatric Behavior Scale (PBS) (Lindgren & Koeppl, 1987) was completed by parents of participants younger than 18 years and assessed, broadly, attention/hyperactivity, conduct, learning problems, mood, anxiety, social skills, and somatic complaints.

**Sleep Quality Questionnaires.** For participants younger than 18 years, a parent filled out the 26-item General Sleep Information questionnaire, the 43-item Children’s Sleep Wake Scale, and the 25-item Children’s Sleep Hygiene Scale (The University of Southern Mississippi Sleep Research Laboratory), which assessed the participants’ behaviors associated with going to bed or waking up in the middle of the night, bedtime and wake time, the child’s bedroom environment, and related other questions. The Pediatric Sleep Questionnaire (PSQ) (Chervin et al., 2000) was also completed by parents of participants younger than 18 years and assessed a variety of sleep-related behaviors and complaints, notably the presence of excessive daytime sleepiness (EDS) as observed by the parent or a teacher.

All participants filled out the Sleep Quality Questionnaire, a short 8-item survey (yes, no don’t know) assessing sleep quality and daytime sleepiness. The Morningness-Eveningness Questionnaire (Horne & Ostberg, 1976) is a multiple choice self-assessment designed to measure chronotype, or “circadian peak time” (morning, evening, or intermediate). Questions assess
preferred bed/wake time, preferred exercise and test-taking time of day, and general feelings of tiredness or refreshment before bed and after waking.

Before bed and after awakening during their time in the sleep laboratory, participants also filled out a brief one-page Scantron-style questionnaire assessing their sleepiness before and sleep quality during the study.

**Physical Activity and Diet Questionnaires.** Participants filled out a Scantron-style Activity Survey assessing participants’ seasonal or year-round participation in particular sports or physical activities. A more detailed Physical Activity Survey assessed the number of hours spent on physical (sports, exercise, etc.) and sedentary (television, homework time, etc.) activities on a typical weekday, typical weekend, and in a typical week, as well as general questions related to lifestyle and attitude toward physical activity. Finally, a detailed diet questionnaire (Harvard Medical School) assessed foods, drinks, vitamins, and added sugars typically consumed daily, weekly, and over a period of several months.

**Epigenetics Study**

During the consent procedure, participants could elect to have an extra tube of blood drawn in the morning for storage and epigenetic (DNA methylation) analysis. Parents also had the option of donating blood for potential future genetic studies. DNA was extracted from peripheral leukocytes and subjected to bisulfite sequencing, which provided single nucleotide resolution of DNA methylation at CpG sites and surrounding regions. The R package methylKit was used in post-processing and analysis to determine sites with $\geq 25\%$ difference in methylation and a q-value (proportion of false positives) $< 0.01$, which were defined, *a priori*, as substantially
differential methylated sites. Genes associated with obesity, sleep-disordered breathing, and other conditions could then be determined and compared.
Chapter 3

OBSTRUCTIVE SLEEP APNEA IN ADOLESCENTS IS ASSOCIATED WITH ELEVATED VISCERAL FAT
3.1. RATIONALE & HYPOTHESIS

Visceral (abdominal) fat is a metabolically active organ. Unlike subcutaneous fat (fat located just below the hypodermal layer), immune dysregulation by resident visceral fat macrophages has been linked to impaired glucose intake and other cardiometabolic morbidities (Hotamisligil et al., 1995). Notably, compared to age- and BMI-matched controls, visceral fat area in obese (Vgontzas et al., 2000; Vgontzas et al., 2008) and even non-obese (Kritikou et al., 2013) men as measured by CT scan is elevated in those with OSA.

In children, there is a lack of agreement regarding clinical and polysomnographic criteria for treatment initiation. It is commonly assumed that most cases of SDB (including OSA and snoring) in children are due to anatomic abnormalities or the presence of enlarged tonsils and adenoids. In fact, SDB is more commonly observed in obese compared to non-obese children (Bixler et al., 2009; Bhattacharjee et al., 2010; O’Brien et al., 2006; Redline et al., 1999). In the Penn State Child Cohort, childhood waist circumference, an indirect measure of abdominal obesity, is a significant predictor of incident SDB in adolescence, and both waist circumference and the more precise DXA-measured visceral fat area are strongly associated with SDB in adolescence cross-sectionally (Bixler et al., 2016). In a study combining the Penn State Child Cohort at baseline with children and adolescents from the Sleep Research & Treatment Center, the synergistic relationship of SDB with overweight and obesity was associated with adverse effects on metabolic health, including inflammation, elevated leptin, reduced adiponectin, and EDS (Tsaoussoglou et al., 2010).

Given the well-established association of visceral fat with OSA in adults and the role of waist circumference in predicting incident SDB from childhood to adolescence, we examined the
cross-sectional association of OSA with DXA-measured visceral fat in adolescents. We hypothesized that, as in adults, visceral fat area was significantly elevated in adolescents with OSA (AHI ≥ 5).

3.2. STATISTICAL ANALYSIS

Participants were categorized into three groups based on previous criteria (Bixler et al., 2016; Redline et al., 2011): no OSA (AHI < 2), mild OSA (2 ≤ AHI < 5) and OSA (AHI ≥ 5). Differences in sociodemographic and physical characteristics in the three groups were assessed using analysis of variance (ANOVA). ANOVA also assessed differences in polysomnographic and subjective sleep characteristics, adjusting for age, gender, BMI percentile, and ethnic minority (non-white/Caucasian) status.

To assess the impact of various measures of body fat composition on OSA, linear regression analyses were performed examining the contributions of BMI percentile, waist circumference, android/gynoid fat ratio, subcutaneous fat area, and visceral fat area separately on AHI, adjusting for age, gender, and ethnic minority status.

Analysis of covariance (ANCOVA) then assessed differences in z-transformed visceral fat and subcutaneous fat area separately between the three OSA groups, adjusting for the confounders age, gender, ethnic minority status, and total body fat area. To assess gender differences, visceral fat area was also examined separately in males and females within each of the three OSA groups, adjusting for age and ethnic minority status. Bonferroni correction was applied to adjust for multiple comparisons.
3.3. RESULTS

Of the 421 participants who followed up as adolescents, 27.3% had mild OSA (2 ≤ AHI < 5) and 10.5% had OSA (AHI ≥ 5), while 62.2% had no OSA (AHI < 2). Compared to those with no OSA, those with at least mild OSA tended to have a higher BMI percentile, waist circumference, more visceral fat area, and were more likely to be male and ethnic minority (non-white) (Table 3.1). Additionally, those with OSA were in a significantly later Tanner (pubertal) stage than the other two groups, though this was primarily driven by older age. Adolescents with OSA also had significantly more visceral fat area (p < 0.01) than even the mild OSA group. Only the OSA group had significantly more total fat area (p < 0.01) and subcutaneous fat area (p < 0.05) compared to the no OSA group.

Table 3.1. Sociodemographic and body fat composition values, stratified by apnea severity.

<table>
<thead>
<tr>
<th></th>
<th>No OSA</th>
<th>Mild OSA</th>
<th>OSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>262 (62.2)</td>
<td>115 (27.3)</td>
<td>44 (10.5)</td>
</tr>
<tr>
<td>AHI</td>
<td>0.9 (0.3)</td>
<td>3.1 (0.4)c</td>
<td>12.1 (0.7)c</td>
</tr>
<tr>
<td>Male (%)</td>
<td>45.5</td>
<td>67.0c</td>
<td>70.5b</td>
</tr>
<tr>
<td>Ethnic minority (%)</td>
<td>17.2</td>
<td>31.3b</td>
<td>21.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.5 (0.1)</td>
<td>17.3 (0.2)b</td>
<td>18.4 (0.3)c</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>4.1 (0.1)</td>
<td>4.2 (0.1)</td>
<td>4.4 (0.1)a</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>60.9 (1.7)</td>
<td>70.1 (2.6)b</td>
<td>79.0 (4.2)c</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.4 (0.8)</td>
<td>83.1 (1.2)£</td>
<td>90.8 (1.9)£</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>91.0 (0.9)</td>
<td>90.2 (1.3)</td>
<td>90.9 (2.1)</td>
</tr>
<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>204.7 (10.1)</td>
<td>225.1 (15.3)</td>
<td>261.8 (24.4)a</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>52.0 (2.4)</td>
<td>67.3 (3.7)b</td>
<td>87.2 (5.9)£</td>
</tr>
<tr>
<td>Total fat area (cm²)</td>
<td>256.7 (12.0)</td>
<td>292.4 (18.4)</td>
<td>349.0 (29.4)b</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM). No OSA = AHI < 2; mild OSA = 2 ≤ AHI < 5; moderate OSA = AHI ≥ 5. BMI percentile = body mass index percentile. *p < 0.05 vs. no OSA; †p < 0.01 vs. no OSA; ‡p < 0.001 vs. no OSA; ‡‡p < 0.1 vs. mild OSA; ‡§p < 0.01 vs. mild OSA; ‡¶p < 0.001 vs. mild OSA.
There were no significant differences between polysomnographic or subjective (complaint of EDS and insomnia) sleep characteristics across groups, adjusting for age, gender, BMI percentile, and ethnic minority status (Table 3.2). There was, however, a trend toward more stage 2 sleep (p = 0.12) and lower slow-wave sleep (p = 0.11) in the OSA group versus those without OSA.

### Table 3.2. Polysomnographic and subjective sleep characteristics, stratified by apnea severity.

<table>
<thead>
<tr>
<th></th>
<th>No OSA</th>
<th>Mild OSA</th>
<th>OSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time (min.)</td>
<td>448.3 (3.5)</td>
<td>445.9 (5.3)</td>
<td>440.2 (8.7)</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>82.9 (0.6)</td>
<td>82.5 (1.0)</td>
<td>81.5 (1.6)</td>
</tr>
<tr>
<td>Sleep onset latency (min.)</td>
<td>25.7 (1.6)</td>
<td>27.4 (2.3)</td>
<td>25.8 (3.9)</td>
</tr>
<tr>
<td>Wake after sleep onset (min.)</td>
<td>68.6 (2.7)</td>
<td>68.6 (4.1)</td>
<td>76.0 (6.8)</td>
</tr>
<tr>
<td>Total wake time (min.)</td>
<td>92.6 (3.5)</td>
<td>94.0 (5.2)</td>
<td>99.2 (8.6)</td>
</tr>
<tr>
<td>Stage 1 (%)</td>
<td>1.0 (0.1)</td>
<td>1.1 (0.1)</td>
<td>0.1 (0.2)</td>
</tr>
<tr>
<td>Stage 2 (%)</td>
<td>53.0 (0.6)</td>
<td>53.9 (0.9)</td>
<td>55.4 (1.4)</td>
</tr>
<tr>
<td>Slow-wave sleep (%)</td>
<td>27.5 (0.5)</td>
<td>26.5 (0.8)</td>
<td>25.2 (1.3)</td>
</tr>
<tr>
<td>REM (%)</td>
<td>18.5 (0.3)</td>
<td>18.6 (0.5)</td>
<td>18.4 (0.8)</td>
</tr>
<tr>
<td>EDS complaint (%)</td>
<td>65.3</td>
<td>64.3</td>
<td>72.7</td>
</tr>
<tr>
<td>Insomnia complaint (%)</td>
<td>34.7</td>
<td>39.1</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM). Adjusted for age, gender, BMI percentile, and ethnic minority status. No OSA = AHI < 2; mild OSA = 2 ≤ AHI < 5; moderate OSA = AHI ≥ 5. REM = rapid eye movement sleep; EDS = excessive daytime sleepiness.

Various measures of body fat were then entered into separate linear regression models to predict AHI, adjusting for age, gender, and ethnic minority status (Figure 3.1). Visceral fat area was most strongly associated with AHI (β = 0.17, p = 0.001), followed by waist circumference (β = 0.15, p = 0.002), BMI percentile (β = 0.14, p = 0.003) and subcutaneous fat area (β = 0.13, p = 0.02).
**Figure 3.1.** Comparison of body fat predictors for apnea/hypopnea index, adjusting for age, gender, and ethnic minority status. BMI percentile = body mass index percentile; AHI = apnea/hypopnea index. Standardized β for predicting AHI reported. * p < 0.05; ** p < 0.01.

When honing in on body fat distribution in OSA, visceral fat ($F_{2,384} = 5.63, p = 0.004$) and subcutaneous fat ($F_{2,384} = 5.63, p = 0.004$) differed between groups. Specifically, z-transformed visceral fat area was significantly highest in the OSA group ($0.15 \pm 0.05$ cm$^2$, $p < 0.01$ vs. all groups) while subcutaneous fat was lowest in the OSA group ($-0.04 \pm 0.01$, $p < 0.01$ vs. all groups), adjusting for age, gender, ethnic minority status, and total fat area (Figure 3.2).
Given known gender differences in body fat composition in adults, we then examined gender differences in visceral fat and OSA in adolescents (Figure 3.3). Overall, adjusting for age and ethnic minority status, visceral fat area was significantly higher in boys (74.29 ± 0.83 cm²) compared to girls (42.77 ± 0.90 cm², p < 0.001). Specifically, in boys, visceral fat area was significantly elevated in the OSA group (87.60 ± 6.38 cm²) compared to no OSA (54.44 ± 3.31 cm², p < 0.001) and mild OSA (62.76 ± 4.08 cm², p = 0.003; F_{2,206} = 10.30 overall). In girls, while visceral fat area was lowest in those with no OSA (53.09 ± 3.50 cm²), they was only a trend difference compared to the mild OSA group (70.68 ± 7.21 cm², p = 0.092; F_{2,175} = 2.79 overall). This was likely driven by variance due to the low sample size (n =12) in girls with OSA, even though the mean visceral fat area was very similar to those with mild OSA (69.36 ± 12.11 cm², p = 0.20 vs. no OSA).
Figure 3.3. Visceral fat area in boys (light gray bars) and girls (dark gray bars) by apnea severity. Adjusted for age and ethnic minority status. Error bars represent SEM. † p < 0.1; ** p < 0.01. Boys: n = 110 with no OSA; n = 72 with mild OSA; n = 20 with OSA. Girls: n = 136 with no OSA, n = 32 with mild OSA, n = 12 with OSA.

3.4. DISCUSSION

Research over the last two decades has begun to hone in on the metabolic impact of fat tissue localization on endocrine and immune function. While all adipocytes produce hormones, cytokines, and other proteins involved in energy homeostasis and cardiovascular function, visceral adipose tissue, in particular, is associated with more severe effects on health compared to subcutaneous adipose tissue. Packed between organs of the abdominal cavity, increased visceral adiposity is associated with elevated risk of developing insulin resistance and Type II diabetes, dyslipidemia, hypertension, cerebro- and cardiovascular disease, and early death (Montague and O’Rahilly, 2000; Kern et al., 2001; Chan et al., 2004; Kuk et al., 2006; Chibu et al., 2007; Karcher et al., 2013). In children and adolescents, there is ample evidence to suggest
that poor diet, lack of physical activity (Stallmann-Jorgensen et al., 2007), genetic factors (Rice et al., 1997; Katzmarzyk et al., 1999), and male gender (Goran et al., 1999) are significant predictors of elevated visceral fat area.

We report a strong association of visceral fat with OSA in boys, but not girls (Figure 3.3). Interestingly, this finding is in accordance with our previous work in middle-aged men and post-menopausal women demonstrating that OSA is strongly associated with visceral fat in males, but global adiposity (total fat area) in females (Kritikou et al., 2013). A recent follow-up of the Cleveland Children’s Sleep and Health Study reported that obesity in childhood predicts OSA in adolescence (Spilsbury et al., 2015), and a follow-up report in the present cohort found that childhood waist circumference and greater $\Delta$BMI percentile from baseline to follow-up predicted OSA in adolescence (Bixler et al., 2016). Similarly, a recent small pilot study in adolescents ($n = 20$) reported an association of AHI with visceral fat area, but not with BMI or subcutaneous fat area (Hannon et al., 2011). This study, however, only included obese adolescents, and potential sex differences in body fat composition or SDB prevalence were not explored.

The link between visceral adiposity and OSA is consistent with decades of research and clinical observation on obesity hypoventilation syndrome. Obesity – particularly central obesity – has been associated with an overall depression in ventilatory control mechanisms. Excess adipose tissue restricts the normal movement of chest and diaphragm muscles, causing them to become more fatigued due to the increased energy needed to move air in and out of the lungs. Furthermore, accumulation of excess fat in the head and neck region narrows the upper airway. Elevated levels of certain growth factors due to obesity may also contribute to soft tissue edema in the neck. Over the long-term, hypoxia can lead to pulmonary vasoconstriction, or the tightening of the small blood vessels of the lung and increased pressure on the pulmonary artery;
this may also contribute to edema due to impaired heart pumping and poor circulation (Mokhlesi & Tulaimat, 2007).

Previous studies, primarily in adults, have proposed opposing views of the association of visceral adiposity, a component of the metabolic syndrome, with sleep apnea – specifically, visceral adiposity leads to OSA vs. OSA causes visceral adiposity (Vgontzas et al., 2005; Chin et al., 1999). The fact that we are observing a robust relationship between visceral adiposity and OSA, similar to adults (Vgontzas et al., 2000), (a) at this young age, and (b) in a non-clinical population further suggests the causative role of abdominal fat in this association. Of note, this is the only large study in this age group to include a more precise measure of obesity, such as a DXA scan, in addition to measures of BMI and waist circumference. In sum, these findings illustrate how visceral adiposity, as young as adolescence, is significantly elevated in OSA.
Chapter 4

INFLAMMATION IS ASSOCIATED WITH OBESITY, AND INDEPENDENTLY ELEVATED IN OBSTRUCTIVE SLEEP APNEA
4.1. RATIONALE & HYPOTHESIS

In adults, levels of proinflammatory cytokines, such as IL-6 and TNFα; acute-phase proteins, including CRP; and adipocyte hormones, like leptin, are positively correlated with body mass index (Vgontzas et al., 2000; Khaodhiar et al., 2004; Ostlund et al., 1996). Visceral fat, in particular, is a metabolically active organ (Einstein et al., 2005), with resident macrophages secreting high levels of cytokines (Fain, 2006). This immune dysregulation has been directly linked to impaired glucose uptake, endothelial dysfunction, and development of other cardiometabolic morbidities (Rask-Madsen et al., 2003; Senn et al., 2002; Jager et al., 2007).

Interestingly, and as seen in adults, elevated levels of CRP and IL-6 have also been reported in children (Tauman et al., 2004; Tauman et al., 2007; Gieles-Hillel et al., 2014) and adolescents (Larkin et al., 2005) with SDB, even after adjusting for BMI. Many studies examining the association between obesity, inflammation, and OSA, however, are small or conducted only in clinical patients.

The aim of this study was to examine the joint effect of body weight and OSA on inflammatory and metabolic markers in a general population of adolescents. We hypothesized that both OSA (AHI ≥ 5) and obesity (BMI ≥ 95th percentile), particularly visceral fat, would be associated with elevated inflammation and leptin, and reduced adiponectin levels.

4.2. STATISTICAL ANALYSIS

Blood samples were provided by 392 of the 421 adolescents (93.1%). Unadjusted mean values of the inflammatory and metabolic markers interleukin-6 (IL-6), interleukin-6 soluble receptor (IL-6 sR), tumor necrosis factor α (TNFα), tumor necrosis factor receptor 1 (TNFR1),
C-reactive protein (CRP), leptin, and adiponectin were calculated, split into no OSA (AHI < 2), mild OSA (2 ≤ AHI < 5), and OSA (AHI ≥ 5) groups.

The Kolmogorov-Smirnov test yielded significance for all markers (all p < 0.001), so data were base-10 logarithmically transformed. Next, mean values of log-transformed markers were assessed, splitting by apnea severity as well as body weight (lean = BMI < 85th percentile; overweight = 85th ≤ BMI percentile < 95th; obese = BMI ≥ 95th percentile), adjusting for age, gender, and ethnic minority status.

To explore the joint effect of both variables on cytokine concentrations, four groups were created based on overweight and mild-to-moderate OSA status: lean, no OSA (BMI < 85th percentile, AHI < 2); lean, AHI ≥ 2; overweight, no OSA (85th ≤ BMI percentile < 95th percentile, AHI < 2); and overweight, AHI ≥ 2. Bonferroni correction was applied to all ANCOVA analyses to adjust for multiple comparisons.

Finally, to examine the relative influence of OSA and body weight on cytokines, linear regression analyses were conducted using visceral fat area, subcutaneous fat area, and OSA (AHI ≥ 5) as predictors, adjusting for age, gender, and ethnic minority status. Standardized β are reported.

Because there was a large range in elapsed time between when blood samples were conducted and when they were assayed (range = 8 – 41 months), analyses were repeated with additional adjustment for time elapsed between sample collection and assay for each cytokine. To also rule out a possible influence of prolonged sleep apnea from baseline on inflammation in adolescence, analyses were also repeated excluding n = 6 who had AHI ≥ 5 at baseline, as well as adjusting for AHI at baseline. In both cases, results were not significantly affected after adjusting for these possible confounds, so they were not included in the final analysis.
4.3. RESULTS

When stratified by apnea severity (Table 4.1), raw values of IL-6, TNFα, and CRP were significantly elevated in adolescents with OSA compared to both no OSA and mild OSA groups (all $p < 0.01$), with a trend increase in TNFR1 in those with OSA compared to no OSA ($p = 0.074$). There was also a marginal increase in leptin in those with OSA compared to the other two groups (both $p < 0.1$). Adiponectin was significantly lower in the OSA group compared to the no OSA group ($p = 0.004$).

Table 4.1. Plasma concentrations of inflammatory cytokines and metabolic hormones, stratified by apnea severity.

<table>
<thead>
<tr>
<th></th>
<th>No OSA (n = 239)</th>
<th>Mild OSA (n = 109)</th>
<th>OSA (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.09 (0.06)</td>
<td>1.08 (0.09)</td>
<td>1.92 (0.14)$^{c,f}$</td>
</tr>
<tr>
<td>IL-6 sR (pg/mL)</td>
<td>44.45 (0.89)</td>
<td>44.18 (1.28)</td>
<td>41.79 (2.02)</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>1.85 (0.08)</td>
<td>1.83 (0.13)</td>
<td>2.48 (0.20)$^{b,e}$</td>
</tr>
<tr>
<td>TNFR1 (ng/mL)</td>
<td>12.51 (0.22)</td>
<td>12.56 (0.31)</td>
<td>13.48 (0.49)$^a$</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.69 (0.07)</td>
<td>0.96 (0.10)$^a$</td>
<td>2.10 (0.15)$^{c,f}$</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>12.23 (0.82)</td>
<td>11.88 (1.20)</td>
<td>16.18 (1.89)$^{d}$</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>8.36 (0.32)</td>
<td>7.30 (0.47)$^a$</td>
<td>6.02 (0.75)$^b$</td>
</tr>
</tbody>
</table>

Data presented as unadjusted mean (SEM). No OSA = AHI < 2; mild OSA = 2 ≤ AHI < 5; moderate OSA = AHI ≥ 5; IL-6 = interleukin-6; IL-6 sR = interleukin-6 soluble receptor; TNFα = tumor necrosis factor alpha; TNFR1 = tumor necrosis factor receptor 1; CRP = C-reactive protein. $^a p < 0.1$ vs. no OSA; $^b p < 0.01$ vs. no OSA; $^c p < 0.001$ vs. no OSA; $^d p < 0.1$ vs. mild OSA; $^e p < 0.01$ vs. mild OSA; $^f p < 0.001$ vs. mild OSA.

Even after adjusting for age, gender, and ethnic minority status (Table 4.2), levels of IL-6, TNFα, CRP, and leptin were significantly elevated in OSA compared to no OSA and mild OSA groups (all $p < 0.05$), with a trend reduction in adiponectin compared to no OSA ($p = 0.08$).

When stratified by body weight, the patterns were similar and, for some biomarkers, more pronounced. Compared to lean and overweight participants, obese adolescents (n = 61) had
significantly elevated IL-6, TNFα, TNFR1, CRP, leptin, and significantly reduced adiponectin (all p < 0.05). Levels of IL-6, CRP, and leptin were all elevated in overweight (n = 76) compared to lean (n = 255) participants as well (all p < 0.05).

Table 4.2. Plasma concentrations of inflammatory cytokines and metabolic hormones, stratified by apnea severity and body weight.

<table>
<thead>
<tr>
<th></th>
<th>No OSA (n = 239)</th>
<th>Mild OSA (n = 109)</th>
<th>OSA (n = 44)</th>
<th>Lean (n = 255)</th>
<th>Overweight (n = 76)</th>
<th>Obese (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log IL-6 (pg/mL)</td>
<td>-0.11 (0.03)</td>
<td>-0.10 (0.04)</td>
<td>0.16 (0.06)</td>
<td>-0.15 (0.02)</td>
<td>-0.03 (0.05)</td>
<td>0.16 (0.05)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log IL-6 sR (pg/mL)</td>
<td>3.71 (0.02)</td>
<td>3.75 (0.03)</td>
<td>3.70 (0.05)</td>
<td>3.73 (0.02)</td>
<td>3.75 (0.04)</td>
<td>3.76 (0.04)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log TNFα (pg/mL)</td>
<td>0.20 (0.02)</td>
<td>0.18 (0.03)</td>
<td>0.29 (0.04)</td>
<td>0.18 (0.02)</td>
<td>0.20 (0.03)</td>
<td>0.30 (0.04)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log TNFR1 (ng/mL)</td>
<td>2.50 (0.02)</td>
<td>2.50 (0.03)</td>
<td>2.55 (0.04)</td>
<td>2.49 (0.02)</td>
<td>2.47 (0.03)</td>
<td>2.58 (0.03)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log CRP (mg/L)</td>
<td>-0.30 (0.03)</td>
<td>-0.20 (0.40)</td>
<td>0.18 (0.06)</td>
<td>-0.31 (0.03)</td>
<td>-0.17 (0.05)</td>
<td>0.12 (0.05)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Leptin (ng/mL)</td>
<td>1.81 (0.07)</td>
<td>1.95 (0.10)</td>
<td>2.74 (0.15)</td>
<td>1.53 (0.05)</td>
<td>2.33 (0.09)</td>
<td>3.19 (0.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log Adiponectin (µg/mL)</td>
<td>1.90 (0.05)</td>
<td>1.84 (0.07)</td>
<td>1.70 (0.11)</td>
<td>1.96 (0.04)</td>
<td>1.85 (0.08)</td>
<td>1.46 (0.09)</td>
</tr>
</tbody>
</table>

Data presented as log-transformed mean (SEM). Adjusted for age, gender, and ethnic minority status. IL-6 = interleukin-6; IL-6 sR = interleukin-6 soluble receptor; TNFα = tumor necrosis factor alpha; TNFR1 = tumor necrosis factor receptor 1; CRP = C-reactive protein. a p < 0.1 vs. no OSA; b p < 0.05 vs. no OSA; c p < 0.01 vs. no OSA; d p < 0.001 vs. no OSA; e p < 0.05 vs. mild OSA; f p < 0.01 vs. mild OSA; g p < 0.001 vs. mild OSA; h p < 0.001 vs. lean; i p < 0.05 vs. overweight; j p < 0.01 vs. overweight; k p < 0.001 vs. overweight.

To explore the joint effect of apnea and body weight on cytokine concentrations (Table 4.3), the sample was divided into four groups based on the presence of mild apnea (AHI ≥ 2) and overweight (BMI percentile ≥ 85th percentile). Levels of IL-6, CRP, and leptin were elevated in those overweight with AHI ≥ 2 compared to the other three groups (all p < 0.1), while adiponectin was significantly lower in the two overweight groups (all p < 0.1). IL-6 and leptin
were also elevated in the overweight group without OSA compared to both lean groups (all \( p < 0.05 \)), and adiponectin was significantly lower (both \( p < 0.1 \)). While overweight was associated with significantly elevated CRP overall (all \( p < 0.001 \) vs. lean groups), even the lean group with \( \text{AHI} \geq 2 \) had elevated CRP compared to the lean group without OSA (\( p = 0.014 \)).

### Table 4.3. Plasma concentrations of inflammatory cytokines and metabolic hormones, stratified by the joint effect of \( \text{AHI} \geq 2 \) and overweight/obese.

<table>
<thead>
<tr>
<th></th>
<th>Lean, no OSA (n = 172)</th>
<th>Lean, AHI ( \geq 2 ) (n = 83)</th>
<th>Overweight, no OSA (n = 67)</th>
<th>Overweight, AHI ( \geq 2 ) (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log IL-6 (pg/mL)</td>
<td>-0.14 (0.03)</td>
<td>-0.17 (0.05)</td>
<td>-0.03 (0.05)(^e)</td>
<td>0.13 (0.05)(^e,g,i)</td>
</tr>
<tr>
<td>log IL-6 sR (pg/mL)</td>
<td>3.72 (0.03)</td>
<td>3.75 (0.04)</td>
<td>3.78 (0.04)</td>
<td>3.73 (0.04)</td>
</tr>
<tr>
<td>log TNF(\alpha) (pg/mL)</td>
<td>0.19 (0.02)</td>
<td>0.17 (0.03)</td>
<td>0.23 (0.04)</td>
<td>0.25 (0.04)</td>
</tr>
<tr>
<td>log TNFR1 (ng/mL)</td>
<td>2.49 (0.02)</td>
<td>2.49 (0.03)</td>
<td>2.51 (0.03)</td>
<td>2.53 (0.03)</td>
</tr>
<tr>
<td>log CRP (mg/L)</td>
<td>-0.36 (0.03)</td>
<td>-0.22 (0.05)(^a)</td>
<td>-0.13 (0.05)(^c)</td>
<td>0.05 (0.05)(^c,g,i)</td>
</tr>
<tr>
<td>log Leptin (ng/mL)</td>
<td>1.52 (0.07)</td>
<td>1.57 (0.09)</td>
<td>2.58 (0.10)(^c,g)</td>
<td>2.85 (0.10)(^c,g,h)</td>
</tr>
<tr>
<td>log Adiponectin (µg/mL)</td>
<td>1.97 (0.05)</td>
<td>1.94 (0.07)</td>
<td>1.73 (0.08)(^e,d)</td>
<td>1.63 (0.08)(^b,f)</td>
</tr>
</tbody>
</table>

Data presented as log-transformed mean (SEM). Adjusted for age, gender, and ethnic minority status. IL-6 = interleukin-6; IL-6 sR = interleukin-6 soluble receptor; TNF\(\alpha\) = tumor necrosis factor alpha; TNFR1 = tumor necrosis factor receptor 1; CRP = C-reactive protein. \(^{a}\)p < 0.05 vs. lean, no OSA; \(^{b}\)p < 0.01 vs. lean, no OSA; \(^{c}\)p < 0.001 vs. lean, no OSA; \(^{d}\)p < 0.1 vs. lean, AHI \( \geq 2 \); \(^{e}\)p < 0.05 vs. lean, AHI \( \geq 2 \); \(^{f}\)p < 0.01 vs. lean, AHI \( \geq 2 \); \(^{g}\)p < 0.001 vs. lean, AHI \( \geq 2 \); \(^{h}\)p < 0.1 vs. overweight, no OSA; \(^{i}\)p < 0.01 vs. overweight, no OSA.

Finally, to examine the relative influence of OSA and body weight on cytokines, linear regression analyses were conducted using visceral fat area, subcutaneous fat area, and OSA (AHI \( \geq 5 \)) as predictors, adjusting for age, gender, and ethnic minority status (Figure 4.1). OSA significantly predicted levels of IL-6 (\( \beta = 0.17, p = 0.002 \)), TNF\(\alpha\) (\( \beta = 0.14, p = 0.02 \)), CRP (\( \beta = 0.28, p < 0.001 \)), and leptin (\( \beta = 0.16, p < 0.001 \)). To a greater degree, visceral fat area predicted levels of IL-6 (\( \beta = 0.33, p = 0.02 \)) and CRP (\( \beta = 0.42, p = 0.003 \)), whereas subcutaneous fat area strongly predicted leptin (\( \beta = 0.78, p < 0.001 \)). Levels of IL-6 sR, TNFR1, and adiponectin were not significantly predicted by the three variables of interest, though there was a trend toward subcutaneous fat predicting lower adiponectin (\( p = 0.10 \)).
Figure 4.1. Comparison of OSA (AHI ≥ 5) and body fat predictors for inflammatory cytokines and metabolic hormones, adjusting for age, gender, and ethnic minority status. Standardized β reported. * p < 0.05; ** p < 0.01; *** p < 0.001.
Of the 392 participants who provided blood, n = 19 (4.8%) reported taking some sort of medication considered to be anti-inflammatory; the vast majority (17 of 19) were asthma treatments or over-the-counter NSAIDs. Repeating the analyses excluding these 19 participants did not significantly alter the results, so they were included in the final analyses to retain the general population nature of the sample. Further adjusting for health comorbidities that could potentially influence inflammation levels (arthritis, asthma, chronic sinusitis/rhinitis, total number of reported health problems, and use of anti-inflammatory medication) did not significantly alter these associations.

4.4. DISCUSSION

Significant elevations in systemic inflammation are observed in adolescents at the level of moderate OSA (AHI ≥ 5), which gives validity to suggested clinical cutoffs for treatment of mild OSA in adults (American Academy of Sleep Medicine, 2014) and previously reported cardiometabolic sequelae, such as insulin resistance and fasting triglycerides in obese adolescents (Watson et al., 2014). Similar to the present study, reports in children and adolescents have described elevated CRP and IL-6 with increasing AHI, arousal index, and/or SpO2 nadir (Tauman et al., 2004; Larkin et al., 2005; Tauman et al., 2007; Gieles-Hillel et al., 2014), even after controlling for BMI. We also observed an elevation of TNFα and leptin in adolescents with OSA, which is in line with studies in both children and adults (Vgontzas et al., 2000; Ip et al., 2000; Gozal et al., 2010; Tsaoussoglou et al., 2010; Canapari et al., 2011). Although we did not observe significantly decreased adiponectin levels in our general population of adolescents with OSA, other studies of clinic populations have (Tsaoussoglou et al., 2010; Kelly et al., 2010).
When considering the association of inflammation with OSA in adolescents, this study is unique in several aspects. For one, our findings from a large non-clinical general population sample of adolescents are more generalizable to public health compared to smaller clinical studies, which are the majority. Second, since we are examining incident cases of OSA (i.e. only those with AHI < 5 at baseline), and even after statistically adjusting for baseline AHI, we know that the inflammation observed is not a result of prolonged childhood exposure to SDB. One limitation of this study is that only a single blood sample was collected in the morning upon awakening. It’s been demonstrated that IL-6, TNFα (Entzian et al., 1996, Vgontzas et al., 2005b), leptin (Langendonk et al., 1998), and adiponectin (Gómez-Abellán et al., 2010) exhibit a circadian pattern of secretion over a 24-hour period. On the other hand, CRP, which was most strongly associated with both visceral fat area and OSA, is not subject to diurnal variation. This feature may also explain why CRP serves as a good marker for cardiovascular risk (Meier-Ewert et al., 2001).

In sum, elevations in systemic inflammation are observed in adolescents at the level of AHI ≥ 5, a cutoff which has been previously associated with cardiometabolic sequelae in this age group. Our findings also give validity to suggested clinical cutoffs for treatment.
Chapter 5

INFLAMMATION MEDIATES THE RELATIONSHIP BETWEEN VISCERAL FAT AND OBSTRUCTIVE SLEEP APNEA IN ADOLESCENTS
5.1. RATIONALE & HYPOTHESIS

Despite the strong association that has been established between OSA and inflammation in adults over the last two decades, the direction of this association is still unclear. Indeed, a major prevailing hypothesis is that inflammation is the result of intermittent hypoxia caused by breathing pauses. This inflammation, in turn, is then the culprit behind the development of metabolic aberrations in those with OSA. This is supported by reports in adults demonstrating that OSA predicts the development of incident cardiometabolic sequelae, such as diabetes (Botros et al., 2009; Appleton et al., 2015); that CPAP reduces inflammation (Steiropoulos et al., 2009), blood pressure (Dimsdale et al., 2000), and improves insulin sensitivity (Feng et al., 2015); and that OSA may be obesogenic, with a positive association between SDB severity and weight gain at follow-up (Brown et al., 2011).

On the other hand, a significant body of literature reports no change in visceral adiposity, insulin resistance, inflammation (Vgontzas et al., 2008), lipids, nor the ratio of patients with metabolic syndrome (Jullian-Desayes et al., 2015). All studies vary in terms of duration and compliance of CPAP use, the timing of the blood samples, and whether or not the confounding factor of visceral adiposity was taken into account in the analysis. Taken together, it is unclear which direction the OSA/inflammation association points, and whether the gold standard treatment, CPAP, can successfully reverse inflammatory sequelae.

In adults, recent prospective findings in the Wisconsin Sleep Cohort indicate that the increased prevalence of OSA has mirrored the surge in obesity over the last two decades (Young et al. 2009). Importantly, a longitudinal study in the same cohort reported that a 10% weight gain predicts a 32% increase in AHI and 6-fold odds of developing moderate-to-severe OSA (Peppard et al. 2000a). A recent study highlighted how rates of SDB have increased between 14% to 55%,
depending on the subpopulation studied, in conjunction with the ongoing obesity epidemic (Peppard et al. 2013).

Notably, compared to age- and BMI-matched controls, visceral fat area in obese (Vgontzas et al. 2000) and even non-obese (Kritikou et al. 2013) men as measured by CT scan is elevated in those with OSA. Furthermore, a recent longitudinal general population study examining incident SDB in adolescents reported that childhood waist circumference was a significant predictor of adolescent SDB, while visceral fat area (measured cross-sectionally) was the strongest predictor of all variables examined (Bixler et al. 2016). As previously described, increases in visceral fat pad size are associated with low levels of TNFα secretion by adipocytes themselves. This results in macrophage recruitment which, in turn, releases more cytokines – IL-6, IL-1β, and TNFα. Levels of the acute phase reactant C-reactive protein (CRP), synthesized by the liver, also rise in response to IL-6 secretion by macrophages and T-cells. In sum, visceral fat is a metabolically active organ associated with significant negative health outcomes.

Based on this, we hypothesized that, in a cross-sectional analysis of a non-clinical sample of adolescents with incident OSA, (a) visceral fat increases the odds of having OSA, and (b) this association is mediated by, rather than results in, inflammation.

### 5.2. STATISTICAL ANALYSIS

Given the lack of significant differences observed in adiposity (Figures 3.2, 3.3) and biomarker concentrations (Table 4.2) in the no OSA and mild OSA groups, OSA was re-defined as a binary variable with two levels: AHI < 5 and AHI ≥ 5. Those who had AHI ≥ 5 at baseline
(n = 6) were excluded from all analyses in order to examine incident cases of OSA to rule out a possible influence of prolonged sleep apnea from baseline on inflammation in adolescence.

Logistic regression was conducted to assess the role of inflammation as a potential mediator of the relationship between visceral fat area and OSA, adjusting for age, gender, and ethnic minority status. Based on the results of the logistic regression, the R package “Mediation” (version 4.4.4) was then used to quantitatively estimate the mediating effects of inflammation in this association.

Because all cytokines measured in the blood (IL-6, IL-6 sR, TNFα, TNFR1, CRP, leptin, and adiponectin) were non-normally distributed, logarithmic transformation was applied.

5.3. RESULTS

Adjusting for age, gender, and ethnic minority status, one standard deviation increase in visceral fat area was associated with a 65% increased odds of moderate OSA in this general population of adolescents (Table 5.1). When IL-6 was introduced in the model (Model 2), the β for visceral fat predicting OSA was attenuated by 46.0% (β = 0.50 decreased to β = 0.27). To a greater degree, replacing IL-6 with CRP (Model 3) attenuated the regression coefficient by 64.0% (β = 0.50 decreased to β = 0.18). Together, these findings suggest that a notable portion of the association between visceral adiposity and OSA can be explained by inflammation, especially IL-6 and CRP. Further adjusting for AHI at the baseline (childhood) sleep study had no effect on these associations.
Table 5.1. Logistic regression examining the relationship between visceral fat area and OSA, with CRP and IL-6 as potential mediators.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ) (SE) OR (95% CI) p</td>
<td>( \beta ) (SE) OR (95% CI) p</td>
<td>( \beta ) (SE) OR (95% CI) p</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>0.50 (0.15) 1.65 (1.23-2.22) 0.001</td>
<td>0.27 (0.16) 1.31 (0.95-1.80) 0.10</td>
<td>0.18 (0.18) 1.19 (0.95-1.80) 0.33</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age, gender, and ethnic minority status.
Model 2: Model 1 + logIL-6
Model 3: Model 1 + logCRP

Mediation analysis was then conducted to assess the proportion of the total effect of visceral fat on moderate OSA that is attributable to systemic inflammation. According to the model, 42% of the association between central obesity and OSA in adolescents is mediated by IL-6 (\( p = 0.03 \)) (Figure 5.1A), while 82% of the association is mediated by CRP (\( p = 0.01 \)) (Figure 5.1B).

![Diagram A](image)

**Figure 5.1.** Model for the association between visceral adiposity and OSA, as mediated by interleukin-6 (A) and C-reactive protein (B). *\( p < 0.05 \).*
5.4. DISCUSSION

In exploring the role of inflammation in the mechanistic relationship between obesity and OSA, 42% of the association between visceral fat and OSA in adolescents is mediated by IL-6, and 82% of the association is mediated by CRP. Thus, these findings point to a model of a feed-forward, vicious cycle (Vgontzas et al., 2005), in which the release of proinflammatory cytokines by visceral adipocytes plays a causative role in the development of OSA. Interestingly, since we are examining incident cases of SDB (only those with AHI < 5 at baseline), and even after adjusting for baseline AHI, we know that the inflammation observed is not a result of a prolonged childhood exposure to SDB. In our study, the mediating effect of CRP was stronger than that of IL-6. IL-6, TNFα (Entzian et al., 1996; Vgontzas et al., 2005), leptin (Langendonk et al., 1998), and adiponectin (Gómez-Abellán et al., 2010) exhibit a circadian pattern of secretion over a 24-hour period. CRP, on the other hand – which was most strongly associated with both visceral fat area and OSA (Figure 4.1) – is not subject to diurnal variation, which may explain why it is the strongest mediating factor.

Our mediational model is supported by a recent study by Kheirandish-Gozal and colleagues demonstrating that anti-inflammatory therapy improved sleep apnea in over 80% of children with mild OSA (1 < AHI < 5) (Kheirandish-Gozal et al., 2014). Furthermore, the Child Adenotonsillectomy Trial (CHAT) reported no significant change in cardiometabolic parameters, including CRP, in the 7-month follow-up period after adenotonsillectomy in children with 2 ≤ AHI < 30 (Quante et al., 2015), suggesting that inflammation in OSA is systemic and not a result of a local inflammatory process. Our findings also add to evidence that dietary and exercise interventions resulting in weight loss, particularly in the abdominal region, are likely to improve
OSA. Indeed, in the adult Wisconsin Sleep Cohort, a 10% weight loss predicted a 26% decrease in AHI (Peppard et al., 2000a). Recent research suggests that high-intensity exercise, specifically, is effective in reducing visceral adiposity (Irving et al., 2008). A weight loss intervention that specifically incorporates high-intensity exercise in patients with OSA would be an interesting future study.

In sum, our findings in a large, representative, non-clinical sample suggest that inflammation, particularly IL-6 and CRP resulting from elevated visceral adipose tissue, is the major mediating link in the relationship between central obesity and sleep apnea, adding to our developing understanding of the pathogenesis and potential treatments for OSA.
Chapter 6

INFLAMMATION IN CHILDHOOD PREDICTS APNEA AND BLOOD PRESSURE REACTIVITY IN ADOLESCENCE
6.1. RATIONALE & HYPOTHESIS

Cross-sectionally, we’ve demonstrated (Chapter 5) that in the association between visceral fat area and OSA, 82% is mediated (explained) by CRP, and 42% is explained by IL-6. This strongly suggests that visceral adipose tissue is a significant source of systemic inflammation, and that this inflammation is strongly associated with OSA in a non-clinical cohort of adolescents. What is not known, however, is whether inflammation during childhood actually contributes to the development of incident OSA in adolescence.

Several studies lend evidence for the causative role of inflammation in OSA. A recent meta-analysis of sham-CPAP randomized controlled trials concluded that CPAP therapy does not significantly alter inflammation nor improve any cardiometabolic biomarkers such as glucose, insulin, or lipids (Jullian-Desayes et al., 2015). Similarly, a recent 6-month trial reported no change in CRP, insulin sensitivity, or serum triglycerides with CPAP, unless combined with weight loss (Chirinos et al., 2014). On the other hand, compared to placebo, a three-week trial of the TNFα antagonist etanercept significantly reduced AHI as well as IL-6 levels in obese men with OSA (Vgontzas et al., 2004a). Given that a major source of this inflammation appears to be visceral adiposity, it is not surprising that Wisconsin Sleep Cohort reported that a 10% weight loss predicted a 26% decrease in AHI at follow-up (Peppard et al., 2000a). Together, these findings suggest that interventions targeting inflammation, rather than simply the breathing abnormalities, may be beneficial in addressing the root cause of the disorder.

In addition, several pre- and perinatal factors have been associated with the development of OSA in children and adolescents, including chorioamnionitis, maternal smoking and weight gain during pregnancy, preeclampsia, gestational diabetes, and complications related to prematurity (Hibbs et al., 2008; Calhoun et al., 2010; Tapia et al., 2016). The exact mechanism
linking these conditions with increased risk for OSA in childhood has not been studied, but a combination of inflammation during this time period, as well as potential changes in DNA methylation, have been hypothesized (Tapia et al., 2016).

In adults, patients with rheumatic diseases, such as rheumatoid arthritis, have been reported to have a higher prevalence of OSA (Taylor-Gjevre et al., 2013). While both share common pathologies of systemic inflammation, the potential direction of this association has not been explicitly examined. Interestingly, a recent study reported a significantly lower prevalence of OSA in patients with spondyloarthritis who take TNF-inhibitors compared to those who do not (Walsh, 2012), suggesting that systemic inflammation may precede the development – or at least the worsening – of OSA.

The aim of this study was to examine the relationship between inflammation and OSA in PSCC participants for whom we have longitudinal data. We hypothesized that higher inflammation levels in childhood were associated with apnea in adolescence. We also hypothesized that inflammation in childhood precedes cardiovascular aberrations, such as blood pressure reactivity, in adolescence.

6.2. STATISTICAL ANALYSIS

Participants from the Penn State Child Cohort with inflammation data at both baseline and follow-up were included in the study. Those with AHI ≥ 2 at baseline (n = 2) were excluded to control for possible effects of prolonged OSA sequelae from childhood. The final sample size was n = 56. Linear regressions were calculated, with both baseline and Δ inflammation as predictors (Δ = cytokine concentration at follow-up minus concentration at baseline).
First, to assess the hypothesis that inflammation precedes OSA, AHI at follow-up was selected as the outcome, with baseline and Δ CRP and TNFα as predictors, adjusting for baseline age, BMI percentile at follow-up, ethnic minority status, and time elapsed between baseline and follow-up time points. Analyses examining Δ inflammation were also adjusted for baseline inflammation (CRP and TNFα, respectively).

Next, to examine the hypothesis that inflammation in childhood predicts later cardiovascular morbidity, systolic and diastolic blood pressure as well as blood pressure reactivity (difference in blood pressure in the standing minus the supine position) were selected as outcomes, with baseline and Δ CRP and TNFα as predictors, adjusting for baseline age, ethnic minority status, and time elapsed between baseline and follow-up time points. Analyses examining Δ inflammation were also adjusted for baseline inflammation.

Given known gender differences in apnea severity in boys and girls at adolescence, and potential gender differences in inflammation as observed in adults (Gaines et al., 2015), all analyses were conducted stratified by gender. Standardized β are reported.

6.3. RESULTS

Baseline and follow-up AHI, follow-up systolic and diastolic blood pressure reactivity, and baseline/follow-up CRP and TNFα are presented in Table 6.1, stratified by gender. Overall, there was an increase in all longitudinal variables at follow-up compared to baseline, except for CRP in girls.
Table 6.1. Sleep, cardiovascular, and inflammation variables at baseline and follow-up, stratified by gender.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Boys (n = 24)</th>
<th>Girls (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
</tr>
<tr>
<td>AHI (events/h)</td>
<td>0.25 (0.07)</td>
<td>2.83 (1.13)</td>
</tr>
<tr>
<td>Systolic BP reactivity (mmHg)</td>
<td>-</td>
<td>2.96 (1.95)</td>
</tr>
<tr>
<td>Diastolic BP reactivity (mmHg)</td>
<td>-</td>
<td>7.06 (0.93)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.23 (0.10)</td>
<td>1.30 (0.49)</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>1.55 (0.24)</td>
<td>1.78 (0.23)</td>
</tr>
</tbody>
</table>

Data presented as mean (SEM). AHI = apnea/hypopnea index; CRP = C-reactive protein; TNFα = tumor necrosis factor alpha.

When examining the impact of inflammation in childhood on apnea in adolescence, \(\Delta CRP\) and \(\Delta TNF\alpha\) significantly predicted AHI at follow-up in boys (\(\beta = 1.02, p < 0.001\) and \(\beta = 1.12, p = 0.04\), respectively) but not girls (\(\beta = -0.09, p = 0.93\) and \(\beta = 0.25, p = 0.25\), respectively), after adjusting for baseline age, BMI percentile at follow-up, ethnic minority status, time elapsed between baseline and follow-up time points, and baseline CRP and TNFα, respectively (Figure 6.1).

![Figure 6.1](image-url)
When examining the impact of inflammation in childhood on cardiovascular outcomes in adolescence, baseline CRP significantly predicted systolic blood pressure reactivity at follow-up in boys ($\beta = 0.56$, $p = 0.02$), with a marginal prediction in girls ($\beta = 0.32$, $p = 0.08$), after adjusting for baseline age, time elapsed between baseline and follow-up time points, and ethnic minority status. (Figure 6.2).

**Figure 6.2.** Baseline C-reactive protein predicting systolic blood pressure reactivity at follow-up, stratified by gender. Adjusted for baseline age, time elapsed between baseline and follow-up time points, and ethnic minority status. Standardized $\beta$ reported. $^T p < 0.1$; * $p < 0.05$.

Finally, $\Delta$CRP significantly predicted diastolic blood pressure reactivity in girls ($\beta = 1.67$, $p = 0.03$) but not boys ($\beta = 0.04$, $p = 0.86$), after adjusting for baseline age, time elapsed between baseline and follow-up time points, ethnic minority status, and baseline CRP (Figure 6.3).
Figure 6.3. C-reactive protein change from baseline to follow-up predicting diastolic blood pressure reactivity at follow-up, stratified by gender. Adjusted for baseline age, time elapsed between baseline and follow-up time points, ethnic minority status, and baseline CRP. Standardized β reported. * p < 0.05.

IL-6, IL-6 sR, TNFR1 were also examined, but were not significant predictors in any analyses.

6.4. DISCUSSION

When assessed prospectively, greater increases of CRP and TNFα levels from baseline to follow-up were significant predictors of AHI at follow-up in boys. Furthermore, baseline CRP levels predicted systolic blood pressure reactivity in boys and were a marginal predictor in girls, while greater increases in CRP across the follow-up period predicted diastolic blood pressure reactivity in girls. Together, these findings demonstrate that inflammation precedes OSA and
cardiovascular aberrations and, moreover, suggest that inflammation may be a causal factor in the development of these conditions.

While the strong association between obesity and OSA is unlikely to be disputed, the mechanism linking inflammation and OSA is more difficult to explain. As detailed in Section 3.4, central obesity has been associated with an overall depression in ventilatory control mechanisms. Excess adipose tissue restricts the normal movement of chest and diaphragm muscles, and accumulation of fat in the head and neck region narrows the upper airway. The mechanism that directly links inflammation to incident OSA is less clear, though it may be the combined effect of, at least, the following two mechanisms. First, because a significant amount of lymph tissue is located in the head and neck, swelling of glands due to chronic systemic inflammation may contribute to upper airway narrowing. Second, inflammation originating from fat tissue and other sources launches a vicious cycle of cardiovascular and metabolic sequelae, including leptin resistance, insulin resistance, elevated lipids and triglycerides, endothelial dysfunction, and elevated blood pressure. Through various mechanisms, these conditions worsen obesity and contribute further to the mechanisms of ventilatory depression mentioned above.

The gender difference we report is interesting to note. The associations of ΔCRP and ΔTNFα with follow-up AHI observed in boys, but not girls, is likely related to (a) significantly more overall visceral adiposity in boys (Figure 3.3), and (b) the overall greater increase in AHI in boys compared to girls (+2.58 events/h vs. +0.87 events/h, Table 6.1). When used as a continuous outcome variable, the larger range in AHI may make it easier to determine such a correlation. The gender differences in the predictive value of CRP when assessing systolic and diastolic blood pressure reactivity, particularly the latter, are difficult to explain. Of note, systolic reactivity was much higher in girls compared to boys (7.76 mmHg vs. 2.96 mmHg). While the
difference in diastolic reactivity was not nearly as pronounced (9.32 mmHg vs. 7.06 mmHg), it was predicted by ΔCRP in girls to a much greater degree, but not boys. Future studies examining this in a larger sample size, as well as during the second follow-up of the PSCC into young adulthood, would be necessary to replicate these findings.

In sum, our findings demonstrate that inflammation precedes OSA and cardiovascular aberrations, adding to accumulating evidence that inflammation is a causal factor in the development of these conditions, even as young as childhood.
Chapter 7

INFLAMMATION IS A MARKER OF THE CARDIOMETABOLIC
AND NEUROCOGNITIVE SEVERITY OF OBSTRUCTIVE SLEEP APNEA
7.1. RATIONALE & HYPOTHESIS

The mechanisms underlying SDB in children and adults – from snoring to upper airway resistance syndrome to OSA – are multifactorial. Anatomic, craniofacial, immunological, neuromuscular, and obesity-related factors have all been shown to play roles (Arens & Marcus, 2004). As important as determining the cause of SDB, however, is identifying and preventing potential cardiometabolic (namely, components of the metabolic syndrome) and neurocognitive comorbidities (such as processing speed, vigilance, distractibility, and working memory) that may accompany the disorder. The emergence of these sequelae cannot be accounted for solely by the severity of the AHI, however, because not all individuals with OSA experience these comorbidities.

Research over the last two decades has established a strong link between OSA and elevated inflammation, even independent of obesity (Vgontzas et al., 1997; Vgontzas et al., 2000; Shamsuzzaman et al., 2000; Vgontzas et al., 2008; Sahlman et al., 2010; Kritikou et al., 2014). Specifically, in children and adolescents with SDB, levels of CRP have been positively correlated with AHI, arousal index, and oxygen desaturation (Tauman et al., 2004; Larkin et al., 2005). In a 2007 study, Gozal and colleagues recruited non-obese children (ages 5-7 years) with and without habitual snoring, dividing the former into OSA (AHI ≥ 2 plus nadir SpO₂ < 92%) and no-OSA (AHI < 1) groups. The OSA group was further divided into those with and without cognitive deficits (defined as a score ≥ 1 standard deviation below the mean on at least two subtests of the Differential Ability Scales [DAS] or the NeuroPsychological Assessment Battery [NEPSY]). While CRP levels were relatively similar between snoring children with OSA/normal cognitive scores and controls without OSA (0.21 ± 0.08 mg/dL vs. 0.19 ± 0.07 mg/dL), children
with OSA and cognitive deficits had more than twice the concentration of CRP (0.48 ± 0.12 mg/dL), as measured by a fasting morning blood draw (Gozal et al., 2007). It is unclear, however, whether specific cognitive domains were more strongly associated with elevated CRP than others, nor whether any cardiometabolic outcomes show similar associations.

While CRP may thus be a reliable predictor of apnea severity, the potential role of inflammation in predicting the medical severity of OSA, in terms of cardiometabolic and neurocognitive sequelae, has not been thoroughly explored. We hypothesized that within adolescents with OSA, markers of inflammation (specifically, CRP) would be positively correlated with individual cardiometabolic sequelae (waist circumference, insulin resistance, triglycerides, low HDL cholesterol, blood pressure) and neurocognitive deficits (particularly those related to attention, memory, or processing speed).

### 7.2. STATISTICAL ANALYSIS

Adolescents with CRP data (n = 372) were included in this study. OSA severity was defined by a dummy variable with three levels: no OSA (AHI < 2), mild OSA (2 ≤ AHI < 5), and OSA (AHI ≥ 5).

Separate linear regressions were conducted within the no OSA (n = 229), mild OSA (n = 102), and OSA (n = 41) groups, with CRP as a predictor and various cardiometabolic and neurocognitive variables as outcomes, adjusting for age, gender, BMI percentile, and ethnic minority status. Standardized β values are reported.

Cardiometabolic outcomes examined in separate linear regressions included waist circumference, insulin resistance as measured by homeostatic model assessment (HOMA = [glucose x insulin] / 405), triglycerides, systolic and diastolic blood pressure, mean arterial
pressure (MAP; defined as [(systolic blood pressure – diastolic blood pressure) / 3] + diastolic blood pressure), HDL cholesterol, and the continuous metabolic syndrome score (cMetS, defined as the sum of Z-transformed waist circumference, MAP, HOMA, HDL cholesterol [multiplied by -1], and triglycerides; Eisenmann et al., 2010).

Neurocognitive outcomes examined were processing speed (from the WISC-IV/WAIS-III Symbol Search task), vigilance (from the Gordon Diagnostic System), working memory (from the WISC-IV/WAIS-III Digit Span – backwards subtest), distractibility (from the Stroop Color and Word Test – interference subtest), visuo-motor integration (from the Developmental Test of Visual-Motor Integration, 5th edition), and the intelligence quotient (IQ) calculation derived from the Wechsler Abbreviated Scales of Intelligence.

7.3. RESULTS

In terms of cardiometabolic health, significant interactions of OSA x CRP were observed for triglycerides (standardized β = 0.23, p = 0.02), cMetS (β = 0.22, p = 0.01), and a trend interaction for HOMA (β = 0.18, p = 0.07). Specifically, CRP significantly predicted waist circumference (β = 0.32, p = 0.008), HOMA (β = 0.60, p < 0.001), triglycerides (β = 0.54, p = 0.001), and cMetS (β = 0.43, p = 0.001) in those with OSA (Figure 7.1). To a lesser degree, CRP predicted HOMA (β = 0.21, p = 0.038) and cMetS (β = 0.18, p = 0.048) in those with mild OSA as well. The associations of CRP with these outcomes in the no OSA group were not significant (all p > 0.10).
Figure 7.1. Relative associations of C-reactive protein with various cardiometabolic variables in adolescents, stratified by apnea severity, adjusting for age, gender, BMI percentile, and ethnic minority status. Standardized $\beta$ reported. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. n = 229 with no OSA; n = 102 with mild OSA; n = 41 with OSA.
In terms of neurocognitive performance, a marginal interaction of OSA x CRP was observed for processing speed deficits (standardized $\beta = 0.12$, $p = 0.15$). Specifically, CRP significantly predicted processing speed deficits in those with OSA ($\beta = 0.40$, $p = 0.01$; Figure 7.2). The associations of CRP processing speed in the no OSA and mild OSA groups were not significant (both $p > 0.37$).

**Figure 7.2.** Relative associations of C-reactive protein with processing speed deficits in adolescents, stratified by apnea severity, adjusting for age, gender, BMI percentile, and ethnic minority status. Standardized $\beta$ reported. * $p < 0.05$. $n = 229$ with no OSA; $n = 102$ with mild OSA; $n = 41$ with OSA.

Analyses were repeated also using IL-6 and TNF$\alpha$ as predictors; however, only CRP significantly predicted the degree of cardiometabolic and cognitive aberrations within OSA.

### 7.4. DISCUSSION

Cross-sectionally, within adolescents with OSA, CRP is a significant indicator of cardiometabolic (waist circumference, insulin resistance, triglycerides, cMetS) and
neurocognitive (processing speed deficits) sequelae. In short, CRP may serve as a biomarker with potential clinical utility in assessing the medical severity of OSA, which may enhance the diagnostic and prognostic utility of AHI.

There is recent evidence suggesting that anti-inflammatory therapy (intranasal and/or oral) in children with mild OSA is effective in reducing apnea severity (Kheirandish-Gozal et al., 2014). In adults, a placebo-controlled, double-blind study that administered the TNFα antagonist etanercept for three weeks reported significantly reduced AHI, decreased plasma IL-6, and reduced objective sleepiness in obese men with severe sleep apnea (Vgontzas et al., 2004a). Thus, it appears that there is an unmet need for an OSA treatment that targets inflammation.

Longitudinal studies should aim to explore how elevations or reductions in inflammation in those with OSA are associated with changes in the severity of cardiometabolic and cognitive sequelae. Furthermore, future work should examine how the association between inflammation and OSA severity on cardiovascular, metabolic, and neurocognitive aberrations progresses during the transition into young adulthood and middle age.

These findings expand on the work of Gozal et al. (2007) by identifying a significant association between CRP and deficits in processing speed, specifically, in adolescents with OSA. We also report the novel findings that CRP levels within individuals with OSA are significantly associated with larger waist circumference, insulin resistance, triglycerides levels, and the composite metabolic syndrome score. In sum, CRP may serve as a biomarker with potential clinical utility in assessing the medical severity of OSA.
Chapter 8

GENERAL DISCUSSION
8.1. SUMMARY OF FINDINGS

In this dissertation, we report five key findings in the Penn State Child Cohort, a representative population sample of 421 adolescents who were followed up in the sleep laboratory eight years after their initial study visit. First, there is a strong association of visceral fat with OSA, particularly in boys, which is consistent with findings in adults by our group and others. Second, we demonstrate that plasma concentrations of inflammatory cytokines and metabolic hormones (namely, IL-6, CRP, and leptin) are elevated while adiponectin is lowered in both OSA and obesity; moreover, these associations remain in OSA independent of obesity. Third, in exploring the mechanistic relationship between obesity and OSA, a significant portion (42%) is explained by IL-6 levels, while an even greater proportion (82%) is mediated by CRP, a non-specific marker of overall inflammation. Fourth, longitudinally, we show how a greater change in inflammation from childhood to adolescence (ΔCRP and ΔTNFα) predicts AHI levels at follow-up in boys; furthermore, higher baseline CRP predicts higher systolic blood pressure reactivity in boys, while greater ΔCRP predicted higher diastolic blood pressure reactivity in girls at follow-up. Finally, cross-sectionally, we demonstrate how CRP levels are associated with higher waist circumference, insulin resistance, triglyceride levels, the continuous metabolic syndrome score, and processing speed deficits in adolescents with OSA.

In sum, our findings support a model in which systemic inflammation – a result of weight gain and, primarily, elevated visceral fat – explains to a great degree the association between obesity and OSA in adolescents. The prospective association between inflammation in childhood with later apnea and cardiovascular aberrations in adolescence adds to the evidence that inflammation plays a primarily causative role in these two outcomes. In turn, OSA is not an
endpoint, but contributes to a progressive worsening of sequelae, including further inflammation, likely a response to intermittent hypoxic conditions. This inflammation – a result of obesity, OSA, or, most likely, a combination of both – provides a potentially useful biomarker for assessing the severity of metabolic and neurocognitive (namely, processing speed) aberrations in adolescents with OSA.
8.2. OBSTRUCTIVE SLEEP APNEA: A CONCEPTUAL MODEL

Based on the results presented in this dissertation combined with previous consistent findings in the fields of sleep, obesity, and immunology, a conceptual model for the development and progression of OSA is proposed below (Figure 8.1).

![Conceptual model of the association between visceral obesity, inflammation, and OSA. Bold text represents the main model tested in this dissertation; unbolded text represents additional findings in this dissertation; italicized text represents hypothesized associations based on previous literature.]

In this model, bold text represents the main model that was hypothesized and tested in this dissertation, unbolded text represents additional findings, and italicized text represents hypothesized mediating factors based on previous literature. To summarize, weight gain from
overnutrition, lack of physical activity, and/or chronic illness results in increased body fat.

Notably, we and others, in both adolescent and adult samples, have reported that visceral fat is more strongly associated with OSA than subcutaneous fat (Shinohara et al., 1997; Vgontzas et al., 2000; Hannon et al., 2011; Kritikou et al., 2013; Harada et al., 2014; Bixler et al., 2016). Through mechanisms including depressed ventilation (Rochester & Enson, 1974), dysfunction of respiratory and upper airway muscles (Deegan & McNicholas, 1995), upper airway narrowing (Lubrano et al., 2012), and edema of soft tissue (Mokhlesi & Tulaimat, 2007), central obesity can contribute directly to upper airway collapse during sleep, manifesting as OSA. In a separate mediational pathway, however, increased inflammation stemming from visceral adipose tissue also explains a significant portion of the relationship between obesity and OSA. These mechanisms may range from upper airway narrowing due to inflammation of the lymph glands in the neck; loss of dilator or respiratory muscle tissue due to inflammation, contributing to respiratory dysfunction (Poelkens et al., 2013); and/or any other local or systemic inflammatory damage that makes one vulnerable to upper airway collapse during sleep, through a combination of mechanisms perhaps not yet identified.

As illustrated, OSA does not represent an endpoint in the model, and rarely are any of the associations unidirectional (Figure 8.1). For one, visceral fat, inflammation, and OSA all have the potential to independently contribute to cardiometabolic sequelae – hypertension, insulin resistance, and dyslipidemia (Hotamisligil & Spiegelman, 1994; Kern et al., 2001; van Hall et al., 2003); in turn, as one’s metabolic profile worsens over time, so do the factors that precipitated cardiometabolic dysfunction in the first place. OSA and inflammation also independently contribute to sleep loss and sympathetic activation (Vgontzas et al., 1997; Pongratz & Straub, 2014), which further promote a vicious cycle of inflammation and worsening outcomes. We also
report the novel finding that inflammation (CRP) is associated with processing speed deficits in adolescents with OSA.

Importantly, this conceptual model also provides an update to the hypothesis of the feed-forward, vicious cycle of OSA first proposed by Vgontzas and colleagues (2005; Figure 1.13) by including a direct arrow between inflammation and OSA (Figure 8.1). Although we do not have baseline DXA measures to claim that, prospectively, visceral fat in childhood precedes OSA in adolescence, we have previously demonstrated that baseline waist circumference, a proxy measure of central obesity, predicted OSA at follow-up in this cohort (Bixler et al., 2016). With obesity having long been recognized as the strongest risk factor for the development of OSA (Bickelmann et al., 1956; Lugaresi, 1975; Section 1.1.3. Risk Factors for Obstructive Sleep Apnea), it would not be surprising if this were the case in this cohort as well. Because of the rapid changes in body composition across childhood and adolescence (Figure 1.9) and the fact that childhood OSA does not appear to be in a continuum with adolescent OSA (Spilsbury et al., 2015; Bixler et al., 2016), however, a longitudinal study of OSA across this age range using precise measure of body fat (such as DXA or CT) would be necessary.

Of course, the proposed model (Figure 8.1) does not account for all cases of OSA. As mentioned in Section 1.1.3. Risk Factors for Obstructive Sleep Apnea, a number of craniofacial features – such as mandibular retrognathia, an inferiorly placed hyoid bone, narrowed posterior airspace, or any condition that causes increased soft tissue volume in the mouth and upper airway – can predispose one to OSA in the absence of central obesity. Thus, Figure 8.1 represents a more systemic mechanistic model for the development and sequelae associated with OSA. It is possible that several adolescents in the PSCC had OSA due to anatomic or local abnormalities; however, the fact that we observed such a robust relationship between visceral adiposity,
inflammation, and OSA in this large, non-clinical cohort provides strong evidence that, in the general population, abdominal obesity plays the primary causative role.

In sum, based on the results presented in this dissertation combined with previous findings in the fields of sleep, obesity, and immunology, we propose a model of a “vicious cycle,” precipitated by weight gain and visceral obesity, which explains to a great degree the development and progression of new-onset OSA in adolescence.
8.3. IMPLICATIONS & FUTURE DIRECTIONS

Given the strong directional association between visceral adiposity, inflammation, and OSA, the findings from this study have potential implications in treatment. For one, given that (a) inflammation appears to play a mediational (Chapter 5) and even perhaps causal (Chapter 6) role in the development of OSA, and that (b) the gold standard treatment, CPAP therapy, does not significantly reduce IL-6, TNFα, CRP, leptin, insulin resistance, nor reverse the metabolic syndrome (Jullian-Desayes et al., 2015), it is evident that the most successful treatment for the majority of OSA cases may involve some sort of anti-inflammatory therapy or, even better, strong patient support through a weight management program. Although CPAP improves sleep quality, daytime sleepiness, and reduces episodes of hypoxia, it does not address or correct the root cause of the disorder. In a recent 24-week randomized trial, improvements in inflammation, serum triglycerides, and insulin resistance were only observed in apneics who combined their CPAP treatment with weight loss (Chirinos et al., 2014). Furthermore, modest reductions in AHI with short-term anti-inflammatory treatment alone have been demonstrated in both adults (Vgontzas et al., 2004) and children (Kheirandish-Gozal et al., 2014). The ideal treatment for many individuals with OSA, particularly those with plasma elevations in proinflammatory cytokines, may combine CPAP with supervised use of an anti-inflammatory medication, such as a TNFα antagonist. Sleep clinics with strong ties to local weight management centers could also be beneficial for many obese patients with OSA.

The results presented in this dissertation may also improve approaches to personalized medicine for patients with OSA. Currently, the study of OSA lacks an ideal sensitive and specific biomarker that predicts OSA-induced organ dysfunction, prognosis, or response to
treatment (Mullington et al., 2016). Although CRP per se provides a rather generalized depiction of systemic inflammation originating from any source in the body, Chapter 7 points to the potential of this protein to serve as a marker of the cardiometabolic (waist circumference, HOMA, triglycerides, cMetS) and neurocognitive (processing speed) severity of the disorder. Because OSA severity – namely, AHI – alone is not a significant predictor of cardiometabolic risk (see Appendix C for details), combining AHI with CRP, or another inflammatory marker(s), may optimize the sensitivity and specificity for differentiating OSA patients with and without cardiometabolic/cognitive aberrations (cross-sectionally) as well as predicting who will develop later health problems (longitudinally). This is a particularly intriguing area for future research that is directly applicable to clinical practice; deciding whether or not to treat OSA in the mild-to-moderate range (5 ≤ AHI < 30) is often a gray area for many physicians, and CPAP compliance is often lowest in this group (Huang et al., 2014). The findings of this dissertation suggest that treating AHI alone (i.e. CPAP or adenotonsillectomy) will not address the root cause of the disorder (obesity and systemic inflammation). A biomarker such as CRP – or one that is discovered to be unique to patients with OSA – will help sleep clinicians better determine a patient’s prognosis and develop personalized treatments. A future research direction and major long-term goal for clinical practice would be to move away from simply treating AHI severity by combining AHI with additional health metrics and considering the influences of age, gender, and body composition.

In line with personalized medicine approaches to treating OSA, further research into genetic and epigenetic markers specific for OSA – particularly those that can be differentiated from obesity-associated polymorphisms – may aid in prognostic evaluation. According to recent work (Riha et al., 2005; Almpanidou et al., 2012; Larkin et al., 2010a; Larkin et al., 2010b) and
the findings presented in this dissertation, a focus on TNFα, IL-6, and CRP genes may be an important area of future focus.

A second follow-up of the PSCC is anticipated as these adolescents transition into young adulthood (ages ~20-30 years). While the phenomenon of childhood OSA is not in a continuum with adolescent OSA (Spilsbury et al., 2015; Bixler et al., 2016), we hypothesize that the majority of adolescent OSA cases will become chronic and persist into young adulthood. In terms of sleep disorders, this transitional period has not yet been investigated, and the second follow-up of the PSCC will provide rich data for study. For example, longitudinal DXA data in this large cohort will allow us to explore how visceral adiposity in adolescence predicts OSA in young adulthood, and to what degree changes in visceral adiposity are related to the severity of OSA. We will also be able to repeat the longitudinal inflammation analyses presented in Chapter 6 in a comparatively larger cohort, as well as explore how inflammation plays a role in more chronic, persistent OSA. Interestingly, in adolescents, we did not detect a gender difference in the level of the various inflammatory and metabolic markers. In healthy adults, however, it has been demonstrated that peripheral levels of CRP (Wener et al., 2000), leptin (Ostlund et al., 1996), and adiponectin (Böttner et al., 2004) are naturally higher in women compared to men, independent of age, race, and body mass index. Furthermore, gender differences in inflammation have been observed in middle-aged adults with OSA, as well as differentially elevated in OSA with comorbid hypertension (Gaines et al., 2015a; see Appendix A and Appendix B). It will be interesting to explore whether gender differences in inflammation begin to emerge during young adulthood, and whether these differences are also related to OSA severity and susceptibility to cardiometabolic aberrations that have been observed during middle age.
There are several limitations to the current study. For one, only a morning (7:00) blood sample was collected. While CRP lacks circadian rhythmicity and levels remain relatively stable regardless of sample collection time (Meier-Ewert et al., 2001), we have previously demonstrated that plasma IL-6 has a pulsatile 24-hour secretion (Vgontzas et al., 2005b), and TNFα (Uthgenannt et al., 1995), leptin (Langendonk et al., 1998), and adiponectin (Gavrila et al., 2003) also display circadian rhythmicity. All participants were in bed from 22:00 until 7:00 with a blood sample taken immediately upon awakening, however, so any external influences affecting inflammation (such as wake time or a state of sleep loss/restriction) were carefully controlled. Another criticism common to large epidemiological sleep studies is the fact that our participants only spent one night in the sleep laboratory. The concept of the “first-night effect,” or the well-recognized observation that participants do not sleep as well during their first night in the laboratory as they would on subsequent nights, has made multi-night studies a common practice despite the time and expense associated with PSG. Importantly, however, it has been recently demonstrated that a single night appears to be useful for reliably classifying one’s sleep duration, thus providing a good general “sense” of one’s sleep in the lab were they to return for multiple nights (Gaines et al., 2015b; see Appendix D).

Our study is unique in several ways. For one, this is the only large study in this age group to include a more precise measure of obesity, such as a DXA scan, in addition to measures of BMI and waist circumference, as well as longitudinal inflammation data from childhood to adolescence. Furthermore, our findings from a large non-clinical general population sample of adolescents are more generalizable to public health compared to smaller clinical studies. It is critical to emphasize that because this is a population-based study of adolescents, the prevalence of disease is relatively low; for example, only five (1.2%) participants had diabetes, the majority
of which were Type 1 (juvenile) cases, and 12 (2.9%) participants took anti-hypertensive medication. Moreover, adjusting for health comorbidities that could potentially influence inflammation – such as arthritis, asthma, chronic sinusitis/rhinitis, total number of reported health problems, and use of anti-inflammatory medication – did not significantly alter any of the associations presented in this dissertation. That said, the fact that we are observing a robust relationship between visceral adiposity, inflammation, and OSA similar to adults (Vgontzas et al., 2000), (a) at this young age, and (b) in a non-clinical population further suggests a causative role of central obesity in this association.

Taken together, the findings presented in this dissertation support a complex vicious cycle in which visceral fat, through primarily inflammatory mechanisms, results in OSA. In turn, OSA contributes to a progressive worsening of cardiometabolic sequelae and neurocognitive deficits. These findings provide a unique perspective and future directions for studying the pathophysiology, prognosis, and potential treatment for OSA in children, adolescents, and adults.


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Appendix A

GENDER DIFFERENCES IN INFLAMMATION IN NON-OBESE ADULTS WITH OBSTRUCTIVE SLEEP APNEA

A.1. RATIONALE & HYPOTHESIS

Obstructive sleep apnea is a prevalent sleep disorder characterized by obstruction of the upper airway during sleep despite breathing effort, as well as an associated reduction in blood oxygen saturation. Seventeen to 24% of men and 5 to 9% of women in general population samples demonstrate an apnea-hypopnea index (AHI) of five or more events per hour of sleep, while 4% of men and 2% of women meet the current clinical and polysomnographic criteria for the diagnosis of sleep apnea warranting immediate therapeutic intervention (Bixler et al., 1998; Bixler et al., 2001; Young et al., 1993). Sleep apnea has been associated with the elevation of several proinflammatory cytokines, independent of obesity (Vgontzas et al., 1997; Vgontzas et al., 2000; Shamsuzzaman et al., 2000; Vgontzas et al., 2008; Sahlman et al., 2010; Kritikou et al., 2014) Among a number of inflammatory pathways, sleep apnea has been especially linked to activation of tumor necrosis factor (TNF)-α receptors, which stimulates secretion of interleukin (IL)-6, in turn triggering the synthesis of C-reactive protein (CRP) in the liver (Akira et al., 1990; Hirano et al., 1990). It is hypothesized that this inflammatory cascade, in addition to insulin resistance, mediates the link between sleep apnea and cardiometabolic complications (Vgontzas et al., 2005a).

Although sleep apnea was traditionally recognized in middle-aged, obese men, its occurrence in women as well as lean individuals is increasingly recognized. The prevalence of sleep apnea increases markedly after menopause, with post-menopausal women having a doubled rate of apnea compared to pre-menopausal women, even after accounting for neck circumference and body mass index (Bixler et al., 2001; Dancey et al., 2001). Also, while the maximum prevalence for obstructive sleep apnea peaks between ages 50-59 in men (Bixler et al., 1998), this peak is not seen in females until after age 65 (Bixler et al., 2001). Furthermore, men
tend to have a higher AHI than women when matched for body mass index (Kapsimalis and Kryger, 2002), are more likely to exhibit the classical symptoms of excessive daytime sleepiness and snoring (Phillips et al., 2008), and the severity of their daytime sleepiness is more likely to be related to lack of regular exercise, depression, and minimum oxygen desaturation than AHI per se (Basta et al., 2008).

In healthy individuals, it has been demonstrated that peripheral levels of CRP (Cartier et al., 2009; Khera et al., 2005; Lakoski et al., 2006; McConnell et al., 2002, Wener et al., 2000), leptin (Couillard et al., 1997, Hellström et al., 2000; Hickey et al., 1996; Kennedy et al., 1997; Ostlund et al., 1996), and adiponectin (Böttner et al., 2004; Saltevo et al., 2009; Song et al., 2014) are naturally higher in women compared to men, independent of age, race, and body mass index. Despite this, and although most studies of sleep apneics statistically control for gender in their analyses, very few have expressly investigated possible gender differences in inflammation.

The aim of our study was to examine potential gender differences in the association of sleep apnea with inflammation and metabolic markers. We hypothesized that the association of these markers with OSA would be stronger in men than in women.

A.2. METHODS

A.2.1. Participants

The study sample consisted of 120 middle-aged, predominantly non-obese mild-to-moderate sleep apneics and controls (62 males, 58 females; mean age = 54.67 ± 0.54 years). Participants were recruited through advertisements in the local community and screened according to research protocols by the Sleep Research and Treatment Center at Penn State
Milton S. Hershey Medical Center (Hershey, PA, USA). All women in the study were post-menopausal (self-reported absence of menses for at least 12 months or total hysterectomy). Exclusion criteria included a history of diabetes mellitus, use of antiglycemic agents and/or fasting blood glucose levels > 126 mg/dL, ongoing infections, rheumatoid arthritis, insomnia, narcolepsy, and use of certain medications (psychotropics, steroids, sympathomimetics, sympatholytics, or hormone therapy for females). The study was approved by the Institutional Review Board at Penn State University College of Medicine and all participants provided written informed consent.

A.2.2. Sleep Laboratory Protocol

During their visit in the laboratory, all participants underwent a clinical history and physical examination, during which height and weight were recorded and body mass index (BMI) calculated (in kg/m²). Blood pressure was also assessed, with hypertension defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or as the use of antihypertensive medication.

Sleep laboratory recordings were conducted in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was monitored continuously for one night for 8 h (22:30-23:00 until 6:30-7:00) using 16-channel polygraph recordings of EEG, electrooculogram (EOG) and electromyogram (EMG). Polysomnography (PSG), respiration (via thermocouple and thoracic strain gauges), and oximeter data were collected using Grass-Telefactor Gamma Sleep Recording software (Middleton, WI, USA). Visual sleep stage scoring was conducted by a registered
polysomnography technologist blind to participant characteristics based on Rechtschaffen and Kales criteria (1968). Apnea-hypopnea index (AHI; number of apneas and hypopneas summed per hour) was also ascertained. An apnea was defined as cessation of airflow for ≥ 10 seconds and an out-of-phase strain gauge movement; a hypopnea was defined as a 50% airflow reduction and associated decrease in SpO2 of at least 4%. In stratifying our study sample, “the presence of sleep apnea” was defined as an AHI ≥ 5 events / hour of sleep.

A.2.3. Blood Sampling

A single fasting blood draw (via venipuncture) was performed at 7:00 immediately after the end of the PSG recording. Blood was collected in EDTA-containing tubes and refrigerated until centrifugation (within 3 h). Blood was stored at -80 °C until assay.

A.2.4. Assays

Plasma interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), and high-sensitivity C-reactive protein (hsCRP) were measured via enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation (CVs) were 7.4% and 7.8% for IL-6, 4.4% and 6.1% for TNFR1, and 5.5% and 11.6% for hsCRP. The lower detection limits were 0.094 pg/mL, 0.043 pg/mL, and 0.124 ng/mL for IL-6, TNFR1, and hsCRP, respectively. Leptin and adiponectin were assessed by commercially-available radioimmunoassays with CVs below 10%.
A.2.5. Statistical Analysis

Two-tailed independent-samples t-tests were used to compare demographic and PSG variables between males and females (between-gender), or between controls and sleep apneics (within-gender). To examine differences in inflammatory and metabolic characteristics between more than two groups (e.g. increasing apnea severity), analyses of covariance (ANCOVA) with Bonferroni correction were conducted. Polynomial linear analysis was also performed to examine the association between increasing apnea severity (i.e. AHI < 5, 5 ≤ AHI < 15, and AHI ≥ 15) and inflammatory markers. Effect size was also assessed by calculating Cohen’s d statistic. Statistical significance was determined using the criterion p < 0.05. All analyses were adjusted for the confounders age and BMI. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY).

A.3. RESULTS

Demographic and PSG parameters of sleep apneic and control males and females are presented in Table A.1. Within gender, the control and sleep apneic groups did not differ in age (all p > 0.05), but sleep apneics tended to have higher BMI (27.79 kg/m2 vs. 26.50 kg/m2 respectively, p = 0.08 for males; 31.09 kg/m2 vs. 28.37 kg/m2, p = 0.03 for females) and waist circumference (100.54 vs. 96.03 cm respectively, p = 0.03 for males; 99.10 vs. 92.33 cm respectively, p = 0.04 for females) than controls. A larger neck circumference was also observed in males with sleep apnea compared to controls (39.87 cm vs. 38.22 cm, p = 0.04), but not in females (p = 0.60). Furthermore, systolic blood pressure was significantly elevated in females with sleep apnea (134.52 mmHg vs. 120.24 mmHg, p = 0.002), but this difference was not
observed for diastolic blood pressure, nor any blood pressure measures in men (all p > 0.15). In this sample, controls and sleep apneics did not differ significantly in any sleep efficiency or architecture parameters.

### Table A.1. Demographic and sleep characteristics of study sample, stratified by gender.

<table>
<thead>
<tr>
<th></th>
<th>Control (AHI &lt; 5)</th>
<th>OSA (AHI ≥ 5)</th>
<th>p</th>
<th>Control (AHI &lt; 5)</th>
<th>OSA (AHI ≥ 5)</th>
<th>p</th>
</tr>
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<td></td>
<td>(n=15)</td>
<td>(n=47)</td>
<td></td>
<td>(n=23)</td>
<td>(n=35)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>52.70</td>
<td>53.46</td>
<td>0.67</td>
<td>55.12</td>
<td>56.86</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>(1.52)</td>
<td>(0.86)</td>
<td></td>
<td>(1.19)</td>
<td>(0.97)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.50</td>
<td>27.79</td>
<td>0.08</td>
<td>28.37</td>
<td>31.09</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(0.63)</td>
<td>(0.35)</td>
<td></td>
<td>(0.94)</td>
<td>(0.76)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.03</td>
<td>100.54</td>
<td>0.03</td>
<td>92.33</td>
<td>99.10</td>
<td>0.04</td>
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<td></td>
<td>(1.78)</td>
<td>(1.02)</td>
<td></td>
<td>(2.47)</td>
<td>(2.00)</td>
<td></td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>101.67</td>
<td>105.04</td>
<td>0.08</td>
<td>108.26</td>
<td>112.50</td>
<td>0.17</td>
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<td></td>
<td>(1.66)</td>
<td>(0.95)</td>
<td></td>
<td>(2.33)</td>
<td>(1.90)</td>
<td></td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>38.32</td>
<td>39.87</td>
<td>0.04</td>
<td>34.45</td>
<td>35.21</td>
<td>0.60</td>
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<tr>
<td></td>
<td>(0.65)</td>
<td>(0.37)</td>
<td></td>
<td>(1.12)</td>
<td>(0.91)</td>
<td></td>
</tr>
<tr>
<td>AHI</td>
<td>2.26</td>
<td>22.94</td>
<td>&lt;0.001</td>
<td>1.76</td>
<td>22.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(4.37)</td>
<td>(2.47)</td>
<td></td>
<td>(2.88)</td>
<td>(2.33)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.53</td>
<td>132.33</td>
<td>0.32</td>
<td>120.24</td>
<td>134.52</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(4.15)</td>
<td>(2.37)</td>
<td></td>
<td>(3.42)</td>
<td>(2.73)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.27</td>
<td>79.11</td>
<td>0.48</td>
<td>73.52</td>
<td>77.24</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(2.23)</td>
<td>(1.27)</td>
<td></td>
<td>(1.97)</td>
<td>(1.57)</td>
<td></td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>368.77</td>
<td>351.54</td>
<td>0.80</td>
<td>368.24</td>
<td>363.87</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>(13.19)</td>
<td>(7.11)</td>
<td></td>
<td>(12.22)</td>
<td>(8.87)</td>
<td></td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>76.65</td>
<td>73.05</td>
<td>0.77</td>
<td>76.57</td>
<td>75.51</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>(2.79)</td>
<td>(1.48)</td>
<td></td>
<td>(2.52)</td>
<td>(1.82)</td>
<td></td>
</tr>
<tr>
<td>Sleep onset latency (min)</td>
<td>21.23</td>
<td>20.00</td>
<td>0.64</td>
<td>25.47</td>
<td>30.90</td>
<td>0.93</td>
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<tr>
<td></td>
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<td>(2.17)</td>
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<td>(4.58)</td>
<td>(3.77)</td>
<td></td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>95.90</td>
<td>114.14</td>
<td>0.83</td>
<td>90.43</td>
<td>92.00</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(12.20)</td>
<td>(7.40)</td>
<td></td>
<td>(9.48)</td>
<td>(7.35)</td>
<td></td>
</tr>
<tr>
<td>Total wake time (min)</td>
<td>112.50</td>
<td>129.73</td>
<td>0.77</td>
<td>112.43</td>
<td>117.97</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>(13.52)</td>
<td>(7.16)</td>
<td></td>
<td>(12.17)</td>
<td>(8.78)</td>
<td></td>
</tr>
<tr>
<td>Stage 1, %</td>
<td>27.02</td>
<td>30.75</td>
<td>0.51</td>
<td>22.43</td>
<td>24.97</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>(2.90)</td>
<td>(1.91)</td>
<td></td>
<td>(1.87)</td>
<td>(1.95)</td>
<td></td>
</tr>
<tr>
<td>Stage 2, %</td>
<td>58.26</td>
<td>52.61</td>
<td>0.47</td>
<td>53.18</td>
<td>50.78</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(2.63)</td>
<td>(1.78)</td>
<td></td>
<td>(1.75)</td>
<td>(1.54)</td>
<td></td>
</tr>
<tr>
<td>Slow-wave sleep, %</td>
<td>4.06</td>
<td>4.81</td>
<td>0.65</td>
<td>12.75</td>
<td>12.88</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>(1.46)</td>
<td>(0.83)</td>
<td></td>
<td>(1.86)</td>
<td>(1.27)</td>
<td></td>
</tr>
<tr>
<td>Rapid eye movement sleep, %</td>
<td>10.67</td>
<td>11.83</td>
<td>0.73</td>
<td>11.63</td>
<td>11.37</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>(1.83)</td>
<td>(0.92)</td>
<td></td>
<td>(1.45)</td>
<td>(1.10)</td>
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</tbody>
</table>

Data are means (SEM). AHI = apnea hypopnea index; BMI = body mass index.
Inflammatory and metabolic characteristics of controls and sleep apneics stratified by gender are presented in Table A.2. Control females had significantly higher levels of TNFR1 (1.23 ng/mL vs. 0.97 ng/mL respectively; *p = 0.01), CRP (1.83 ng/mL vs. 0.90 ng/mL, p=0.005), leptin (28.52 ng/mL vs. 6.83 ng/mL; p < 0.001) and adiponectin (13.38 ng/mL vs. 5.70 ng/mL; p < 0.001) compared to control males. These gender differences were seen in sleep apnea as well; female apneics had significantly higher levels of CRP (2.81 ng/mL vs. 1.56 ng/mL, p = 0.002), leptin (32.96 ng/mL vs. 5.54 ng/mL, p < 0.001), adiponectin (11.53 ng/mL vs. 5.13 ng/mL, p < 0.001), and a trend toward higher TNFR1 (1.26 ng/mL vs. 1.13 ng/mL, p = 0.08) compared to apneic males.

Because of these basal gender differences, the remaining analyses were conducted separately in males and females (within-gender). Sleep apneic males had a significantly higher concentration of plasma TNFR1 than controls (1.13 ng/mL vs. 0.97 ng/mL; p = 0.04) and a trend towards higher CRP (1.56 ng/mL vs. 0.90 ng/mL; p = 0.06) and IL-6 (1.14 pg/mL vs. 0.82 pg/mL; p = 0.11). In females, only CRP was elevated in sleep apneics compared to controls (2.81 ng/mL vs. 1.83 ng/mL; p = 0.04) (Table A.2).

Table A.2. Inflammatory and metabolic markers in participants with and without OSA, stratified by gender.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (AHI &lt; 5)</td>
<td>OSA (AHI ≥ 5)</td>
<td>Control (AHI &lt; 5)</td>
<td>OSA (AHI ≥ 5)</td>
</tr>
<tr>
<td></td>
<td>(n=15)</td>
<td>(n=47)</td>
<td>(n=23)</td>
<td>(n=35)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.82 (0.16)</td>
<td>1.14 (0.09)</td>
<td>1.57 (0.28)</td>
<td>1.38 (0.22)</td>
</tr>
<tr>
<td>TNFR1 (ng/mL)</td>
<td>0.97 (0.06)</td>
<td>1.13 (0.04)*</td>
<td>1.23 (0.06)a</td>
<td>1.26 (0.05)</td>
</tr>
<tr>
<td>CRP (ng/mL)</td>
<td>0.90 (0.30)</td>
<td>1.56 (0.16)</td>
<td>1.83 (0.36)b</td>
<td>2.81 (0.28)b*</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>6.83 (0.91)</td>
<td>5.54 (0.50)</td>
<td>28.52 (3.31)c</td>
<td>32.96 (2.65)c</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>5.70 (0.76)</td>
<td>5.13 (0.42)</td>
<td>13.38 (1.49)c</td>
<td>11.53 (1.19)c</td>
</tr>
</tbody>
</table>

Data are means (SEM). Inflammatory and metabolic variables are adjusted for age and BMI. a: p < 0.05, b: p < 0.01, c: p < 0.001 for males vs. females; *: p < 0.05 for controls vs. OSA.
We then examined the association of increasing apnea severity (AHI < 5, 5 ≤ AHI < 15, and AHI ≥ 15) with inflammatory markers in men and women. A dose-response was observed with CRP in both genders (p-linear = 0.04; Figure A.1). Men with AHI ≥ 15 (n = 27) had significantly higher CRP than control men (n = 15; 1.67 ng/mL vs. 0.90 ng/mL respectively; p = 0.04; Cohen’s d = 0.69). Similarly, women with AHI ≥ 15 (n = 20) had significantly higher CRP than control women (n = 23; 2.96 ng/mL vs. 1.82 ng/mL; p = 0.04; Cohen’s d = 0.67). No other differences were observed between the three sleep apnea severity groups in terms of plasma IL-6, TNFR1, leptin, or adiponectin in males or females.

**Figure A.1.** C-reactive protein levels are elevated with increasing OSA severity. Mean C-reactive protein concentrations in males and females, stratified by sleep apnea severity. Data adjusted for age and BMI. Error bars represent SEM. AHI = apnea/hypopnea index. CRP = C-reactive protein. * p < 0.05.
A.4. DISCUSSION

This is the first large study to examine gender differences in the profiles of inflammatory and metabolic markers in middle-aged, predominantly non-obese sleep apneics and controls. Although healthy women tend to have significantly higher values of TNFR1, CRP, leptin, and adiponectin than men, men with apnea appear to have a more severe inflammatory response, independent of age and body mass index. CRP is consistently elevated in both genders, and appears to be a significant predictor of apnea severity. Our findings suggest that the inflammatory and metabolic abnormalities in sleep apnea should be examined separately in men and women, and CRP may be a clinically useful and reliable marker of sleep apnea severity in both genders.

Previous work in the cardiovascular field supports our finding that peripheral CRP (Cartier et al., 2009; Khera et al., 2005; Lakoski et al., 2006; McConnell et al., 2002; Wener et al., 2000;), leptin (Couillard et al., 1997; Hellström et al., 2000; Hickey et al., 1996; Kennedy et al., 1997; Ostlund et al., 1996), and adiponectin levels (Böttner et al., 2004; Saltevo et al., 2009; Song et al., 2014) are naturally higher in women compared to men, independent of age, race, and body mass index. Our study expands these findings in demonstrating that other inflammatory markers, such as TNF, are also higher in women than in men. Although gender differences in inflammatory and metabolic markers among healthy individuals have mostly been observed within pre-menopausal women and age-matched males, our findings indicate that these differences persist at least several years beyond menopause. All women in our study were post-menopausal, and the mean age of our control females was five years older than the widely cited median age (50 years) of menopause onset in American women (Kato et al., 1998; National Institute on Aging, 2008). These higher levels of inflammatory and metabolic markers in women
compared to men are likely related to the fact that women, independent of BMI, tend to have more body fat than men (Ley et al., 1992). Furthermore, previous work has demonstrated that in males and females matched for body fat content, women tend to have higher plasma concentrations of CRP (Khera et al., 2005) and leptin (Couillard et al., 1997; Kennedy et al., 1997) per unit fat mass. Interestingly, a recent study by Doran et al. (2013) in a large, representative U.S. population sample demonstrated that men with serum CRP > 3.0 mg/L have significantly higher odds of both cardiovascular and all-cause mortality, whereas neither outcome was observed in women with the same CRP cut-off. For these reasons, inflammatory and metabolic markers should be examined separately in men and women, and different cut-off points should be applied for the two genders.

In the present study, we found that men with sleep apnea showed significantly elevated TNFR1 levels and a trend toward higher CRP and IL-6 levels as compared to control men. In females, however, only CRP was significantly elevated in sleep apneics (p < 0.04). Together, these data suggest that, in middle-aged individuals, the inflammatory and metabolic aberrations in males with mild-to-moderate sleep apnea appear to be more severe than that of females. This can be explained by previous work showing that even within a relatively non-obese sample of men, visceral fat is the strongest predictor of sleep apnea, whereas total fat in women is most strongly associated with sleep apnea (Vgontzas et al., 2000; Kritikou et al., 2013). Due to the nature of visceral fat and its propensity to accumulate more in males (Lemieux et al., 1993), this may explain why men are more susceptible to a poorer metabolic and proinflammatory profile.

Compared to subcutaneous fat, visceral fat has reduced sensitivity to insulin (Saltiel and Kahn, 2001) and is more vascularized, innervated, and contains a larger number of inflammatory and immune cells, making it more vulnerable to metabolic risks (Ibrahim, 2010). Although we did
not examine specific fat types in the present study, it is likely that the sleep apneic men exhibited a greater proportion of this more harmful fat type than their female counterparts, accounting for the different inflammatory profiles between men and women. Interestingly, we did not observe significantly elevated leptin or reduced adiponectin in sleep apneics compared to controls in either males or females. Previous work by our group has demonstrated that obese and non-obese male sleep apneics have significantly higher plasma leptin levels (Kritikou et al., 2013; Kritikou et al., 2014; Vgontzas et al., 2000). However, in the present study, we examined only mild-to-moderate sleep apneics in contrast to more severe apnea, and only a single morning sample was used compared to twice a day or 24-hour blood sampling in prior studies.

In sum, our findings suggest that the inflammatory and metabolic abnormalities in sleep apnea should be examined separately in men and women, and that CRP may be a clinically useful marker of sleep apnea severity in both genders.
Appendix B

C-REACTIVE PROTEIN IS ELEVATED IN OBSTRUCTIVE SLEEP APNEA WITH COMORBID HYPERTENSION

B.1. RATIONALE & HYPOTHESIS

A number of studies in large general population samples, both prospective and cross-sectional, have demonstrated a clear association between sleep apnea and hypertension (Bixler et al., 2000; Nieto et al., 2000; Peppard et al., 2000b). Subsequent studies in large community-based cohorts have further demonstrated that men with sleep apnea have a significantly increased risk for hypertension (Hedner et al., 2006; Mohsenin et al., 2009) and stroke (Redline et al., 2010) compared to women. Additionally, a recent large study reported that men with serum CRP > 3.0 mg/L have significantly higher odds of both cardiovascular and all-cause mortality compared to women with the same CRP cut-off (Doran et al., 2013).

No study to date, however, has explored the link between gender and apnea-associated cardiovascular outcomes, such as hypertension, in the context of the inflammatory response. We hypothesized that inflammation, particularly CRP, would be a marker of comorbid hypertension in men and women with OSA.

B.2. STATISTICAL ANALYSIS

The study sample, methods, and statistical analysis described in Appendix A were also applied in this study. To assess the association of sleep apnea associated with the comorbid cardiovascular outcome (i.e., hypertension) and inflammation, we examined differences between three groups: controls without hypertension, sleep apneics without hypertension, and sleep apneics with hypertension. Effect size was also assessed by calculating Cohen’s d statistic. Statistical significance was determined using the criterion $p < 0.05$. All analyses were adjusted
for the confounders age and BMI. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY).

B.3. RESULTS

We assessed the association of sleep apnea with a comorbid cardiovascular outcome (hypertension) by examining differences in inflammatory markers between three groups (non-sleep apneic/non-hypertensive controls, sleep apneics without hypertension, and sleep apneics with hypertension). Importantly, AHI did not differ between sleep apneics with or without hypertension in both men (AHI = 20.87 vs. 24.92 respectively; p = 0.43) and women (AHI = 20.80 vs. 24.44; p = 0.49). A dose-response was observed with CRP in both genders, although this association was stronger in women (p-linear = 0.005) than in men (p = 0.09) (Figure B.1). Sleep apneic women with hypertension (n = 20) had the highest CRP levels (3.20 ng/mL vs. 1.38 ng/mL in controls; p = 0.005; Cohen’s d = 1.09). In men, sleep apneics with hypertension (n = 23) also had the highest CRP levels (1.72 ng/mL vs. 0.97 ng/mL in controls; p = 0.09; Cohen’s d = 0.64). No other associations were observed between hypertension and any of the inflammatory or metabolic variables in either men or women.
**Figure B.1.** Association of C-reactive protein with OSA and comorbid hypertension in men and women. Mean C-reactive protein concentrations of controls, sleep apneics without hypertension, and sleep apneics with hypertension. Data adjusted for age and BMI. Error bars represent SEM. HTN = hypertension. $^T_p < 0.1; **p < 0.01$.

**B.4. DISCUSSION**

No studies to date have explored the synergistic effect of apnea and hypertension on inflammatory and metabolic outcomes in men and women. Three of the larger, population-based studies in the field, in fact, reported no effect of gender on sleep apnea-associated hypertension prevalence in middle-aged participants (Bixler et al., 2000; Nieto et al., 2000; Peppard et al., 2000b). In contrast, a population-based study has shown that men in the highest AHI tertile have a 3.7-fold risk of hypertension, whereas women in the highest tertile only have a 1.6-fold increased risk (Hedner et al., 2006). Furthermore, men within the highest BMI quartile have been shown to have a 2-fold higher risk for hypertension than BMI-matched women, independent of sleep apnea (Mohsenin et al., 2009). Another large prospective study reported a significantly increased stroke risk in the highest AHI quartile in men, but not women (Redline et al., 2010).
Interestingly, there is also evidence of a gender difference in hypertension risk in response to intermittent hypoxia in an animal model; Hinojosa-Laborde and Mifflin (2005) demonstrated that female rats subjected to intermittent hypoxia were at a lower risk for developing elevated blood pressure than males. Interestingly, a recent study reported an association between blunted nocturnal blood pressure decline (“non-dipping”) and sleep apnea with elevated serum CRP, but this association was not observed in sleep apneics with a normal dipping pattern; however, this study did not analyze the genders separately (Ishikawa et al., 2008). Our results, showing a more severe inflammatory profile in men than women, suggest that future work should examine carefully the differential association of sleep apnea with cardiovascular complications between the two genders.

When we examined the association between inflammatory and metabolic markers with sleep apnea severity, only CRP emerged with a significant dose-response trend in both men and women (Figure A.1). In order to further assess the relationship of inflammatory markers and sleep apnea with clinically-meaningful adverse outcomes, we examined the association of the various inflammatory and metabolic markers with hypertension, controlling for severity of apnea. Once again, a significant dose-response association was observed most strongly with CRP relative to other inflammatory and metabolic markers. In females, independent of age and BMI, sleep apneics with hypertension had significantly higher levels of plasma CRP compared to controls and sleep apneics without hypertension. The same linear trend was observed in men, but was not as strong, due most likely to the higher variability of the values in the male control group (Figure B.1). Finally, the stronger and consistent association of CRP with severity of sleep apnea and associated cardiovascular morbidity, compared to the other markers, may reflect that CRP levels do not exhibit a circadian influence (Meier-Ewert et al., 2001).
There are several limitations to our study that may impact its generalizability. First, the study was conducted in middle aged, mild-to-moderate sleep apneics who were relatively non-obese. Additional studies should explore the profiles of inflammatory and metabolic markers in individuals comprising a larger age range, within a larger sample of obese individuals, and in those with more severe sleep apnea. Second, we only performed a blood draw in the morning after awakening (7:00). We have previously demonstrated that plasma IL-6 has a pulsatile 24-hour secretion (Vgontzas et al., 2005b), and TNFα (Uthgenannt et al., 1995), leptin (Langendonk et al., 1998), and adiponectin (Gavrila et al., 2003) also display circadian rhythmicity. Because this phenomenon is not detected in CRP (Meier-Ewert et al., 2001), combined with our consistent observation of elevated CRP with increasing apnea severity and cardiovascular problems, this suggests that CRP may be the best preclinical marker to assess the degree of inflammation in sleep apnea when only a single blood draw is possible. Finally, within some of our groups (e.g. control males), we have a small sample size, resulting in larger variability. While, overall, we examined a large sample of 120 participants, breaking them into discrete categories limited our statistical power. Despite this, Cohen’s d calculations suggest that the effect size of both apnea severity (Figure A.1) and hypertension (Figure B.1) on plasma CRP levels are moderate to large in both genders (Cohen’s d range = 0.64 – 1.09). Future studies should aim to recruit equal numbers of sleep apneics and controls.

In summary, our findings suggest that the inflammatory and metabolic abnormalities in sleep apnea should be analyzed and interpreted separately in men and women. Not only are there contrasting profiles between genders in controls vs. sleep apneics, but the impact of inflammation on cardiovascular risk also differs between men and women. Although healthy women have higher levels of plasma inflammatory and metabolic markers than their male
counterparts, the inflammatory response to obstructive sleep apnea does not appear to be as severe in females as it is in males. While the women of our sample are post-menopausal; it is likely that the protective effects of female hormones may still be in effect within these early stages. This is supported by work in a large population cohort suggesting that apnea in women peaks at age 65, roughly 15 years after the onset of menopause (Bixler et al., 2001). Finally, CRP, compared to other inflammatory markers, may be a clinically useful and reliable marker of apnea severity and comorbid cardiovascular problems in both men and women because its secretion is not influenced by circadian factors.
Appendix C

SYSTEMIC INFLAMMATION IS A BETTER PREDICTOR OF CARDIOMETABOLIC RISK THAN APNEA/HYPOPNEA INDEX IN MILD-TO-MODERATE OBSTRUCTIVE SLEEP APNEA
C.1. RATIONALE & HYPOTHESIS

A recent study of a middle-aged Icelandic general population cohort demonstrated that there is a large (36.6%) and relatively asymptomatic (i.e. no complaints of EDS, fatigue, or impaired cognition) population of individuals with OSA in the mild-to-moderate range (5 ≤ AHI < 30; Arnardottir et al., 2016). While most sleep clinicians would agree on when to treat more severe OSA (American Academy of Sleep Medicine, 2014), the decision of when to treat milder apnea continues to be a clinically gray area. When comparing the prevalence of OSA based on AHI criteria solely in men, the prevalence is two to three times higher compared to the prevalence when considering AHI plus the presence of clinical symptoms (Bixler et al., 1998). Furthermore, when examining AHI alone, the prevalence of OSA increases linearly in both men and women, reaching its peak in old age; on the other hand, the prevalence of OSA based on AHI plus clinical symptoms peaks in middle age (55 years for men, 65 years for women) then declines steeply into old age (Bixler et al., 1998; Bixler et al. 2001). It is unclear, then, which guidelines for diagnosis and treatment of OSA are most appropriate for clinicians to follow.

It is also known that the association of AHI with cardiometabolic outcomes is influenced by age, gender, obesity, depression, and other comorbid conditions. For instance, the association of apnea with hypertension decreases with age in men and women, suggesting that OSA in older individuals is not an independent risk factor for hypertension (Bixler et al., 2000). Furthermore, most longitudinal studies report little to no association of OSA with mortality, stroke, or heart failure until the threshold of AHI ≥ 30 (Marin et al., 2005; Arzt et al., 2005; Young et al., 2008; Punjabi et al., 2009; Redline et al., 2010; Gottlieb et al., 2010). Moreover, more recent studies suggest that measures of overnight oxygen desaturation, as measured by pulse oximetry, may be
better predictors of incident carotid plaques (Gunnarsson et al., 2015) and stroke (Stone et al., 2016) than AHI alone. Thus, there is a need for a biomarker to enhance the diagnostic accuracy and assess individual risk for cardiometabolic aberrations associated with mild-to-moderate OSA. Given the strong association of inflammation with cardiovascular and metabolic disease risk, we were interested in exploring the potential of CRP in this role.

The aim of this study was to examine the relative contribution of AHI and CRP in predicting cardiometabolic risk in individuals with mild-to-moderate OSA. We hypothesized that CRP would be more strongly associated with cardiometabolic aberrations than AHI in individuals with mild-to-moderate OSA.

C.2. METHODS

Middle-aged, relatively non-obese men and post-menopausal women (n = 62; 55.6% male, mean age 55.0 ± 0.7 years, mean BMI 29.2 ± 0.5 kg/m²) underwent an 8-hour polysomnography study. Mild-to-moderate OSA was defined as 5 ≤ AHI < 30.

Blood pressure was assessed in the evening before bed. A single fasting blood draw was taken upon awakening and assessed for CRP (via ELISA). Additional samples were sent to Quest Diagnostics™ for fasting glucose and total cholesterol analyses.

Because CRP concentrations were non-normal in the sample, this variable was base-10 logarithmically transformed. Linear and logistic regression models assessed the relative contribution of AHI and CRP in predicting the cardiometabolic outcomes fasting glucose and blood pressure. ANCOVA assessed differences in groups defined by combinations of low vs.
high CRP and AHI based on clinically relevant cut-offs (CRP ≥ 1.5 mg/L and AHI ≥ 10). All analyses were adjusted for age, gender, and BMI.

C.3. RESULTS

AHI alone was not a significant predictor of fasting glucose (p = 0.34), but CRP was (β = 0.34, p = 0.03). When both AHI and CRP were simultaneously entered into the model, the association of fasting glucose with CRP remained (β = 0.31, p = 0.05), even after adjusting for age, gender, and BMI (Table C.1).

<table>
<thead>
<tr>
<th></th>
<th>AHI</th>
<th>logCRP</th>
<th>AHI</th>
<th>logCRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.14 (p = 0.34)</td>
<td>0.34 (p = 0.03)</td>
<td>0.07 (p = 0.65)</td>
<td>0.31 (p = 0.05)</td>
</tr>
</tbody>
</table>

Three linear regressions: AHI as a predictor (#1), logCRP as a predictor (#2), and both AHI and logCRP (#3) as predictors of glucose in mild-to-moderate OSA (5 ≤ AHI < 30) (n = 53). Adjusted for age, gender, BMI. Standardized β reported.

Similarly, when examining the associations of AHI and CRP with clinically-elevated fasting glucose levels (≥ 100 mg/dL), AHI alone was not a significant predictor (p = 0.12), while CRP was (OR = 17.81, p = 0.01). When both AHI and CRP were simultaneously entered into the model, the association of elevated glucose with CRP remained (OR = 18.32, p = 0.02), even after adjusting for age, gender, and BMI (Table C.2).
Table C.2. AHI and CRP as predictors of elevated fasting glucose in mild-to-moderate OSA.

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AHI</td>
<td>logCRP</td>
<td>AHI</td>
</tr>
<tr>
<td>Elevated glucose</td>
<td>1.08 (p = 0.12)</td>
<td>17.81 (p = 0.01)</td>
<td>1.07 (p = 0.24)</td>
</tr>
</tbody>
</table>

Three logistic regressions: AHI as a predictor (#1), logCRP as a predictor (#2), and both AHI and logCRP (#3) as predictors of raised glucose in mild-to-moderate OSA (5 ≤ AHI < 30) (n = 53; n = 18 with elevated glucose). Controlled for age, gender, BMI. OR reported. Elevated glucose defined as ≥ 100 mg/dL. Odds ratio reported.

Although neither AHI nor CRP were significant predictors of systolic and diastolic blood pressure in the full sample, stratifying by gender revealed an association of CRP with diastolic blood pressure. While AHI alone did not predict blood pressure in men and women (all p > 0.05), CRP predicted diastolic blood pressure in men (β = 0.45, p = 0.02). When both AHI and CRP were simultaneously entered into the model, the association of CRP with diastolic blood pressure remained (β = 0.45, p = 0.02), adjusting for age and BMI (Table C.3).

Table C.3. AHI and CRP as predictors of systolic and diastolic blood pressure in mild-to-moderate OSA, stratified by gender.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>AHI</td>
<td>logCRP</td>
<td>AHI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n = 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.21 (p = 0.25)</td>
<td>0.12 (p = 0.63)</td>
<td>0.12 (p = 0.55)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0.22 (p = 0.24)</td>
<td>0.45 (p = 0.02)</td>
<td>0.03 (p = 0.17)</td>
</tr>
</tbody>
</table>

Women (n = 28)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AHI</td>
<td>logCRP</td>
<td>AHI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-0.15 (p = 0.48)</td>
<td>0.17 (p = 0.45)</td>
<td>-0.18 (p = 0.39)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.38 (p = 0.06)</td>
<td>-0.32 (p = 0.17)</td>
<td>-0.34 (p = 0.09)</td>
</tr>
</tbody>
</table>

Three linear regressions: AHI as a predictor (#1), logCRP as a predictor (#2), and both AHI and logCRP (#3) as predictors of blood pressure in mild-to-moderate OSA (5 ≤ AHI < 30) (n = 34 men, n = 28 women). Controlled for age, BMI. Standardized β reported.

To further explore the potential role of AHI in predicting blood pressure, fasting glucose, and total cholesterol, the sample was split into two groups using the clinically-meaningful cut-off
of AHI = 10: ↓ AHI (AHI < 10; n = 26) and ↑ AHI (AHI ≥ 10; n = 36). Adjusting for age, gender, BMI, and logCRP, there were no significant differences, however, between the two groups in terms of the cardiometabolic outcomes examined (all p > 0.44; Table C.4).

**Table C.4.** Cardiometabolic outcomes in adults with mild-to-moderate OSA, stratified by AHI severity.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↓ AHI (n = 26)</td>
<td>↑ AHI (n = 36)</td>
<td></td>
</tr>
<tr>
<td>AHI (events/h)</td>
<td>7.96 (0.73)</td>
<td>18.66 (0.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.88 (0.30)</td>
<td>2.47 (0.26)</td>
<td>0.15</td>
</tr>
<tr>
<td>Systolic BP (n = 61) (mmHg)</td>
<td>135.29 (3.32)</td>
<td>133.16 (2.89)</td>
<td>0.64</td>
</tr>
<tr>
<td>Diastolic BP (n = 61) (mmHg)</td>
<td>79.39 (1.86)</td>
<td>77.79 (1.62)</td>
<td>0.53</td>
</tr>
<tr>
<td>Glucose (n = 53) (mg/dL)</td>
<td>99.66 (3.92)</td>
<td>101.85 (3.28)</td>
<td>0.68</td>
</tr>
<tr>
<td>Cholesterol (n = 48) (mg/dL)</td>
<td>206.63 (9.79)</td>
<td>216.69 (8.23)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Mean (SEM) blood pressure, glucose, and total cholesterol values based on groups defined by clinically-meaningful AHI cut-offs in mild-to-moderate apneics (5 < AHI ≤ 30; n = 62). Adjusted for age, gender, BMI, and logCRP. (↓ AHI = < 10; ↑ AHI = ≥ 10)

To further explore the potential role of CRP in predicting blood pressure, fasting glucose, and total cholesterol, the sample was split into two groups using the clinically-meaningful cut-off of CRP = 1.5 mg/L: ↓CRP (CRP < 1.5 mg/L; n = 25) and ↑ CRP (CRP ≥ 1.5 mg/L; n = 37).

Adjusting for age, gender, BMI, and AHI, total cholesterol was significantly elevated in the ↑ CRP group (225.16 ± 7.35 mg/dL vs. 191.40 ± 9.61 mg/dL, p = 0.009). Of note, while fasting glucose was not significantly higher in the ↑ CRP group (p = 0.18), it was clinically elevated (103.83 ± 3.39 mg/dL) according to the definition of the metabolic syndrome (Alberti et al., 2006) compared to the ↓ CRP group (95.78 ± 4.61 mg/dL) (Table C.5).
Table C.5. Cardiometabolic outcomes in adults with mild-to-moderate OSA, stratified by CRP levels.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 ↓ CRP (n = 25)</th>
<th>Group 2 ↑ CRP (n = 37)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI (events/h)</td>
<td>12.32 (1.33)</td>
<td>15.45 (1.08)</td>
<td>0.08</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.96 (0.25)</td>
<td>3.08 (0.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (n = 61) (mmHg)</td>
<td>135.49 (3.52)</td>
<td>133.08 (2.94)</td>
<td>0.61</td>
</tr>
<tr>
<td>Diastolic BP (n = 61) (mmHg)</td>
<td>79.22 (1.91)</td>
<td>77.76 (1.60)</td>
<td>0.63</td>
</tr>
<tr>
<td>Glucose (n = 53) (mg/dL)</td>
<td>95.78 (4.61)</td>
<td>103.83 (3.39)</td>
<td>0.18</td>
</tr>
<tr>
<td>Cholesterol (n = 48) (mg/dL)</td>
<td>191.40 (9.61)</td>
<td>225.16 (7.35)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Mean (SEM) blood pressure, glucose, and total cholesterol values based on groups defined by clinically-meaningful cut-offs in mild-to-moderate apneics (5 < AHI ≤ 30; n = 62). Adjusted for age, gender, BMI, and AHI. ↓ CRP = < 1.5 mg/L; ↑ CRP = ≥ 1.5 mg/L.

Finally, to examine the joint impact of AHI and CRP on cardiometabolic outcomes, the sample was split into four groups based on combinations of the AHI and CRP cut-offs used above. There was a significant interaction between AHI ≥ 10 and CRP ≥ 1.5 mg/L on cholesterol (p = 0.009). Specifically, adjusting for age, gender, and BMI, the group with ↑AHI and ↑ CRP had significantly higher total cholesterol (231.86 ± 2.63 mg/dL) than the group with ↑AHI and ↓ CRP (176.14 ± 14.23 mg/dL; p < 0.01), with a trend elevation in both ↑ CRP groups compared to the ↓ CRP groups (p < 0.1). There was also a trend (p < 0.1) elevation in both ↑ CRP groups compared to the ↓ AHI/↓ CRP group in terms of fasting glucose levels which, of note, were clinically elevated according to the definition of metabolic syndrome (Table C.6).
Table C.6. Cardiometabolic outcomes in adults with mild-to-moderate OSA, stratified by the joint effect of AHI and CRP levels.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 15)</th>
<th>Group 2 (n = 10)</th>
<th>Group 3 (n = 11)</th>
<th>Group 4 (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI (events/h)</td>
<td>↓ AHI, ↓ CRP</td>
<td>↑ AHI, ↓ CRP</td>
<td>↓ AHI, ↑ CRP</td>
<td>↑ AHI, ↑ CRP</td>
</tr>
<tr>
<td></td>
<td>7.73 (1.00)</td>
<td>19.86 (1.25)</td>
<td>8.43 (1.18)</td>
<td>18.16 (0.76)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.75 (0.31)</td>
<td>1.28 (0.38)</td>
<td>3.45 (0.36)</td>
<td>2.92 (0.23)</td>
</tr>
<tr>
<td>Systolic BP (n = 61) (mmHg)</td>
<td>134.83 (4.51)</td>
<td>136.52 (5.64)</td>
<td>133.77 (5.34)</td>
<td>132.74 (3.59)</td>
</tr>
<tr>
<td>Diastolic BP (n = 61) (mmHg)</td>
<td>80.41 (2.44)</td>
<td>77.31 (3.05)</td>
<td>77.74 (2.89)</td>
<td>78.10 (1.94)</td>
</tr>
<tr>
<td>Glucose (n = 53) (mg/dL)</td>
<td>90.96 (5.65)</td>
<td>104.57 (7.66)</td>
<td>106.12 (6.31)</td>
<td>102.72 (3.99)</td>
</tr>
<tr>
<td>Cholesterol (n = 48) (mg/dL)</td>
<td>203.81 (12.25)</td>
<td>176.14 (14.23)</td>
<td>211.56 (12.72)</td>
<td>231.86 (2.63)</td>
</tr>
</tbody>
</table>

Mean (SEM) blood pressure, glucose, and total cholesterol values based on groups defined by clinically-meaningful AHI and CRP cut-offs in mild-to-moderate apnoeics (5 < AHI ≤ 30; n = 62). Adjusted for age, gender, BMI. ↓ AHI = < 10; ↑ AHI = ≥ 10; ↓ CRP = < 1.5 mg/L; ↑ CRP = ≥ 1.5 mg/L. "p < 0.1 vs. Group 1; "p < 0.1 vs. group 2; "p < 0.01 vs. Group 2

C.4. DISCUSSION

CRP was a stronger predictor of fasting glucose levels and elevated glucose (≥ 100 mg/dL) as well as diastolic blood pressure (men) than AHI in individuals with mild-to-moderate OSA. Elevated total cholesterol and fasting glucose levels were also observed as function of elevated CRP levels, but not elevated AHI. In sum, incorporating a measure of systemic inflammation, such as CRP, in assessing the medical severity of mild-to-moderate OSA strongly enhances the diagnostic utility of AHI, which may have implications in how OSA is diagnosed and treated.

Although CPAP remains the gold standard treatment for OSA, compliance is a significant problem. In a recent long-term study of CPAP use, 31% of patients with OSA who were prescribed CPAP never commenced therapy after diagnosis and titration, and an additional 15% abandoned their CPAP after an average of 10 months of use (Wolkove et al., 2008). Importantly, it has been demonstrated that CPAP does not significantly reduce IL-6, TNFα, TNFR1 (Vgontzas et al., 2008; Arias et al., 2008), CRP (Kohler et al., 2009), leptin (Hoyos et al., 2012;
Kritikou et al., 2014), nor fasting insulin and glucose (Vgontzas et al., 2008), even after comparing groups with low vs. high CPAP compliance (Kritikou et al., 2014). A sham-controlled study also demonstrated no reversal of metabolic syndrome after 12 weeks of CPAP use (Hoyos et al., 2013).

On the other hand, a placebo-controlled, double-blind study that administered the TNFα antagonist etanercept for three weeks reported significantly reduced AHI, decreased plasma IL-6, and reduced objective sleepiness in obese men with severe sleep apnea (Vgontzas et al., 2004a). Similarly, there is recent evidence suggesting that anti-inflammatory therapy (intranasal and/or oral) in children with mild OSA is effective in reducing apnea severity (Kheirandish-Gozal et al., 2014). Thus, it appears that there is an unmet need for an OSA treatment that targets inflammation.

Based on these findings, a focus on the degree of inflammation, rather than the severity of the AHI, may be a better predictor of the cardiometabolic risk associated with OSA. Specifically, when a physician is presented with a case of mild (5 ≤ AHI < 15) or moderate (15 ≤ AHI < 30) OSA and is unsure of whether, when, or how to treat, a measure of plasma CRP may provide guidance. Regardless of whether or not CPAP is deemed necessary, (co)treatment with an anti-inflammatory agent may be able to reduce AHI, lower cardiometabolic disease risk, or both. Interestingly, and unlike other markers of inflammation, CRP lacks diurnal rhythmicity (Meier-Ewert et al., 2001), which may explain why CRP has emerged as a marker of considerable interest in prospective cardiovascular and epidemiological research (Ridker, 2003).

In sum, our findings suggest that CRP is more strongly associated with cardiometabolic aberrations than AHI in individuals with mild-to-moderate OSA, and that the use of a biomarker
like CRP may enhance diagnostic accuracy and better assess individual risk for future cardiometabolic problems.
Appendix D

A SINGLE NIGHT OF POLYSOMNOGRAPHY IS USEFUL FOR RELIABLY CLASSIFYING ONE’S HABITUAL SLEEP

D.1. RATIONALE & HYPOTHESIS

In the realm of sleep research, one of the most global and hotly-debated questions has long been, “How many nights are enough?” Due to the time and expense associated with in-laboratory polysomnography (PSG), sleep researchers and clinicians often have to record participants for a single night only, especially in large epidemiological studies. The issue of night-to-night variability in some components of sleep, however, raises the question of how many nights in the laboratory will provide a sufficient representation of one’s habitual sleep patterns.

The concept of the “first-night effect,” or the well-recognized observation that participants do not sleep as well during their first night in the laboratory as they would on subsequent nights, has made multi-night studies a common practice. Agnew and colleagues first recognized this phenomenon in 1966 in 43 participants who spent four consecutive nights in the laboratory. Compared to the following nights, the first night’s sleep was significantly more disrupted, with more total wake time and a higher proportion of stage 1 sleep, as well as significantly less REM and delayed slow-wave sleep (Agnew et al., 1966). Subsequent work by Merica and Gaillard in the 1980s suggested that only stage 4 sleep produced stable results across consecutive study nights (Merica and Gaillard, 1985), and more recent work using quantitative EEG (QEEG) methods has proposed that, compared to visual scoring, only certain components of QEEG-scored sleep exhibit stability across nights (Tan et al., 2000; Tucker et al., 2007; Geiger et al., 2011; Tarokh et al., 2011; Israel et al., 2012). Statistical projections have even suggested that it may take between one and three weeks to achieve stability of certain sleep components (Larsen et al., 1995; Wohlgemuth et al., 1999). Many of these studies, however, have been confounded by participants of older age, the use of ad libitum sleep schedules, or did
not take obesity into account—factors that critically affect night-to-night stability of sleep (Zheng et al., 2012). However, Lorenzo and Barbanoj (2002) reported that, in healthy volunteers across separate sessions of consecutive nights in the lab, only the “very first night” was significantly different, and only in REM-related variables. Their findings suggest that, once familiar with the PSG equipment, participants exhibit stability in sleep across consecutive nights.

The issue of night-to-night variability in the sleep of insomniacs has also raised questions as to how many nights are enough; however, research on the utility of a single night of PSG has been mixed. A large study of controls, insomniacs, and patients with movement and behavioral disorders observed a significant first-night effect in all four groups, although it was most pronounced in insomniacs (Newell et al., 2012). Similarly, a two-week actigraphy study of older adults reported that those with insomnia complaints exhibited significantly more night-to-night variability than those without (Kay et al., 2013). However, Vallières and colleagues have demonstrated that even when insomniacs are clustered into three groups based on sleep diary variability, the groups do not differ on PSG variables across consecutive nights (Vallières et al., 2011). Furthermore, Edinger reported that night-to-night variability across three nights in the laboratory do not differ between insomniacs and controls (Edinger et al., 1997), and have suggested that a single night of PSG may be sufficient when the goal is to determine a diagnosis of insomnia (Edinger et al., 1991). Additionally, in the last several years, studies have begun to utilize a single night’s sleep duration as a predictor of the cardiometabolic risks and neuropsychological deficits associated with chronic insomnia. Specifically, sleep duration extracted from one night has been used in large epidemiological samples to classify participants into “short” and “normal” sleepers (Vgontzas et al., 2009a; Vgontzas et al., 2009b; Vgontzas et al., 2010; Fernandez-Mendoza et al., 2010; Fernandez-Mendoza et al., 2012; Vgontzas et al.,
The accuracy and stability of a single night in categorizing participants into these groups, however, has not been assessed.

Our aim was to evaluate sleep duration stability over the short-term (consecutive nights) in relatively young, non-obese samples of both insomniacs and controls, as well as over the long-term (across several years) in a general population sample of men. Our other major goal was to examine the persistence of subjects’ classification as “short” or “normal” sleep duration from the first night to subsequent nights in the laboratory. We hypothesized that while night-to-night stability would be low to moderate, one night in the sleep lab would be a reliable marker of one’s classification as a “short” or “normal” sleeper.

D.2. METHODS

D.2.1. Participants

The study sample consisted of 150 insomniacs and 151 normal-sleeping controls who spent multiple consecutive nights in the sleep laboratory (“short-term” cohort), as well as 95 men who visited the sleep laboratory twice, with several years between visits (“long-term” cohort). Insomniacs (52.0% male, mean age 36.6 ± 1.1y) were recruited from both the local community and the Sleep Disorders Clinic at the Penn State Milton S. Hershey Medical Center, complained of chronic difficulty (> 6 months) in initiating or maintaining sleep (Kales et al., 1991; Vgontzas et al., 1994; Vgontzas et al., 1995), and were not receiving treatment or medication for their insomnia at the time of the study. Controls (56.3% male, mean age 36.0 ± 1.2y) were non-apneics who reported no sleep complaints and were drawn from screening and baseline nights of
studies on sleep restriction (n=36) (Vgontzas et al., 2004b; Pejovic et al., 2013), total sleep deprivation (n=41) (Pejovic et al., 2010), or served as controls in one of two studies examining the effect of continuous positive airway pressure on obstructive sleep apnea (n=74) (Vgontzas et al., 2007; Vgontzas et al., 2008; Kritikou et al., 2013; Kritikou et al., 2014). In addition, a subsample of 95 men (mean age 51.1 ± 1.1y) from the Penn State Adult Cohort, a random general population sample of 1,741 adults (Bixler et al., 1998), visited the sleep laboratory on two separate occasions. All studies were approved by the University Institutional Review Board (Pennsylvania State University College of Medicine) and all participants provided written informed consent.

D.2.2. Sleep Laboratory Protocol

During their visit in the laboratory, all participants underwent a physical examination, during which height and weight were recorded and body mass index (BMI) calculated (in kg/m²). Sleep laboratory recordings were conducted in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was monitored continuously for 8 h (22:30-23:00 until 6:30-7:00) using 16-channel polygraph recordings of EEG, electrooculogram (EOG) and electromyogram (EMG). Polysomnography (PSG), respiration (via thermocouple and thoracic strain gauges), and oximeter data were collected using Grass-Telefactor Gamma Sleep Recording software (Middleton, WI, USA). Insomniacs and controls spent three consecutive nights in the laboratory, and men from the general population cohort spent two single nights, with an average of 2.6 years between each visit. Visual sleep stage scoring was conducted by a registered polysomnography technologist.
based on Rechtschaffen and Kales criteria (1968). Apnea-hypopnea index (AHI, or the number of apneas and hypopneas summed per hour) was also ascertained; an apnea was defined as cessation of airflow for ≥ 10 seconds and an out-of-phase strain gauge movement, while a hypopnea was defined as a 50% airflow reduction and associated decrease in SpO2 of at least 4% (Bixler et al., 1998). Those with an AHI of ≥ 5 were excluded from the control and insomnia samples.

**D.2.3. Statistical Analysis**

For descriptive purposes, sociodemographic and sleep characteristics were compared using independent-samples t-tests and paired-samples t-tests (two-tailed) in insomniacs/controls and the longitudinal cohort, respectively. Specifically, differences in the PSG variables total sleep time (TST), sleep efficiency (SE), sleep onset latency (SOL), wake after sleep onset (WASO), and percentage of stages 1, 2, slow-wave sleep (SWS), and rapid eye movement (REM) sleep were assessed between groups (controls/insomniacs) or across time (longitudinal cohort). When appropriate, Pearson’s r examined associations between sociodemographic variables and sleep outcomes.

Given their consistent association with cardiometabolic and insomnia-related outcomes in the sleep literature, we were then particularly interested in examining within-subjects short-term (in insomniacs and controls) and long-term (longitudinal cohort) stability of variables associated with sleep duration (TST, SOL, and WASO). Intraclass correlation coefficients (ICC), which incorporate both between- and within-subjects variance and are commonly used in test-retest reliability analyses, were computed separately for each outcome. Specifically, under the
framework of analysis of variance, the total variance in the data can be attributed to two major systematic sources: between- and within-subjects variances. ICCs are interpreted as the proportion of total variance explained by between-subjects variance; in our study, ICCs reflect the proportion of variance in sleep characteristics explained by differences between participants. As the study participants are considered a random sample of the larger population, the between-subjects factor is treated as a random factor in the analysis. To retain the generalizability of the results, the nights included in the ICC analyses were also considered a random subset of all possible nights. Therefore, two-way random-effects ICCs were calculated to examine the short- and long-term stability of sleep characteristics. To explore the utility of one or more nights to attain stability, both “single measures” (ICC[2,1]) and “average measures” (ICC[2,k]) were calculated based on a single night of data versus the average of multiple (k) nights of data, respectively (Shrout et al., 1979). Coefficients and their 95% confidence intervals (truncated when < 0) were interpreted using Landis and Koch (1977) benchmark values: 0.00 – 0.20 = “poor stability,” 0.21 – 0.40 = “slight stability,” 0.41 – 0.60 = “moderate stability,” 0.61 – 0.80 = “substantial stability,” and 0.81 – 1.00 = “almost perfect stability.” For simplicity, and in line with previous studies (Landis and Koch 1977; Israel et al., 2012), we denote ICC ≥ 0.60 as variables demonstrating “reliable” short- or long-term stability. By using these cut-offs and incorporating two forms of ICCs, we were then able to examine the utility of either a single night (“single measures”) or multiple nights (“average measures”) in attaining stable measures of sleep duration. Between- and within-subjects variances were also calculated from ANOVA output as the between- or within-people mean square minus the mean square error, divided by sample size.

To examine the stability of one’s sleep duration classification, individuals were categorized as “short” or “normal” sleepers for each of their nights in the laboratory as defined
by the median TST for Night 1 as well as the median for the average TST of subsequent nights (< median TST = “short sleeper”; ≥ median TST = “normal sleeper”). Separate cut-offs were used for Night 1 and subsequent nights in our three study samples to take into account the first-night effect, the heterogeneity of the groups, and to address the issue of regression to the mean. Persistence of the individuals’ first-night classification on subsequent nights was assessed through contingency tables. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 20.

D.3. RESULTS

D.3.1. Short-term sleep stability

Sociodemographic and PSG characteristics of controls and insomniacs are shown in Table D.1. Though they did not differ in age or gender distribution, the control group had a higher body mass index. Controls slept significantly more over three nights on average compared to insomniacs and had significantly higher sleep efficiency, while insomniacs had a much longer SOL. Of note, controls experienced more WASO by 8.1 minutes, which was significantly associated with increased BMI in controls (Pearson’s r = 0.45, p < 0.001), but not insomniacs (r = -0.06, p = 0.52). Additionally, compared to controls, insomniacs had less stage 1 and slow-wave sleep, but more stage 2 and REM (Table D.1).
Table D.1. Sociodemographic and polysomnographic sleep characteristics of controls and insomniacs.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=151)</th>
<th>Insomniac (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Male</td>
<td>56.3</td>
<td>52.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.0 (1.2)</td>
<td>36.6 (1.1)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5 (0.4)</td>
<td>24.1 (0.4)</td>
</tr>
<tr>
<td>TST (min.)</td>
<td>416.7 (3.0)</td>
<td>392.4 (3.8)</td>
</tr>
<tr>
<td>SE (%)</td>
<td>86.8 (0.6)</td>
<td>81.7 (0.8)</td>
</tr>
<tr>
<td>SOL (min.)</td>
<td>17.0 (1.1)</td>
<td>49.2 (3.1)</td>
</tr>
<tr>
<td>WASO (min.)</td>
<td>46.5 (2.7)</td>
<td>38.4 (2.5)</td>
</tr>
<tr>
<td>Stage 1 (%)</td>
<td>8.7 (0.4)</td>
<td>6.4 (0.3)</td>
</tr>
<tr>
<td>Stage 2 (%)</td>
<td>60.2 (0.8)</td>
<td>65.0 (0.6)</td>
</tr>
<tr>
<td>SWS (%)</td>
<td>12.5 (0.7)</td>
<td>6.8 (0.6)</td>
</tr>
<tr>
<td>REM (%)</td>
<td>18.6 (0.5)</td>
<td>21.8 (0.4)</td>
</tr>
</tbody>
</table>

Mean values reported with standard error of the mean (SEM) in parentheses. PSG variables represent three consecutive nights in controls and insomniacs. TST = total sleep time, SE = sleep efficiency, SOL = sleep onset latency, WASO = wake after sleep onset, SWS = slow-wave sleep, REM = rapid eye movement sleep. \(^a p < 0.05; ^bp < 0.001\)

Intraclass correlation coefficients for TST, SOL, and WASO in controls and insomniacs across three consecutive nights are shown in Table D.2. Single-measure TST was moderately stable in controls (ICC = 0.50) and insomniacs (ICC = 0.43), while average-measure TST was substantially stable (ICC = 0.75 and 0.69, respectively). Similarly, single-measure SOL was slightly stable in controls (ICC = 0.39) and moderately stable in insomniacs (ICC = 0.57), but demonstrated substantial stability when average measures were applied (ICC = 0.66 and 0.80, respectively). Single-measure WASO demonstrated moderate stability in controls (ICC = 0.59) and slight stability in insomniacs (ICC = 0.37), though average-measure WASO was substantially to almost perfectly stable (ICC = 0.81 and 0.64, respectively). Of note, within-subjects variances were consistently lower than between-subjects variances for all sleep outcomes examined (Table D.2).
Table D.2. Intraclass correlations (ICC) for sleep outcomes in controls and insomniacs.

<table>
<thead>
<tr>
<th></th>
<th>ICC Nights 1-3 Single Measure</th>
<th>ICC Nights 1-3 Average Measure</th>
<th>Between-Subjects Variance</th>
<th>Within-Subjects Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 151)</td>
<td>TST</td>
<td>0.50 (0.40-0.59)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75 (0.67-0.81)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>990.28</td>
</tr>
<tr>
<td></td>
<td>SOL</td>
<td>0.39 (0.29-0.49)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 (0.55-0.74)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.27</td>
</tr>
<tr>
<td></td>
<td>WASO</td>
<td>0.59 (0.50-0.67)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81 (0.76-0.86)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>922.17</td>
</tr>
<tr>
<td><strong>Insomniacs</strong></td>
<td>TST</td>
<td>0.43 (0.32-0.53)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69 (0.59-0.77)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1540.54</td>
</tr>
<tr>
<td>(n = 150)</td>
<td>SOL</td>
<td>0.57 (0.48-0.65)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 (0.73-0.85)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1198.69</td>
</tr>
<tr>
<td></td>
<td>WASO</td>
<td>0.37 (0.27-0.47)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64 (0.52-0.73)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>594.74</td>
</tr>
</tbody>
</table>

ICC represents three consecutive nights in controls and insomniacs, with 95% CI in parentheses. TST = total sleep time, SOL = sleep onset latency, WASO = wake after sleep onset. <sup>a</sup>p < 0.001.

When examining sleep stages (stages 1, 2, slow-wave sleep [SWS] and rapid eye movement [REM] sleep), single-measure ICCs were substantially stable in controls and slightly to moderately stable in insomniacs; stability was improved to almost perfect (controls) and moderate to substantial (insomniacs) when examining average measures (Table D.3).

Interestingly, in terms of SWS, a single night yielded almost perfect stability in both normal-sleeping controls and insomniacs (both ICC = 0.89).

Table D.3. Intraclass correlations (ICC) for sleep stage outcomes in controls and insomniacs.

<table>
<thead>
<tr>
<th></th>
<th>ICC Nights 1-3 Single Measure</th>
<th>ICC Nights 1-3 Average Measure</th>
<th>Between-Subjects Variance</th>
<th>Within-Subjects Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 151)</td>
<td>Stage 1</td>
<td>0.74 (0.67-0.79)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89 (0.86-0.92)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.93</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>0.81 (0.76-0.85)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93 (0.90-0.95)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.90</td>
</tr>
<tr>
<td></td>
<td>SWS</td>
<td>0.89 (0.86-0.92)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96 (0.95-0.97)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.43</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>0.70 (0.63-0.76)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 (0.83-0.91)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.90</td>
</tr>
<tr>
<td><strong>Insomniacs</strong></td>
<td>Stage 1</td>
<td>0.34 (0.24-0.45)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61 (0.49-0.71)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.10</td>
</tr>
<tr>
<td>(n = 150)</td>
<td>Stage 2</td>
<td>0.62 (0.51-0.70)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83 (0.76-0.88)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.53</td>
</tr>
<tr>
<td></td>
<td>SWS</td>
<td>0.89 (0.86-0.92)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96 (0.95-0.97)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.02</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>0.47 (0.33-0.60)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 (0.60-0.82)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.63</td>
</tr>
</tbody>
</table>

ICC represents three consecutive nights in controls and insomniacs, with 95% CI in parenthesis. SWS = slow-wave sleep, REM = rapid eye movement sleep. <sup>a</sup>p < 0.001.
D.3.2. Long-term sleep stability

Sociodemographic and PSG characteristics of the longitudinally-studied general population sample of 95 men are shown in Table D.4. The average time between sleep studies was 2.6 years, and neither BMI nor apnea-hypopnea index changed significantly over time (all \( p > 0.05 \)). There were also no differences in TST, SE, SOL, WASO, or any of the sleep architecture variables in this group after 2.6 years, except that stage 2 was significantly reduced by 1.9%.

Table D.4. Sociodemographic characteristics of longitudinal sample (n = 95 men).

<table>
<thead>
<tr>
<th></th>
<th>Night 1</th>
<th>Night 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.1 (1.1)</td>
<td>53.7 (1.1)</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>30.2 (0.6)</td>
<td>30.6 (0.7)</td>
</tr>
<tr>
<td>AHI (events/hour)</td>
<td>9.7 (2.4)</td>
<td>5.9 (1.0)</td>
</tr>
<tr>
<td>TST (min.)</td>
<td>356.4 (7.1)</td>
<td>354.7 (6.6)</td>
</tr>
<tr>
<td>SE (%)</td>
<td>74.2 (1.5)</td>
<td>73.9 (1.4)</td>
</tr>
<tr>
<td>SOL (min.)</td>
<td>24.4 (3.1)</td>
<td>24.8 (2.9)</td>
</tr>
<tr>
<td>WASO (min.)</td>
<td>98.5 (6.1)</td>
<td>99.0 (5.7)</td>
</tr>
<tr>
<td>Stage 1 (%)</td>
<td>15.7 (0.8)</td>
<td>16.7 (0.8)</td>
</tr>
<tr>
<td>Stage 2 (%)</td>
<td>68.1 (0.7)</td>
<td>66.2 (0.8)</td>
</tr>
<tr>
<td>SWS (%)</td>
<td>0.62 (0.1)</td>
<td>0.45 (0.1)</td>
</tr>
<tr>
<td>REM (%)</td>
<td>15.6 (0.5)</td>
<td>16.6 (0.6)</td>
</tr>
</tbody>
</table>

Mean values reported with standard error of the mean (SEM) in parentheses. TST = total sleep time, SE = sleep efficiency, SOL = sleep onset latency, WASO = wake after sleep onset, SWS = slow-wave sleep, REM = rapid eye movement sleep. *\( p < 0.01 \); †\( p < 0.001 \).

Table D.5 shows intraclass correlation coefficients for the sleep duration variables TST, SOL, and WASO across the two nights. Single-measure TST and WASO showed moderate long-term stability (ICC = 0.50 and 0.44, respectively); however, there was a poor correlation in SOL between the two time points (ICC = 0.18). Average-measure SOL was slightly stable across
several years (ICC = 0.30), while TST and WASO showed substantial stability (ICC = 0.67 and 0.61, respectively). Again, within-subjects variances were consistently lower than between-subjects variances for all sleep outcomes examined (Table D.5).

<table>
<thead>
<tr>
<th>Male Cohort</th>
<th>TST</th>
<th>ICC Nights 1, 2 Single Measure</th>
<th>ICC Nights 1, 2 Average Measure</th>
<th>Between-Subjects Variance</th>
<th>Within-Subjects Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 95)</td>
<td>SOL</td>
<td>0.50 (0.34-0.64)</td>
<td>0.67 (0.51-0.78)</td>
<td>1493.95</td>
<td>13.89</td>
</tr>
<tr>
<td></td>
<td>WASO</td>
<td>0.18 (0.00-0.37)</td>
<td>0.30 (0.00-0.54)</td>
<td>99.39</td>
<td>4.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44 (0.26-0.59)</td>
<td>0.61 (0.41-0.74)</td>
<td>958.82</td>
<td>12.23</td>
</tr>
</tbody>
</table>

ICC represents two nights with 2.6 years between visits, with 95% CI in parentheses. TST = total sleep time, SOL = sleep onset latency, WASO = wake after sleep onset. ^p < 0.05; ^p < 0.001.

In terms of sleep stages, single-measure ICCs were moderately stable, and became substantially stable when assessing average-measures (Table D.6). SWS stability, however, demonstrated only slight to moderate stability; this is likely due to the loss of SWS that occurred between the initial visit and follow-up several years later (Table D.4).

<table>
<thead>
<tr>
<th>Male Cohort</th>
<th>Stage 1</th>
<th>ICC Nights 1, 2 Single Measure</th>
<th>ICC Nights 1, 2 Average Measure</th>
<th>Between-Subjects Variance</th>
<th>Within-Subjects Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 95)</td>
<td>SWS</td>
<td>0.29 (0.09-0.46)</td>
<td>0.45 (0.17-0.63)</td>
<td>0.51</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>REM</td>
<td>0.51 (0.34-0.64)</td>
<td>0.67 (0.51-0.78)</td>
<td>17.22</td>
<td>0.34</td>
</tr>
</tbody>
</table>

ICC represents two nights with 2.6 years between visits, with 95% CI in parentheses. SWS = slow-wave sleep, REM = rapid eye movement sleep. ^p < 0.01; ^p < 0.001
Persistence of Night 1 classification on subsequent nights

Median sleep durations for Night 1 as well as the average of subsequent nights in the sleep laboratory are presented for controls, insomniacs, and the longitudinal cohort of men in Table D.7. In controls, when Nights 2 and 3 were averaged and their median used as a cutoff, 71.4% of “short” and “normal” sleepers on Night 1 retained these classifications over two subsequent nights. Similarly, 74.7% of insomniacs remained in the same category over three nights in the laboratory. In the longitudinal sample, 72.6% of the group retained their “short” or “normal” classifications over 2.6 years.

Table D.7. Stability of sleep classification in controls, insomniacs, and the longitudinal sample.

<table>
<thead>
<tr>
<th></th>
<th>Short-Term</th>
<th>Night 1</th>
<th>Long-Term</th>
<th>Night 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 151)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Short” sleep duration</td>
<td></td>
<td>&lt; 425.0</td>
<td>“Short” sleep duration</td>
<td>&lt; 367.9</td>
</tr>
<tr>
<td>(min.)</td>
<td></td>
<td>&lt; 430.3</td>
<td>(min.)</td>
<td>&lt; 365.0</td>
</tr>
<tr>
<td>“Normal” sleep duration</td>
<td></td>
<td>≥ 425.0</td>
<td>“Normal” sleep duration</td>
<td>≥ 367.9</td>
</tr>
<tr>
<td>(min.)</td>
<td></td>
<td>≥ 430.3</td>
<td>(min.)</td>
<td>≥ 365.0</td>
</tr>
<tr>
<td>Persistence of Night 1 classification</td>
<td></td>
<td></td>
<td>Persistence of Night 1 classification</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.4%</td>
<td></td>
<td>72.6%</td>
</tr>
<tr>
<td><strong>Insomniacs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 150)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Short” sleep duration</td>
<td></td>
<td>&lt; 391.0</td>
<td>“Short” sleep duration</td>
<td>&lt; 367.9</td>
</tr>
<tr>
<td>(min.)</td>
<td></td>
<td>&lt; 412.5</td>
<td>(min.)</td>
<td>&lt; 365.0</td>
</tr>
<tr>
<td>“Normal” sleep duration</td>
<td></td>
<td>≥ 391.0</td>
<td>“Normal” sleep duration</td>
<td>≥ 367.9</td>
</tr>
<tr>
<td>(min.)</td>
<td></td>
<td>≥ 412.5</td>
<td>(min.)</td>
<td>≥ 365.0</td>
</tr>
<tr>
<td>Persistence of Night 1 classification</td>
<td></td>
<td></td>
<td>Persistence of Night 1 classification</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>74.7%</td>
<td></td>
<td>72.6%</td>
</tr>
</tbody>
</table>

“Short” and “normal” sleep classification as determined by median sleep duration in controls, insomniacs, and a longitudinal general population sample.
D.4. DISCUSSION

We examined the short-term stability of sleep duration in 150 insomniacs and 151 normal-sleeping controls. We also assessed long-term sleep stability over several years in a longitudinal general population sample of 95 middle-aged men. Overall, while the variables total sleep time, sleep onset latency, and wake after sleep onset were only slightly to moderately stable according to single-measures intraclass correlations, the average of three consecutive nights in the laboratory, or two nights separated by several years, produced moderately to very strongly stable (ICC ≥ 0.60) within-subject assessments of sleep duration. Furthermore, the majority of controls (71.4%) and insomniacs (74.7%) who were classified objectively as “short” or “normal” sleepers during their first night in the lab retained these classifications over consecutive nights. Similarly, 72.6% of the longitudinal sample remained “short” or “normal” sleepers across several years. In sum, our findings suggest that while a single night in the laboratory may not yield the most reliable, reproducible measures, three consecutive nights, or two single-night recordings separated by several years, are sufficient. Importantly, however, a single night may be useful for reliably classifying one’s sleep duration over the short-term and long-term.

Few studies employing visually-scored sleep have examined stability across consecutive nights. In Merica and Gaillard’s (1985) analysis of 147 healthy adults aged 16 to 71 years, only stage 4 sleep produced reliable results over several recordings. Extending these findings in 50 older adults (ages 54 to 82 years), Larsen and colleagues (1995) reported that, compared to spectral power scoring techniques, visually-scored slow-wave sleep (stages 3 and 4) had a significantly lower correlation over two consecutive nights. Using a Spearman-Brown formula to
approximate projected slow-wave sleep stability over more nights, Larsen estimated that it would take six nights of visually-scored slow-wave sleep to attain the same reliability as two nights of computer-analyzed sleep (Larsen et al., 1995).

Similar to the present study, Wohlgemuth and colleagues (1999) studied controls and insomniacs for three consecutive nights in the laboratory with PSG. Using statistical projections, the authors concluded that a week of visually-scored PSG recordings is required to achieve adequate stability in the variables TST, SOL, WASO, SE, and time in bed (TIB) in normal sleepers; for their insomniac counterparts, however, more than two weeks were needed to achieve SOL stability, and at least three weeks were necessary for stable WASO. We should note, however, that this study allowed for habitual sleep periods, as evidenced by variability in TIB between participants. Without standardizing this variable across all participants, the assessment of short-term stability in sleep continuity may be compromised. Furthermore, more stringent criteria were placed in terms of what was considered “stable.” Wohlgemuth et al. calculated G coefficients, which can be interpreted analogously to ICC, and used a threshold of 0.80 to define “adequate stability.” In line with others (Landis and Koch, 1977; Israel et al., 2012), we had elected an ICC threshold of 0.60 as demonstrating “reliable” short- or long-term stability. Interestingly, when comparing the stability coefficients of Wohlgemuth’s control and insomniac participants to our own, many results—both for a single night and three nights—are quite comparable. Finally, the average age of participants in Wohlgemuth’s study was close to 70 years. A large meta-analysis by Ohayon and others (2004) has demonstrated an exponential decrease in deep sleep and increase in WASO, as well as linear increase in stage 1 and decrease in REM sleep with aging (Ohayon et al., 2004). As such, it is possible that instability of sleep duration over consecutive nights could be confounded by fragmented sleep due to older age.
Israel and colleagues (2012) recently examined short-term stability in 54 adult insomniacs and 22 normal-sleeping controls, employing both visual scoring and QEEG across three consecutive nights. Power spectral analysis yielded significantly higher intraclass correlation coefficients than visually-scored sleep, with the exception of slow-wave sleep. Visually-scored TST, SOL, and WASO only ranged from ICC = 0.34 – 0.56 (“slight” to “moderate” stability) compared to QEEG-scored variables (0.55-0.88) in both groups. Like Wohlgemuth et al., it is important to note that this study also did not standardize TIB. On the other hand, a three-night home-based, ad libitum PSG study by Coates and colleagues (1982) in 12 insomniacs and 12 normal sleepers (age range = 20-60 years) achieved substantial stability of SOL and WASO in both insomniacs (0.70 and 0.67) and controls (0.58 and 0.72, respectively). While these findings are in agreement with what we observed, the investigators omitted the first night from analysis. As such, extending these findings to a typical single-night research setting with fixed TIB may be troublesome.

Overall, the variable TST appeared to fare best in terms of both short-term and long-term stability, particularly when examining persistence of sleep duration classification; in turn, this may be consistent with the view that sleep duration is, to some degree, driven by biological factors (Klei et al., 2005; Barclay et al., 2013). Studies of mono- and di-zygotic twins, for example, suggest that sleep efficiency and wake time after sleep onset are highly heritable ($h^2 > 0.97$) (Kuna et al., 2012), and that a significant portion of variance in stages 2, 4, and delta sleep appears to be genetically determined (Linkowski, 1999). Although the difference in median TST between the first night and subsequent nights was larger in insomniacs than controls (21.5 minutes vs. 5.3 minutes; Table D.7), which is consistent with previous work (Newell et al., 2012), both groups still demonstrated substantial stability (ICC = 0.69 and 0.75, respectively) in
sleep duration when averaged across three nights. Interestingly, our findings are also in agreement with a study employing wrist actigraphy, which demonstrated little internight variability in TST and WASO across seven consecutive nights (Otte et al., 2011). The slightly greater stability of SOL compared to TST in volunteer research insomniacs may reflect the fact that a long self-reported sleep latency (≥ 45 minutes) was the primary quantitative selection criterion (Kales et al., 1991). On the other hand, the relatively low long-term stability and wide confidence interval of SOL across several years in the longitudinal cohort suggests that this variable may be more influenced by an individual’s “state” (i.e. how they felt that day, sleeping conditions during that particular night, and other fluctuating circumstances). Whereas such state-dependent variables (such as familiarity with the room, smells, general procedures, and the PSG technologist) are relatively controlled across consecutive nights, the increased time between visits in this longitudinal study may explain why certain variables, like latency to fall asleep, are vulnerable to instability and the first-night effect. Therefore, Agnew et al.’s (1966) description of decreased sleep efficiency, increased stage 1 sleep, and decreased REM as a result of the first-night effect would not be surprising to observe at both time points in this longitudinal sample. It’s of interest to note that regardless of intraclass correlation coefficient, variance is consistently lower within- than between-subjects, suggesting relatively good overall stability of TST, SOL, and WASO across nights; this is consistent with previously published large studies suggesting that inter-individual differences in sleep stability are fairly stable and robust (Tucker et al., 2007; Van Dongen et al., 2005).

Another major finding in our study is that a single-night sleep recording is valid and clinically useful in classifying participants in terms of “short” and “normal” sleep duration (Table D.7). In turn, this finding strengthens the validity of our approach in our previous reports
that insomnia with short sleep duration, based on a single-night recording, is consistently associated with cardiometabolic morbidity and mortality (Vgontzas et al., 2009a; Vgontzas et al., 2009b; Vgontzas et al., 2010; Fernandez-Mendoza et al., 2010; Fernandez-Mendoza et al., 2012; Vgontzas et al., 2013).

There are, however, several fundamental differences between the present study and previous studies on the topic. Firstly, our large sample size is a critical factor in our statistical approach. The intraclass correlation is a test-retest reliability method designed to quantify the degree to which related individuals (e.g. insomniacs) resemble each other in regards to a quantitative trait (e.g. total sleep time) (Koch et al., 1982). ICC is calculated by dividing the between-subject variance of this trait by the sum of between-subject trait variance plus within-subject pooled variance. The value of the ICC is positively associated with number of observations (i.e. number of nights studied in the laboratory), as more trials reduce within-subject variance. However, ICC is negatively associated with sample size due to increasing between-subject variance (Bonett, 2002; Shoukri et al., 2004). The fact that our large sample size (95, 150, and 151 participants per group) continued to yield strong ICC values compared to similar studies with 50 or fewer participants per group (Agnew et al., 1966; Larsen et al., 1995; Wohlgemuth et al., 1999; Tan et al., 2000; Tan et al., 2001; Buckelmüller et al., 2006Tucker et al., 2007; Israel et al., 2012) suggests that well-defined populations not only sleep similarly across nights, but also to one another. This conclusion is also in agreement with our data suggesting that, relative to others, one’s sleep classification (“short” vs. “normal”) is reasonably persistent across nights (Table D.7). In making these comparisons, it is especially important to note that our control and insomnia groups were of similar sample size and sociodemographic characteristics.
Additionally, focusing on visually-scored sleep, as opposed to more sophisticated spectral analytic methods, allows us to extend our findings to the clinical setting and/or large research samples, where visual scoring is the norm. Our design is also unique in that it mimics typical multi-night sleep study protocols, which aim to habituate participants to the laboratory setting. In many cases, the first night of sleep is omitted from analysis due to the first-night effect. Comparing both single- and average-measures stability allows us to observe the impact of this phenomenon. As multi-night PSG studies are expensive, time-consuming, and often not even considered for use in large epidemiological studies, it is important to understand if and how one night differs from subsequent nights. Our multi-night approach also addresses hypotheses from previous research which estimate that it takes a week or more to achieve adequate stability in variables related to sleep duration in adults (Larsen et al., 1995; Wohlgemuth et al., 1999). These conclusions were not based on studies employing multiple nights of PSG, but were rather approximations based upon statistical projections.

Finally, our comparison of sleep stability between insomniacs and controls does not have the confounding variables of older age or obesity that have been shown to affect day-to-day variation in sleep duration (Ohayon et al., 2004; Vgontzas et al., 1998) or quality (Zheng et al., 2012). Controls and insomniacs did not differ in terms of gender distribution, mean age, nor included any subjects older than 70 years, and body mass index did not exceed the “obese” threshold in either group. Although our longitudinal general population sample was relatively older (mean age 51.1 ± 1.1 years), no differences in sleep duration over time could be attributed to changes in body mass index or apnea-hypopnea index (Table D.4).

There are several limitations to the present study that may impact its generalizability to certain research samples. First, our control and insomniac groups included mostly non-Hispanic
Caucasians. Although a number of studies have identified racial/ethnic disparities in sleep architecture (Rao et al., 1999; Profant et al., 2002; Redline et al., 2004), no studies to date have explored the short- and/or long-term stability of sleep duration across ethnic groups. Additionally, although our longitudinal sample of 95 participants was derived from a representative general population sample, it consisted entirely of men due to the primary focus of our previous work. As studies examining the long-term stability of PSG-measured sleep are limited, future work should explore gender effects on stability of sleep over time, particularly in a middle-aged to older population such as ours. Examining the role of physiologic changes, such as gain or loss in BMI over time, would also be an interesting extension to this study. Finally, regression to the mean is an issue to keep in mind when interpreting the persistence of “short” and “normal” sleep duration, particularly as these groups were defined simply by a median split. Re-defining the medians from Night 1 to subsequent nights, however, may protect against this effect to some degree.

Despite these limitations, our multi-night, in-laboratory design, which was not confounded by older age, obesity, or ad libitum sleep schedules, demonstrated relatively high short-term and long-term stability of visually-scored sleep across three consecutive nights, or two nights assessed longitudinally. We conclude that a single night in the laboratory may provide reliable measures, particularly in the context of classifying one’s sleep duration both in the short-term and long-term.
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Education
2011 Bachelor of Arts (BA), Biology (Neuroscience minor), Cum laude | St. Mary’s College of Maryland
2016 Doctor of Philosophy (PhD), Neuroscience | Penn State College of Medicine

Honors & Awards
2011 Neuroscience Department Award, St. Mary’s College of Maryland
2014 Science Journalism Student Award, Society for Neuroscience
2014 Front Line Scholar, TEDMED
2014, 2015 Class of 1971 Endowed Scholarship, Penn State College of Medicine
2015 Inspired Award for Innovation in Research, Penn State Milton S. Hershey Medical Center
2015 Don Stabile Alumni Doctoral Scholarship, St. Mary’s College of Maryland
2016 Helen F. Holt Scholarship for Early Career Women in Science, AAAS
2016 Travel Award, European Sleep Research Society

Peer-Reviewed Publications & Book Chapters

Scientific Communication, Outreach, Advocacy, & Service
2011 – present Freelance science blogger/journalist
2013 – 2016 Founder, Editor-in-Chief, *Lions Talk Science Graduate Student Blog: Penn State College of Medicine*
2014 Annual Meeting Blogger, Society for Neuroscience; Washington, DC
2014 Speaker, TED@NYC 2014; New York City, NY
2014 – present Student Member, Institutional Biosafety Committee, Penn State College of Medicine
2015 Keynote Speaker, *Pennsylvania Society for Biomedical Research Annual Awards Dinner*
2015 – present Science Policy & Government Relations Chairperson, Hershey Medical Consulting Group
2015 – present Associate Editor, *Journal of Science Policy & Governance*
2016 – present Early Career Science Policy Ambassador, Society for Neuroscience