OXIDATIVE STRESS AND CELLULAR ADHESION MOLECULES IN
OBSTRUCTIVE SLEEP APNEA

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES
(Craniofacial Science)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

February 2021

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Oxidative Stress and Cellular Adhesion Molecules in Obstructive Sleep Apnea

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the degree of Doctor of Philosophy
in Craniofacial Science

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Abstract

**Background:** Obstructive Sleep Apnea (OSA) is the most common respiratory disorder during sleep. OSA is an independent risk factor for developing cardiovascular diseases (CVD). Although risk is increased, it is still challenging to identify which patients will develop CVD as standard disease metrics are not that helpful. Circulating biomarkers could be useful to risk stratify OSA patients. However, current evidence in this regard is limited. Oxidative stress biomarkers and cellular adhesion molecules might be particularly useful as these are elevated in OSA patients and in patients with CVD.

**Thesis Objectives:**

1. Identify and summarize the existing evidence on prognostic biomarkers in OSA (Chapters 1-2).
2. Evaluate the association between cellular adhesion molecules, oxidative stress markers and OSA (Chapters 3-4).
3. Evaluate whether levels of cellular adhesion molecules and oxidative stress markers predict incident CV events in an OSA-cohort (Chapter 5-6).
4. Discuss the implications and future directions of the findings (Chapter 7).

**Methods:** Adult patients (≥19 years old) referred for suspected OSA to the University of British Columbia Hospital Sleep Disorder Laboratory for inpatient polysomnography (PSG) were studied. Fasting blood (15 ml) was collected on the morning after PSG, and plasma was stored in a -80°C freezer. Plasma levels of 8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG), superoxide dismutase (SOD), intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1
(VCAM-1) and E-selectin (endothelial selectin) were assessed. Incidence of CV events was assessed by deterministic linkage through Popdata-BC.

**Results:** OSA severity was independently associated with higher circulating E-selectin and 8-isoprostane levels. In patients with suspected OSA, ICAM-1 was an independent predictor of incident CV events (OR=4.12 95% CI 1.47-11.55). In moderate to severe OSA patients, E-selectin was independently associated with CV events (OR = 4.07 95% CI 1.06 – 15.61), but not in patients without OSA. Oxidative stress markers were not associated with incident CV events.

**Conclusion:** E-selectin and 8-isoprostane were independently associated with OSA. Cellular adhesion molecules such as ICAM-1 and E-selectin were associated with incident CV events and might be promising markers in CV disease prediction in OSA. Oxidative stress markers were not associated with incidence of CV events.
Lay Summary

Obstructive Sleep Apnea (OSA) is a disease where the person stops breathing during sleep. OSA is linked to serious complications, such as strokes, heart attacks and deaths. However, we currently don’t know which patients will develop such complications. It has been proposed that certain blood molecules can be useful to predict patient risk.

We reviewed the literature to find potential molecules that could be associated with OSA and its consequences. Also, we recruited patients referred to the UBC Sleep Disorders Clinic for possible OSA. After their overnight sleep test, patients answered a questionnaire and donated blood. The patients were followed up using provincial health databases (PopData BC) and cardiovascular events were obtained.

OSA is a predictor of inflammatory molecules that are also common in cardiovascular diseases. Two of these markers were also risk factors for CV events. Inflammation may be involved in how OSA and cardiovascular events are linked.
Preface

This thesis is the result of my research work in the evaluation of circulating biomarkers in Obstructive Sleep Apnea (OSA). I was responsible for most of the design, data collection and cleaning, preparation of databases, analysis, interpretation of results and writing of all manuscripts. This study was approved by the University of British Columbia Research Ethics Board (H13-00346) and Vancouver Coastal Health Research Institutes (V11-80199).

All inferences, opinions and conclusions drawn in this dissertation are those of the authors, and do not reflect the opinions or policies of the Data Stewards(s). Data extracts were provided by the British Columbia Ministry of Healthy and WorkSafe BC. These extracts are cited below in compliance with Population Data BC protocols.


This dissertation is an original and an independent work by the author, Bernardo Urbanetto Peres. It was divided in 7 chapters, as shown below:

Chapter 1 – Introduction. A summary of current literature and concepts in Obstructive Sleep Apnea explored in this thesis. This chapter is original, unpublished, independent work by the thesis author.
Chapter 2 – Circulating biomarkers to identify cardiometabolic complications in patients with Obstructive Sleep Apnea: A systematic review. A version of the original study can be found as Peres BU, Hirsch Allen A, Fox N, et al. Circulating biomarkers to identify cardiometabolic complications in patients with Obstructive Sleep Apnea: A systematic review. *Sleep Med Rev.* 2019;44:48-57. doi:10.1016/j.smr.2018.12.004. AJ Hirsch Allen, Nurit Fox provided insights and helped during data collection. Najib Ayas and Fernanda Almeida reviewed the study design, reviewed data collection, analysis and final manuscript. All other co-authors reviewed the data and manuscript. I was involved in all steps of this study and wrote the manuscript for the published paper.

Chapter 3 – Association between Obstructive Sleep Apnea and Oxidative Stress. This chapter is based on a work developed at the Heart Lung Innovation laboratory at Saint Paul’s Hospital. I was responsible for study design, most of the data collection, data analysis, interpretation, and writing of manuscript. A version of this material has been published as: Peres BU, Allen AH, Shah A, Fox N, Laher I, Almeida F, et al. Obstructive Sleep Apnea and Circulating Biomarkers of Oxidative Stress: A Cross-Sectional Study. *Antioxidants.* 2020 Jun 2;9(6):476. [https://doi.org/10.3390/antiox9060476](https://doi.org/10.3390/antiox9060476). AJ Hirsch Allen, Nurit Fox and Ismail Laher provided insights and helped during data collection. Najib Ayas and Fernanda Almeida reviewed the study design, reviewed data collection, analysis and final manuscript. All other co-authors reviewed the data and manuscript. I was involved in all steps of this study and wrote the manuscript for the published paper.

Chapter 4 – Obstructive Sleep Apnea Severity, Body Mass Index, and Circulating Levels of Cellular Adhesion Molecules. This chapter was done in collaboration with Dr. Stephan Van Eeden, also at the Heart Lung Innovation laboratory at Saint Paul’s Hospital. I was responsible
for study design, data analysis, interpretation, and writing of manuscript. A version of this material has been published as: Peres BU, Allen AJ, Kendzerska T, Shah A, Fox N, Laher I, Almeida F, Jen R, Sandford A, van Eeden S, Ayas N. Obstructive Sleep Apnea Severity, Body Mass Index, and Circulating Levels of Cellular Adhesion Molecules. Lung (2020). https://doi.org/10.1007/s00408-020-00401-x. AJ Hirsch Allen, Nurit Fox, Aditi Shah provided insights and helped during data collection. Tatiana Kendzerska helped with statistical analysis and final editing of the tables included in the paper. Najib Ayas and Fernanda Almeida reviewed the study design, reviewed data collection, analysis and final manuscript. All other co-authors reviewed the data and manuscript. I was involved in all steps of this study and wrote the manuscript for the published paper.

Chapter 5 – Circulating Levels of Cell Adhesion Molecules and Future Risk of Cardiovascular Events in Obstructive Sleep Apnea. This chapter is the continuation of the previous work from chapter 4 and extensive work along with Population Data BC. Help with programming the analysis was provided by Mr. Patrick Daniele. I was responsible for study design, most of the data collection, data analysis, interpretation, and writing of manuscript. A version of this manuscript is being submitted for publication.

Chapter 6 - Oxidative Stress and Risk of Incident Cardiovascular Events in Obstructive Sleep Apnea Patients. This chapter is the continuation of previous work and my current work at the Heart Lung Innovation laboratory at Saint Paul’s Hospital. It also involved extensive work along with Population Data BC. Help with programming the analysis was provided by Mr. Patrick Daniele. I was responsible for study design, most of the data collection, data analysis, interpretation, and writing of manuscript.
Chapter 7 - Discussion, Future Directions, and Conclusions. This chapter is original, unpublished, independent work by the author, Bernardo Urbanetto Peres.
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List of Abbreviations

AASM: American Academy of Sleep Medicine;
ACE: Angiotensin Converting Enzyme;
Adj: Statistical Adjustment;
ADMA: Asymmetric dimethylarginine;
AFIB: Atrial Fibrillation;
AHI: Apnea Hypopnea index;
BC: British Columbia;
BMI: Body mass index;
BP: Blood pressure;
CABG: Coronary Artery Bypass Graft;
CAD: Coronary artery disease;
CAM: Cell adhesion molecules
CCI: Canadian Classification of Health Interventions;
CHF: Congestive Heart Failure;
CI: Confidence Interval;
CPAP: Continuous positive airway pressure;
CRP: C-reactive protein;
CRT-P: Cardiac Resynchronization Therapy Pacemaker;
CSA: Central Sleep Apnea;
cTnT: Cardiac troponin T;
CV: Cardiovascular;
CVD: Cardiovascular Disease;
DNA: Deoxyribonucleic acid;
ECG: Electrocardiogram;
EDS: Excessive daytime sleepiness;
EEG: Electroencephalogram;
ELISA: Enzyme-linked immunosorbent assay;
EMG: Electromyogram;
ESRD: End stage renal disease;
ESS: Epworth Sleepiness Scale;
FiO₂: Fraction of inspired oxygen;
GPR83: G protein-coupled Receptor 83;
GWAS: Genome Wide Association Studies;
HbA1c: Hemoglobin A1c;
HDL: High density lipoprotein;
HIF1-α: Hypoxia Induced Factor 1 – alpha;
HMG CoA: Hydroxymethylglutaryl coenzyme A;
HOMA-IR: Homeostasis Model Assessment of Insulin Resistance;
hs-CRP: High sensitivity c-reactive protein;
ICAM: Intercellular adhesion molecule;
ICD-10: International Classification of Diseases-10;
IH: Intermittent Hypoxia;
IHD: Ischemic Heart Disease;
IL-1β: Interleukin 1 beta;
IL-1Ra: Interleukin 1 receptor antagonist;
IL-6: Interleukin 6;
IL-8: Interleukin-8;
IQR: Interquartile Range;
LDL: Low-density lipoprotein;
LVEF: Left Ventricle Ejection Fraction;
MetS: Metabolic Syndrome;
MI: Myocardial Infarction;
miRNA: microRNA;
MMA: Maxillomandibular Advancement;
NADPH: Nicotinamide Adenine dinucleotide phosphate;
NFkB: Nuclear factor kappa B;
NOS: Newcastle-Scale;
NR: Not reported;
NREM: non-rapid eye movement
NS: Not significant;
ODI: Oxygen desaturation index;
OHdG: oxo-deoxyguanosine
OR: Odds Ratio;
OS: Oxidative Stress;
OSA: Obstructive Sleep Apnea;
PCI: Percutaneous Coronary Intervention;
Pcrit: Critical Pressure;
PD: Peroxides;
PICO(S): Population Intervention Control Outcome (Study design);

PON-1: Paraoxonase-1;

PRISMA: Preferred reporting items for systematic reviews;

Pro-BNP: pro-Brain Natriuretic Peptide

PSG: Polysomnography;

RANTES: Regulated on Activation Normal T Cell Expressed and Secreted;

RDI: Respiratory disturbance index;

REM: rapid eye movement

RNA: Ribonucleic acid;

ROS: Reactive oxygen species;

RR: Relative Risk;

SaO₂: Oxygen saturation;

SAVE: Sleep Apnea Cardiovascular Endpoints;

SD: Standard deviation;

sEng: Soluble Endoglin;

sFlt-1: Soluble fms-like tyrosine kinase-1;

sICAM: soluble intercellular Adhesion Molecule;

SOD: Superoxide dismutase;

SREBP1: Sterol regulatory element-binding protein 1;

T2DM: Type 2 Diabetes Mellitus;

TBARS: Thiobarbituric reactive substances;

TIA: Transient Ischemic Attack;

TNF-α: Tumor Necrosis Factor alpha;
UA: Upper airway;

UBC: University of British Columbia;

UPPP: Uvulopalatopharyngoplasty;

VCAM: Vascular cell adhesion molecule;

VT: Ventricle Tachycardia;

WHO: World Health Organization;

XO: Xanthine Oxidase;
Acknowledgements

First, I would like to thank my family. This program would never be possible without your great support and love.

I would like to thank my supervisory committee, Dr. Benjamin Pliska, Dr. Fernanda Almeida and Dr. Najib Ayas. Thank you for your patience, constant feedback and support during all these years.

I also would like to thank Dr. Ismail Laher for his great support and time during my comprehensive exam.

I would like to thank Dr. AJ Hirsch Allen. Thank you for being patient and serving as an example during all these years.

Finally, I would like to express my gratitude to all students and staff from the Faculty of Dentistry and Medicine of the University of British Columbia.
Dedication

I would like to dedicate this PhD to my beloved nephew and godson, Pedro; to my brother, Raphael Urbanetto Peres; to my father, Paulo Edelvar Corrêa Peres; and to my mother, Rosanara Pacheco Urbanetto. Thank you for your unwavering support and encouragement during the past five years. I love you all!
Chapter 1: Introduction

1.1 Obstructive Sleep Apnea

1.1.1 Definition and Diagnosis

Sleep is essential for human life and development. Sleep regulates several physiological functions, such as growth, cognition, performance, vigilance and immune response[1]. It is defined as “a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment”[2]. Under normal conditions, sleep presents two distinct phases: a rapid eye movement (REM) state, and a non-rapid eye movement state (NREM). In 2007, the American Academy of Sleep Medicine started using the term N for NREM sleep stages and R for REM. N1 and N2 are the terminology used for previously called stages 1 and 2 respectively. N3 indicates the sum of stages 3 and 4[3]. Stage N1 corresponds to the shallowest and N3 to the deepest. The deeper the sleep stage, the harder it is to wake up from it. NREM sleep is mostly characterized by little brain activity and a movable body. REM sleep, in opposition to NREM, is where the brain will be mostly active, with clear signals from the electroencephalogram (EEG) and the muscles will be atonic[4].

A healthy young adult, sleeping on average 8 hours will start sleeping in NREM and achieve REM sleep approximately every 90 minutes. In general, NREM sleep is usually 75 to 80% of total sleep, with stage N1 contributing to 2-5%, stage N2: 45-55%, stage N3: 18-25%. REM sleep is about 20-25% of sleep and will happen 4 to 6 times during a sleep cycle, being predominant in the last third of the night. The gold standard method to assess sleep is by using a level I polysomnography (PSG). Briefly, there are four different kinds sleep studies available. A level I PSG will capture EEG (frontal, central and occipital – to capture brain waves), EOG (electrooculogram, to capture eye movements), ECG (electrocardiogram), recordings of airflow,
respiratory effort, oxygen saturation, and limb EMG (Electromyogram). In a level I PSG a technician will be monitoring the study during the whole study. Level IIIs are essentially a PSG without a technician present, they can be portable or not. Level IIIs will be portable without EEGs and levels IVs usually measure oxygen saturation only[4].

Alterations in sleep that can cause problems with daytime functioning, or any changes to these normal patterns mentioned above are known as Sleep Disorders. According to the third edition of the International Classification of Sleep Disorders, there are seven major diagnostic sections: Insomnia, Sleep-related breathing disorders, Central disorders of hypersomnolence, Circadian rhythm sleep-wake disorders, Parasomnias, Sleep-related movement disorders, and Other sleep disorders. Sleep-related breathing disorders are divided in Obstructive Sleep Apnea (OSA), Central Sleep Apnea (CSA) syndromes, Sleep-related hypoventilation disorders, and sleep-related hypoxemia disorder[5].

OSA is characterized by recurrent collapse of the upper airway, leading to intermittent hypoxia and sleep fragmentation. The severity of OSA is assessed by an apnea-hypopnea index (AHI), meaning the number of times per hour that the airway narrows (hypopnea) or closes (apnea). Apneas and Hypopneas are scored from PSG. The American Academy of Sleep Medicine considers apneas as ≥90% drop in oronasal thermal sensor signal for ≥10 seconds. Hypopneas are scored as a drop in nasal flow of ≥30% for ≥10 seconds associated with a ≥3 or 4% oxygen desaturation or an arousal pre-event baseline[3]. The diagnosis of OSA syndrome includes an AHI≥5 associated with signs/symptoms (such as sleepiness, fatigue, snoring, comorbidities), or an AHI≥15 with not necessarily associated signs/symptoms. Usually, an AHI higher than or equal to five is considered mild OSA, 15-30 moderate and 30 or higher as severe[6].
1.1.2 Pathophysiology and Risk Factors

The upper airway (UA) is a multipurpose structure that lacks bony support and consequently relies heavily on neuromuscular control to allow for swallowing, breathing and speaking. However, its mobile nature naturally creates a risk for potential collapse points[7]. The key concept to understand how OSA develops and worsens relies on the functional interaction between anatomical and local factors in an upper airway that fails to maintain patency during sleep.

OSA is considered a heterogeneous condition[8]. Currently, there are four endotypes that contribute either alone or in combination to the establishment of OSA. An endotype reflects a subgroup or subtype of a condition with a specific pathophysiological mechanism. The first one refers to anatomical features on the pharyngeal area, the second relates to the functionality of the muscles recruited to maintain airway patency, the third includes factors related to arousals threshold during sleep, and the fourth refers to ventilatory control stability also known as high loop gain (i.e.: tendency of a small disturbance in the feedback mechanism of respiratory drive to cause a significant unstable breathing pattern that leads to higher collapsibility). These four concepts are explained in detailed below:

1.1.2.1 Collapsibility of the airway – Anatomy, airway and surrounding tissues

Anatomy plays a role in pathophysiology of OSA. Any condition that leads to a narrowing of the airway can increase the chances of airway collapse. To measure how easily or how difficult an UA collapses a continuous positive airway pressure (CPAP) device is used. Basically, the pressure is reduced or even reversed (suctioning air) and the pressure where the UA collapses is called critical pressure (Pcrit). A negative critical pressure is considered normal,
usually around \(-5\) cmH\(_2\)O. Although 20% of OSA patients have similar Pcrit values to healthy controls\([9]\), there is strong evidence that higher Pcrit is found among OSA patients when compared to people without OSA\([10,11]\).

Intuitively, a narrower UA is more likely to collapse. The cross-sectional area of the UA is influenced by compromised craniofacial features and the surrounding soft tissues. Consequently, risk factors associated with significant impact on the lumen of the UA are common findings in OSA patients. For example, OSA was prevalent in 85% of infants with Pierre Robin\([12]\) and 46% in Treacher Collins syndrome\([13]\). The average neck circumference is also used as a clinical measure to assess the risk for OSA. A 17 inch neck circumference is an independent risk factor for the presence of OSA in men and 16 inches in women\([14]\).

Another anatomical feature to be investigated is the oropharyngeal anatomy. During an oral examination, a crowded (small) airway area is represented by high Mallampati Scores, associated with nasal obstruction showed a significant correlation with AHI\([15]\). Central obesity also greatly affects the chances of collapse of the UA. Tongue volume, tongue fat percentage along with an increase in the parapharyngeal fat pads are highly correlated with the presence of OSA\([16]\). For instance, obese patients with similar BMI will have significantly different AHIs, depending on their percentage of fat in their tongue. Patients with a BMI of 35 Kg/m\(^2\) and tongue fat percentage of 42% will have an AHI of 59, while the ones with a tongue fat percentage of 24% will show an AHI of 9.6\([17]\). For similar reasons, edema in the UA region will also contribute to narrowing of the airway. Conditions such as end-stage renal disease and congestive heart failure are known to increase daytime leg fluid retention. During sleep, the excessive fluid shifts rostrally accumulating on the neck region, exerting pressure on the UA and
increasing the surface tension on the lining mucosa of the UA. This increased collapsibility can lead to OSA[18].

**1.1.2.2 Muscle responsiveness**

Upper airway muscle recruitment is essential to maintain airway patency, avoid collapsibility and consequently OSA[19]. Although it is difficult to scrutinize the individual effects of each sleep stage in each muscle, it is accepted by the literature that overall muscular activity is decreased during sleep. Examples of muscles that are considered to play a significant role in UA dilation and stiffening include primarily the genioglossus and secondarily the tensor veli palatini. Interestingly, genioglossus activity is increased in OSA patients during awake and sleep. It is believed that rather than a loss of muscle excitation, OSA patients exhibit a noticeable reflex inhibition[20]. The presence of edema also influences muscle responsiveness. Mucosal edema affects chemoreceptor stimulation and hinders the mechanical translation of the efferent output[7].

**1.1.2.3 Arousal threshold**

Historically, arousals from sleep were thought to play an independent role in restoring airway after collapse events. The rationale relies on the high prevalence of arousals associated with hypopneas and apneas, and the correlation of longer arousals with severe obstructions. However, not all patients need an arousal to reestablish airway patency[20]. In fact, when comparing OSA patient and healthy controls, OSA patients were able to regain airway opening without an arousal, but to a lesser degree[21]. This suggests that the development of OSA would be related to a low arousal threshold. A low arousal threshold means that very little stimuli is needed to wake up, as opposed to a high arousal threshold, where a significant stimulus is needed to wake one up. A higher arousal threshold might lead to more severe desaturation and longer
obstructive events. OSA is more likely to happen in patients with low arousal threshold for two reasons; first, shallower stages of sleep are associated with a less stable breathing pattern, increasing the chances of airway collapse; second, arousals are associated with higher controller gain (hypercapnic) during the ventilatory response, an exacerbated response in loop gain leads to a less stable breathing, which increases the chances of airway collapse[20].

1.1.2.4 High loop gain

Loop gain is a concept used widely in electrical engineering, but also in biology that refers to the sum of gain around a feedback loop. It determines if the output will be stable or unstable depending on the feedback. In sleep medicine, loop gain is used to describe how likely the ventilatory control system is to develop unstable oscillations when presented with breathing disturbances (e.g.: hypopneas, apneas, arousals). A high loop gain system is considered to be unstable because a similar stimulus (hypercapnia) would yield a strong response, that is a hypersensitive ventilatory control system[20]. When comparing OSA patients with control subjects at a similar Pcrit, loop gain tended to be almost double in those who develop hypopneas and apneas[9]. Also, severe OSA patients have higher loop gain than moderate and mild cases[22].

1.1.2.5 Risk factors

Obstructive sleep apnea has three classic risk factors: age, obesity and male sex. Currently, there is clear evidence that menopause, genetics (family history), smoking, alcohol consumption before sleep and nighttime nasal congestion also are risk factors for OSA[23]. The role of nasal congestion and alcohol intake offer biological plausibility and strong evidence relating it to snoring. However, there is still need for further evidence to classify these as risk factors for OSA[24].
Obesity is by far the most important risk factor in OSA. For every one standard deviation increase in body mass index, there is an increase of 4.17 odds of having OSA[25]. The prevalence of OSA seems to be remarkably increased as BMI increases. A 40% prevalence of OSA can be expected in healthy overweight individuals, while a prevalence close to 80% of OSA can be seen in BMI’s above 40 kg/m2[26,27]. As mentioned above, fat deposition around the upper airway and the tongue likely play roles. In addition, lung volume is greatly affected by abdominal and thoracic fat deposition. A reduced lung volume causes reduction of the traction exerted in the trachea, leading to higher collapsibility of the UA[7].

Age is also supported by many studies as a risk factor for obstructive sleep apnea. The prevalence of moderate to severe OSA in a community based-cohort is almost double (17.4%) in adults between the age of 50-70, when compared to adults between the age of 30-49 (9.5%)[28]. However, OSA tends not to be further increased after the age of 65[29]. The mechanisms by which age affects prevalence of OSA are variable. Some of the mechanisms are thought to be differential fat deposition around UA with age, reduction on muscle tone and connective tissue of the pharynx, and reduction of muscle reflex and muscle activity[20].

Male sex has also been recognized as a risk factor for OSA. OSA is more commonly found in males when compared to females. For instance, there is a 4.6-fold difference between male and female sex, independent of age, BMI and other confounding factors. The prevalence of moderate to severe OSA in the same BMI (30-39.9 Kg/m²) and age range (30-49 years old) is 16.6% in men and 3.6% in women. However, hormonal changes due to menopause modifies the risk for OSA. The same prevalence of 3.6% for women between 30-49 years old have a 3.8-fold increase (13.9%) for women between 50-70 years old[28]. Therefore, postmenopausal female sex is also a risk factor for OSA[23]. Men are believed to experience more OSA than women.
because men are more likely to experience visceral and hepatic fat accumulation. This fat accumulation pattern correlates with waist circumference and central obesity, which contribute to OSA pathophysiology[30].

Family history is also a risk factor for OSA. Familial history can be explained in two different ways: first, poor eating habits and bad health life style choices would lead to obesity, which predisposes to OSA; second, genetic factors could be responsible for OSA. Genetics are thought to increase OSA risk by four different pathways. First, by regulating genes related to fat accumulation, distribution, gain, inflammatory response and establishment of metabolic syndrome. Second, by establishing craniofacial and upper airway anatomy. Third, by regulating ventilatory control and lastly, by influencing sleep and circadian rhythm. However, these all indicate relationships with traits found in OSA. It is still unclear if there are specific genes related to OSA[31]. This is a common issue in complex and heterogeneous diseases such as OSA. Genome-wide association studies (GWAS) were successful in identifying potential loci for narcolepsy and restless leg syndrome, but in OSA the literature is scarce[32,33]. There is anecdotal evidence around gene GPR83 (a gene expressed in the brain in areas related to ventilatory control) [34]. In addition to that, duration of apneas and hypopneas have been found to be significant in areas of the genome close to pathways related to HIF-1α (a key regulator of ventilatory response) and SREBP1 (responsible for lipid metabolism). It is believed that episodes of intermittent hypoxia (a hallmark of OSA) can affect SREBP1 and lead to fat redistribution[35].

Smoking is believed to increase OSA risk by inducing inflammatory response on the upper airway and modulating sleep stability. There is no causal association between smoking and OSA; however, there is a dose-response relationship indicating heavy smokers are at the highest
increase risk of experiencing OSA. In a logistic regression model adjusted for sex, education, age, BMI, and alcohol intake, the odds ratio of experiencing moderate OSA was 4.44 in current versus never-smokers. Also, smokers that consumed less than 20 cigarettes had 3.94 the odds of having moderate OSA when compared to never smokers, while those who smoked 40 cigarettes or more had 40.47 the odds of experiencing OSA[36].

1.1.3 Prevalence, Impact and Clinical Phenotypes

OSA is the most common sleep-related breathing disorder. Despite several efforts to increase awareness and recognize this condition as a serious public health matter, OSA remains highly undiagnosed and untreated. Historically, there have been different reports of the prevalence of OSA worldwide. Each study had different sex ratios, age range and most importantly not all used objective assessment (PSG) to diagnose OSA. This made almost impossible to compare the true prevalence between different countries. Recently, Benjafie and collaborators [37] performed a literature-based analysis to estimate the global prevalence of OSA in a population range of 30-69 years old. Currently, it is estimated that between 711 to 961 million people suffer from obstructive sleep apnea (AHI≥5)[37].

The underdiagnosis of OSA has been mentioned as a hidden health crisis. The estimated impact of OSA is considered to be close to 150 billion dollars in the United States. This includes workplace accidents, loss of productivity, motor vehicle accidents and comorbid diseases. The same report from the AASM considered the cost of diagnosing and treating OSA to be close to 12.4 billion dollars. The report also predicted that a potential 100.1 billion dollars could be saved if every OSA patient were to be diagnosed and treated[38,39].

To better understand the different facets of OSA, clinical phenotypes were created based on different parameters. Clinical characteristics, physiological measures and imagining features
are all being used to characterize OSA patients[40]. For instance, clinical clusters of “minimally symptomatic”, “disturbed sleep” and “excessive daytime sleepiness” have been identified and validated. Excessively sleepy OSA patients are at increased risk of developing cardiovascular disease (hazard ratio 95% CI 1.7-2.4)[41], in comparison to other subtypes. Although clinical phenotypes are currently being studied for cardiovascular prognosis in OSA, biochemical phenotypes have been poorly explored[42].

1.1.4 Treatment

OSA is considered a chronic and complex disease. Therefore, treatment modalities should consider long-term adherence to treatment, effectiveness and patient preference[43]. Continuous positive airway pressure (CPAP) is considered the first choice of treatment for OSA, especially in severe cases. The mechanism of action relies on a pneumatic splint opening the upper airway preventing it from collapse during sleep. [44]. Although highly effective, adherence to CPAP is challenging. Studies suggest that 46 to 83% of patients are unable to tolerate CPAP[45].

Oral appliances (OA) are considered the first line of treatment for mild to moderate OSA, and for patients intolerant to CPAP. It works by advancing the mandible forward during sleep, increasing the cross-sectional area of the upper airway and decreasing the collapsibility of the upper airway. The mean effect size of reduction in AHI regardless of OSA severity is 9.29 with a confidence interval of 12.28 to 6.3[44]. Although considered less effective, the adherence to treatment is remarkably higher than CPAP. Oral appliances have been reported to be used 92% of the time, with an average time of 6.7 hours per night[46]. Because of the difference between efficacy and adherence of both treatments, and similar effects on common comorbidities[47], some authors suggest that the mean disease alleviation of OA is similar to CPAP[46].
Cognitive behavior therapy, exercise-training and dietary interventions (life-style interventions) are extensively used in OSA, especially because of the close relationship between sleep-disordered breathing and obesity. A network meta-analysis compared aerobic exercise training, dietary weight loss, oral appliances, and CPAP. While all interventions showed a significant reduction on AHI, CPAP showed the highest efficacy. Interestingly, exercise training was comparable (no statistical difference) to oral appliances and CPAP[48]. It’s important to emphasize that the exercises involved one-hour of aerobics combined with resistance training, supervised by an experienced professional for 2 months, 6 times a week[49]. Finally, bariatric surgery also leads to a significant reduction in AHI. In a systematic review, all studies that reported pre and post-op AHI showed a significant reduction in OSA severity[50].

Positional therapy is also considered a viable treatment for OSA, especially in patients with supine dependent OSA. It consists of different strategies (e.g.: sewing a tennis ball to the back of a t-shirt) to avoid supine sleep. It has been shown to reduce mean supine AHI from 38 to 8.5 events/hour[51].

OSA can also be treated surgically. Uvulopalatopharyngoplasty (UPPP) and maxillomandibular advancement (MMA) are common procedures in the surgical approach. UPPP tend to have a low success rate (24 to 33%), regardless of BMI[52]. MMA osteotomy has been shown to be remarkably more successful. The average success rate (defined as 50% reduction in AHI) in a meta-analysis of 518 patients showed a success rate of 85.5%[53]. However, the average advancement of the maxillomandibular complex has to be on average 10mm[54].

Another approach involves hypoglossal nerve stimulation by an implanted device that delivers electrical pulses to aid in UA patency during sleep[55]. It consists of a neurostimulator
with two arms: a sensing lead and a stimulation lead. The stimulation lead is attached to the hypoglossal nerve through an incision at the inferior border of the mandible. The sensing lead is inserted in the pleura, at the fourth intercostal region. A 12-month follow up randomized trial in 124 patients showed a mean reduction of AHI from 32 to 15.3[55].

In addition to neural stimulation, some authors suggest the use of medications that could affect arousal thresholds[56]. This could be particularly effective especially in OSA endotypes mainly characterized by low arousal threshold. Benzodiazepines result in detrimental effects on breathing (reduced oxygen saturation) probably due to the muscle relaxation effect of it[57], on the other hand, selective serotonin reuptake inhibitors (e.g: tradozone) significantly change increase the arousal threshold, allowing OSA patients to tolerate higher levels of hypercapnia without muscle relaxation. However, altering a non-anatomical trait doesn’t seem to translate in major changes in AHI. Evidence suggests that patients with positive or close to zero Pcrits did not benefit from pharmacological intervention, while patients with Pcrit of -2cmH2O had 35% reduction in AHI[58]. This evidence supports the need for a better understanding of OSA endotypes. OSA is a disease with multiple presentations and a personalized approach should be considered for novel and future treatments[40].

1.2 Obstructive Sleep Apnea and Cardiovascular Diseases

1.2.1 Cardiovascular Diseases, Risk Factors and Inflammation

Cardiovascular diseases (CVD) are a group of conditions that affect the heart and the circulatory system, and it is considered one of the most significant public health concerns. Although it has decreased over the last few years, it still is the leading cause of death globally[59]. The World Health Organization (WHO) estimates that 17.7 million people died from CVD in 2015, which represents 31% of all deaths in the world. In Canada, CVD
represented a direct cost of 11.7 billion dollars (including physician care, drugs, and hospital care)[60]. Also, it is estimated that 2.4 million Canadians live with a diagnosed heart condition and of those, twelve die every hour[61].

The conventional risk factors for CVD are smoking, diabetes, hyperlipidemia and hypertension. Eighty percent of patients with coronary heart disease will have at least one of these 4 risk factors[62]. All these factors are known to produce an inflammatory response in the endothelium, leading to future CVD[63]. Inflammation is also considered a risk factor for the development of CVD. Patients treated with statins, which reduces the production of cholesterol by the liver, (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) show a significant reduction in the incidence of CVD also by lowering levels of C-reactive protein, a marker of inflammation[64]. Also, anti-inflammatory therapy with interleukin 1-β inhibitor leads to significant lower rates of recurrent cardiovascular events (hazard ratio 0.85, 95% CI 0.74-0.98) compared to placebo, regardless of lipid-level lowering[65]. Inflammation can also be assessed by oxidative stress and cellular adhesion molecules levels.

Oxidative stress is a state that occurs because of an imbalance between oxidants (such as reactive oxygen and nitrogen species) and antioxidants produced by cells, such as enzymes like superoxide dismutase or vitamins. Secondary effects of oxidative stress include endothelial dysfunction (reduced nitric oxide bioavailability and excessive asymmetric dimethylarginine, causing impaired capacity of vasodilation and inhibition of normal signaling within the endothelium), oxidation of DNA, proteins, and increase lipid peroxidation[66]. Oxidative stress markers found in the blood, saliva and urine are elevated in CVD and correlate with traditional CVD risk factors. For instance, when 3000 patients from the Framingham Heart Study were studied, urinary isoprostanes were significantly elevated in obese and smokers[67]. Also, when
comparing patients with CVD versus without, higher levels 8-isoprostanee (fourth quartile vs. first) were associated with 2 times the odds of developing cardiovascular disease[68].

Cellular adhesion molecules are also involved in the inflammatory process responsible for cardiovascular disease[69]. Cell adhesion molecules (CAMs) are proteins involved in the recruitment and binding of other cell types (leukocytes) to the endothelium. Under healthy conditions, leukocytes will not adhere to endothelial cells; however, under pro-inflammatory conditions there is an increase in CAMs. The upregulation of CAMs come as a response from initial build-up of oxidative low-density lipoproteins, increased shear stress induced by increased blood pressure, reduced oxygen saturation and other building blocks of atherosclerosis. The migration of leukocytes and newly recruited monocytes to endothelial cells propagates the inflammatory response, causing increased degradation of extracellular matrix and unstable atherosclerotic lesions. This leads to damage to the vessel wall and thromboembolic events[70].

Circulating CAMs, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are associated with incident cardiovascular events in non-OSA populations. For instance, high levels of ICAM-1 are associated with a 5.53 increase in the odds of incident coronary heart disease[71].

1.2.2 Obstructive Sleep Apnea, Cardiovascular Disease, Oxidative Stress and Inflammation

OSA is an independent risk factor for CVD. Patients with severe OSA are at a higher risk of developing coronary artery disease, atrial fibrillation, myocardial infarction, heart failure, diabetes, obesity, and metabolic syndrome[72]. Data from observational studies strongly indicate that OSA is implicated in the development of cardiovascular disease. For instance, Marin et al[73] followed 235 patients with untreated severe OSA, 264 healthy participants and 372
patients treated with CPAP for ten years. The authors found that in a fully adjusted model when comparing healthy controls to patients with untreated severe OSA, the OSA group had 2.87 (95% CI 1.17 – 7.51) the odds of suffering from a fatal CVD event and 3.17 (1.12 – 7.51) the odds of suffering from non-fatal CVD events.

In obstructive sleep apnea, oxidative stress and cellular adhesion molecules are also increased[74]. Patients with OSA, analogous to what happens with ischemic/reperfusion injury[75], will experience significant and repetitive drops in their oxygen levels (hypoxic events) followed by reoxygenation[76], which is known as intermittent hypoxia (IH). IH is the mechanism by which OSA contributes to oxidative stress[76]. IH produces reactive oxygen species (ROS) through leakage of free radicals from the mitochondria, activation of XO (xanthine oxidase) and activation of NADPH (nicotinamide adenine dinucleotide phosphate) in phagocytic and non-phagocytic leukocytes[76].

OSA also increases cellular adhesion molecules via intermittent hypoxia[77]. Hypoxia reduces oxygen flow, affecting hypothalamic modulators. There is increased release of inflammatory cytokines (e.g.: IL-6), and adhesion molecules (e.g.: ICAM, VCAM, selectins). This induce an environmental change characterized by increased attachment of leukocytes to the endothelium, a hypercoagulability state and significant increase in oxidative stress. The excess of reactive oxygen species (hallmark of oxidative stress), perpetuate the inflammatory cycle by activating transcription factors (e.g.: NFκB and HIF-1-α) that produce further inflammatory and vasoactive proteins[78].

Because both OSA is an independent risk factor for CVD and because OSA and CVD are associated with increased oxidative stress and cellular adhesion molecules (figure 1.1) it would be reasonable to investigate the clinical utility of such markers in CVD and OSA.
If OSA is associated with cardiovascular disease, then treating OSA with CPAP should prevent cardiovascular events in patients with OSA. This hypothesis was the focus of a multi-centered randomized trial, the SAVE study. Briefly, patients from 89 clinical centers in 7 countries were recruited. Patients with a history of cardiovascular disease (coronary artery and cerebrovascular disease) and moderate to severe OSA were randomized to either CPAP in addition to routine care (n=1346), when compared to conventional care alone (n=1341) did not have a reduced rate of death from cardiovascular causes, myocardial infarction, stroke, hospitalization for heart failure, unstable angina, and transient ischemic attack. Their hazard ratio was 1.10 (with a 95% confidence interval of 0.91 – 1.32). It was argued that the adherence to CPAP was not ideal and that could have affected the results. Still, when comparing patients that used CPAP above 4 hours/night with
usual care, the hazard ratio was 0.8 (with a 95% confidence interval of 0.6 – 1.07)[79]. Smaller trials also supported negative results of the effect of treating OSA in consequent cardiovascular outcomes[80]. However, as the SAVE study was the largest multicentered trial in the field, the results were disappointing. It also raised the question of future direction on how to effectively treat patients with obstructive sleep apnea. Alternative clinical conducts besides the generic, costly and labor-intensive CPAP are clearly needed to help to identify which patients are at higher risk of developing cardiovascular events than others.

1.3 Circulating Biomarkers

A biomarker is defined as an objective indication of a medical state that can be precisely and reproducibly measured. They can be used as surrogates for clinical endpoints if they consistently and accurately predict a clinical outcome[81]. This thesis focused on measuring human circulating biomarkers, i.e. molecules present in a patient’s blood sample, from their circulation.

1.3.1 OSA Biomarkers

In the obstructive sleep apnea literature, biomarkers have been poorly explored[82]. Biomarkers can be diagnostic or prognostic. The majority of the publications in OSA explored diagnostic biomarkers, in other words, the markers that detect the presence of OSA. This thesis focused on prognostic biomarkers of OSA which are the markers that can help evaluating with risk stratification for consequences of OSA. The following sub-section presents diagnostic and prognostic biomarkers to demonstrate the concepts of an ideal biomarker in OSA and review the current literature on the topic.

An ideal biomarker in OSA should be disease-specific, meaning reaching high values of sensitivity and specificity for one specific disease without confounders. Also, it should correlate
directly with severity of disease (either by apnea-hypopnea index or oxygen desaturation index). Additionally, it should respond to treatment, so it would serve as measure for efficacy of interventions. And finally, the ideal biomarker should be in a causal pathway, where changes in that marker would predict outcomes or even improvements in outcome[83]. Considering such extensive list, it is currently recognized that there is no ideal biomarker in OSA. Such facts led to a significant effort from the scientific community in sleep to identify potential markers in the OSA population[82,84,85]. For instance, De Luca Canto et al[85] reported that in the adult population, inflammatory markers (IL-6 and IL-10) could be useful to facilitate the diagnosis of OSA. Montesi et al[84] grouped potential markers that could be used in the OSA population. Markers were divided in inflammatory, metabolic, hypertension, carotid intimal-medial thickness and oxidative stress markers. Among all these options, markers of oxidative stress emerged as a unifying paradigm between OSA and the myriad of comorbidities that can be associated with OSA, specially cardiovascular disease[86]. Most of these studies have looked at biomarkers that would help to diagnose OSA. If one considers that OSA is a multifactorial condition that shares many pathological pathways with many other diseases, it is unlikely that a single biomarker will be able to diagnose OSA, and OSA only. Although potential markers have been identified there isn’t any consensus on the literature about OSA specific markers.

As proposed, a potential biomarker should also correlate with treatment response. Several studies measured the effects of CPAP treatment on different cardiometabolic biomarkers. However, the literature remains conflicting. The presence of confounding factors and a significant heterogeneity in study designs can explain why there still no consensus. A systematic review by Julian-Desayes and collaborators[87] analyzed the effects of CPAP treatment on sympathetic activation markers, lipid and glucose metabolites, inflammatory and oxidative stress
markers, hepatic enzymes and coagulation factors. They found that, in terms of blood and urine catecholamines, half of the studies reported some reduction of norepinephrine (roughly 25%) either by decreased levels or increased clearance. The other half found no changes in sympathetic markers when CPAP was used. Regarding lipid metabolites, two out of six studies found a reduction of total cholesterol levels within 24 hours of CPAP. The effects of CPAP on glucose control were negative in 88% of the trials included in the review. It is important to mention that there is evidence from well-designed trials that continuous use of CPAP for 8 hours for 14 days is capable of improving glucose response[88]. Whether is possible or not to achieve such this high adherence in a regular patient care scenario is debatable. CPAP seems to generate good responses in terms of oxidative stress and endothelial function markers, but not in inflammatory markers (such as IL-6, CRP, and others). Finally, in regards to liver enzymes and coagulation factors, the majority of studies are negative[87].

The literature in mandibular advancement devices and OSA biomarkers have been extremely scarce. One study showed improvement of some inflammatory markers in OSA patients after 3 and 6 months of active therapy, despite residual apneas[89]. A recent randomized controlled trial looked at CRP, IL-6, TNF-α, adiponectin, leptin, p-selectin, glucose and lipid metabolism, and N-terminal pro-brain natriuretic peptide (pro-BNP). They showed that despite a high adherence to treatment, and reduction of AHI, the was no effect on circulating biomarkers after 2 months of treatment[90].

Finally, biomarkers could also be used as a prognostic tool in OSA, which is the focus of this thesis. Potential marker candidates would share common pathological pathways between OSA and pertinent outcomes such as cardiovascular disease and be able to identify which patients are at increased risk of developing new or subsequent adverse events. Once more, there
is limited literature that examined potential prognostic markers in OSA. In 2016, Maeder et al[91] reviewed the literature comparing patients with and without OSA, looking at markers that were already well established in the cardiovascular literature. The conclusions pointed to BNP and cardiac troponins as potential prognostic markers in OSA. However, these markers were correlated with presence and severity of OSA, not necessarily further increased when OSA and a cardiometabolic condition was present[73]. More recently, Khalyfa et al[92] explored the expression of exosomal microRNAs (miRNAs) in untreated OSA patients with hypertension. When comparing normal dippers to reverse dipping hypertensive patients, they found two completely different clusters of miRNAs in non-dippers versus reverse dippers. There is a significant increase in miRNAs expressions in reverse dip hypertension subjects. This was supported by correlation with gene expressions that regulate pathways associated with endothelial dysfunction (e.g.: HIF-1). They provided evidence that a set of circulating exosomal miRNAs may have a pivotal role in nocturnal hypertension in OSA patients. Therefore, exosomal miRNAs may be potential prognostic biomarkers of cardiometabolic conditions in OSA populations[92]. Still, none of these studies were prospective. There are no studies conducted within an OSA population that used biomarkers to predict cardiometabolic consequences.

1.4 Research Goals, Gaps in the literature and Summary of Projects

The overall goal of this PhD was to explore the potential clinical utility of OS and CAM biomarkers in OSA, particularly to predict incident CVD. We first systematically reviewed the literature to identify biomarkers that have been used to identify cardiometabolic disease in OSA. We then identified that OS and CAM markers were promising candidates. After that, we explored the association with OSA adjusting for potential confounding factors in a large sample.
Finally, we then tested if any of the markers associated with OSA could be helpful identifying cardiovascular events in an OSA cohort.

The studies in this thesis aimed to fill different gaps in the literature. First, as seen in literature reviews[85,93] and in the background information presented above, there are no well-established circulating biomarkers in OSA populations to predict health outcomes. Second, the majority of studies in OSA are limited in sample size and lack adjustment of fundamental confounding factors such as obesity. Third, to provide prognostic utility to potential biomarkers, studies should be prospective in nature. Currently there are no prospective cohort studies that assessed the clinical utility of biomarkers to identify risk for cardiometabolic conditions in OSA patients.
Chapter 2: Circulating biomarkers to identify cardiometabolic complications in patients with Obstructive Sleep Apnea: A Systematic Review


2.1 Summary

Untreated Obstructive sleep apnea (OSA) is associated with an increased risk of cardiometabolic diseases such as diabetes and myocardial infarction. However, it is difficult to predict which patients are at particularly high risk. This systematic review aimed to identify potentially useful circulating biomarkers that could predict cardiometabolic complications in OSA. We searched Cochrane (EBM), EMBASE, Medline, PubMed, and Web of Science databases. Search concepts included: “Obstructive Sleep Apnea”, “Biomarkers” and “Risk-Stratification”. Manuscripts were included if they studied adults with OSA, circulating (blood) markers, and relationships with clinical outcomes. After screening, 10 were included. Studies addressed cardiovascular disease, type 2 diabetes, end-stage renal disease and metabolic syndrome. In general, levels of inflammatory markers, adhesion molecules, and vascular proteins were associated with the presence of cardiometabolic disease in OSA patients. Although studies regarding prognostic circulating biomarkers in OSA are limited, a number of potentially promising biomarkers were identified in our review. However, more research is needed using prospective cohorts to determine which biomarkers are most robustly associated with and useful in predicting future cardiovascular and metabolic sequelae in OSA patients. Identification of
such biomarkers could guide more selective and targeted therapy for OSA in an emerging era of precision-based medicine.

2.2 Introduction

The goal of this chapter is to identify and summarize studies that examined prognostic biomarkers in OSA. We wanted to see if there are any substantial gaps in the literature and also list potential markers that could be promising candidates to be further explored in the context of OSA.

OSA is the most common respiratory sleep disorder and is characterized by recurrent closure of the upper airway leading to intermittent hypoxia and arousals. OSA causes many adverse physiologic and biochemical sequelae including: activation of the sympathetic nervous system, systemic inflammation, oxidative stress, endothelial dysfunction, hypercoagulability, and metabolic dysregulation[94–97]. In addition, patients with OSA are at increased risk of developing cardiometabolic diseases [98] including cardiovascular disease (CVD), atrial fibrillation, hypertension, heart failure, diabetes, stroke, and metabolic syndrome[99–101]. For example, men with severe untreated OSA have approximately a three times greater risk of experiencing a fatal or non-fatal cardiovascular (CV) event compared to healthy controls[73].

One current challenge in the management of OSA is to distinguish which patients are at highest long-term risk of developing these complications. The ability to identify high-risk patients is important both in terms of providing prognostic information, and to provide the opportunity for a more targeted approach to OSA treatment (precision medicine). For example, if a patient could be identified as being at high risk of subsequent myocardial infarction, more aggressive management of OSA and other cardiac risk factors (e.g., hypertension) could be considered. Levels of circulating biomarkers may provide such information.
Many biomarkers have been linked to OSA and may be useful to predict cardiometabolic sequelae: these include inflammatory markers, oxidative stress markers, adhesion molecules, lipids, and catecholamines [84]. The purpose of this systematic review was to identify potentially useful circulating biomarkers that could predict cardiometabolic complications in OSA.

2.3 Materials and Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis PRISMA Checklist[102]. Our PICO(S) question was: In adult (>18 years old) patients with diagnosed OSA (P), will the presence or occurrence of a cardiovascular or metabolic complication (I) be associated with elevated levels of circulating biomarkers (O) in comparison with patients with OSA who do not have a cardiometabolic condition (C) within any study design (S)? Appropriate subject headings and keywords with different truncation and word combination were selected for various databases (Appendix A). The concepts included “Obstructive Sleep Apnea” and “Biomarkers.” The third concept “Risk Stratification” was not readily accessible as subject headings or keywords; therefore, two authors (BUP and AHA) screened each full-text checking for “risk stratification” concept, which was identified as any paper containing an OSA population, with and without a diagnosable comorbidity. Of note, the purpose of this review was not to identify biomarkers that have been associated with sleep apnea[103], but rather to identify biomarkers that may be useful in predicting future risk of cardiometabolic complications in patients with OSA.
2.3.1 Eligibility Criteria

2.3.1.1 Inclusion Criteria

Studies published in English with a full-text available, including adult subjects (18 years old or older), diagnosed with OSA (not central or mixed sleep apnea), and with blood samples drawn, were eligible for inclusion. Additionally, articles were required to report clinical outcomes associated with OSA (excessive daytime sleepiness and obesity were not considered).

2.3.1.2 Exclusion Criteria

Studies including children, infants or adolescents (less than 18 years old) were excluded from the review. Also, any other source of biological material other than blood (i.e., urine, saliva, exhaled breath condensation) was not considered. Histological and nonhuman studies, reviews, letters, expert opinions/interviews, and studies published as an abstract only were not included. Finally, studies without a control group within the OSA population were not considered.

2.3.2 Search Strategy and Study Screening

EBM Reviews, EMBASE, Medline, PubMed, and Web of Science databases were utilized (Appendix A.1). All references were exported using RefWorks (ProQuest, Bethesda, MD). EBM Reviews was included to confirm the existence of previous reviews regarding cardiometabolic biomarkers in OSA. Grey literature search, expert consult and manual search in references from included papers were verified to minimize the possibility of omitting any relevant study. The search process started in July 2\textsuperscript{nd}, 2016 and finished July 26\textsuperscript{th}, 2017. Initially, duplicates were removed through RefWorks, then two independent reviewers (BUP and AHA) applied inclusion and exclusion criteria to screen: first, for titles only, and then abstracts. After the initial screening, the same criteria was applied to full-text manuscripts. In case of any disagreement, a consensus was reached by consulting a third reviewer (NA).
studies were imported into a second citation management software (Zotero, Virginia, US), and had data extracted by one author (BUP). Variables extracted from all studies included: year of study, first author, country where study was conducted, study design, type of cohort (community vs hospital), criteria for diagnosis of OSA, sample size, sex, mean age, body mass index, mean AHI, mean and standard deviation or median and interquartile range of levels of biomarkers, time of blood collection, p-values comparing the OSA group with the OSA and comorbidity group, the laboratory technique that was used to process the samples, and time to follow-up. A modified Newcastle-Scale (NOS) was used to determine the of risk bias in selecting the studies that were reviewed.

2.4 Results

A total of 2,733 papers were identified. After duplicates were removed, and application of inclusion and exclusion criteria to titles, 1,143 papers remained. After abstract screening, 206 full-text articles were obtained. From these, we were able to identify prognostic markers in 14 studies. As part of grey literature search and manual inclusion of relevant papers, an additional 14 papers were identified; of these, only 1 study fit the criteria for inclusion in the final review (Appendix A.2 and A.3). As the primary focus was on cardiovascular and metabolic disorders, a total of 10
articles were included in the final analysis (Figure 2.1).

Figure 2.1 - Flow Diagram

2.4.1 Studies Characteristics

Of the 10 papers included in the final review (Tables 2.1 – 2.3), two were from China[104][105] and the remaining countries of origin varied with representation from Japan[106], USA[107], Turkey[108], Israel[109], Germany[110], France[111], Sweden[112] and Bulgaria[113]. The sample size ranged from 18[110] to 432[105]. Although one study used a community-based sample[112], the majority collected data from clinic-based samples.
There was substantial variation across the studies regarding sleep diagnostic testing for OSA. Some studies used portable monitors[112], while others used full polysomnography[107–109,111]. Oxygen desaturation criteria for OSA also varied; some used a 4% drop in the oxygen saturation to score hypopneas[107] while others used a 3% desaturation [113]. Some studies considered OSA as an AHI above 5/hr [104,108], while others only included severe cases (AHI>30)[107]. The modified Newcastle-Ottawa scale (Table 2.7) indicated that the majority of studies (90%) scored five or more out of nine in this quality assessment tool, which reflects an overall moderate quality of the evidence.

All study designs were either case-control or cross-sectional. In other words, none of the studies examined the use of biomarkers to prospectively identify the development of future complications, but rather studied the presence of concurrent OSA and the cardiac or metabolic outcome.

2.4.2 Biomarkers and Cardiovascular Complications

Most of the studies (6/10) investigated cardiovascular (CV) complications (Tables 2.1 and 2.4). Cardiovascular outcomes had a wide range of definitions; for example, some studies considered hypertension as a cardiovascular event, while others used composite outcomes including ischemic heart disease, heart failure, coronary artery disease, stroke and transient ischemic attack.

Table 2.4 describes the biomarkers tested in these studies. As can be appreciated, a broad range of markers were studied in this context. These included markers of oxidative stress and inflammation, levels of adhesion molecules, vascular proteins, catecholamines, lipids/lipoproteins, and glucose. Data should be interpreted cautiously given that most of the studies had small sample
sizes and were not prospective in design. In addition, none of the studies (6/6) reported levels of biomarkers after adjustment for possible confounding factors.

Nevertheless, inflammatory markers may be the most promising biomarkers to risk-stratify OSA patients, as in general, levels were elevated in patients with CVD. The most frequently studied molecule was C-reactive protein (CRP), which is produced by the liver with an important role in immune and inflammatory responses [114]. The other potentially important marker was YKL-40, also known as human cartilage glycoprotein 39 or chitinase-3-like protein 1. It is also involved in immune and inflammatory responses, and elevated YKL-40 levels are related to cell migration and remodeling after endothelial disruption. [115]. YKL-40 was significantly elevated in 134 OSA patients with coronary artery disease[104]. Finally, several other biomarkers such as hs-CRP, IL-1Ra, IL-8, RANTES and TNF-α were higher in fifteen OSA patients who had an acute cardiovascular event, compared to fifteen non-obese OSA with similar age, BMI and OSA severity[111].

Vascular proteins and adhesion molecules are also well-established markers for atherosclerosis and subsequent CVD in non-OSA cohorts. We identified two studies that examined these markers in OSA patients; both these studies were small. One of these molecules, intercellular adhesion molecule, is a glycoprotein that plays a role in leukocyte adhesion to injured endothelium and could be useful for risk stratification in OSA populations [32]. In our review, soluble intercellular adhesion molecule (sICAM) was positively associated with CVD in 15 OSA patients with acute coronary syndrome or cerebrovascular ischemic accidents[111]. Also, endoglin (a marker of angiogenesis that is involved in the progression of CVD) [33] and fms-like tyrosine kinase-1 (a regulatory marker of vascular endothelial growth) [34] were significantly elevated in eleven (n=11) hypertensive OSA patients [19].
Oxidative stress can occur because of an imbalance between oxidants and antioxidants. Oxidative stress plays a pivotal role in the initiation and progression of atherosclerosis in non-OSA populations[66,75,95]. OSA, which is categorized by intermittent hypoxia, can result in the development of oxidative stress, analogous to what happens with ischemic/reperfusion injury. In this review, oxidative stress markers such as thiobarbituric reactive substances (TBARS), peroxides, paraoxonase-1[109] and nitrite/nitrate[107] were not increased in OSA patients with CVD. However, it must be noted that only two studies examined these markers.

In terms of lipids and lipoproteins, there is a single report linking low-density lipoproteins (LDL) levels to OSA and coronary artery disease[104]. Cholesterol, high-density lipoprotein (HDL), LDL and triglycerides were no further elevated if a cardiovascular condition was accompanied by OSA[104,109].

Finally, the repetitive arousals and hypoxemia characteristic of OSA activate the sympathetic nervous system, which may also contribute to the development of hypertension and other cardiometabolic disease. However, we only identified one study that examined the utility of catecholamines such as epinephrine and norepinephrine as a prognostic marker. In this 22 patient study, catecholamine levels were not significantly elevated in OSA patients with hypertension compared to those without hypertension. [107].

2.4.3 Biomarkers and Metabolic Outcomes

There were only three papers that assessed metabolic outcomes in patients with OSA (Table 2.2 and 2.5) and their association with circulating biomarkers. In general, inflammatory markers were elevated more than other circulating biomarkers in patients with the metabolic outcome. However, lipid levels were not significantly elevated.
The most recent study[105] aimed to determine whether inflammatory markers such as YKL-40 and C-reactive protein were useful to predict the development of Type 2 diabetes (T2DM) in OSA patients. The relatively robust sample size yielded a modest statistical signal in a multivariate logistic regression model. The odds ratio (95% confidence interval) was 1.012 (1.003 – 1.019) for CRP and 1.023 (1.018 – 1.028) for YKL-40. The other study that investigated T2DM was identified by reviewing grey literature[113]. Resistin, a hormone that links obesity to diabetes, was significantly elevated in severe OSA patients with T2DM, after adjusting for age, BMI and homeostasis model assessment of insulin resistance. Finally, Shiina et al[106] included 41 patients with an AHI above 15 (using a 4% oxygen desaturation to score respiratory events). After adjusting CRP levels for age, gender, smoking status, cholesterol levels, antihypertensive drugs, statins, BMI and mean blood pressure there was no significant difference between OSA patients with and without metabolic syndrome.

2.4.4 Biomarkers and Renal Outcomes

Finally, one paper investigated kidney disease associated with OSA (Tables 2.3 and 2.6). End stage renal disease (ESRD)[110] was studied in a small sample (n=18) of OSA patients (AHI >10). CRP, and troponin levels were significantly higher in OSA patients with ESRD than OSA patients with normal renal function suggesting these may be reasonable markers of poor kidney outcomes to study in the future.
## Table 2.1 – Characteristics of cardiovascular studies

<table>
<thead>
<tr>
<th>Author (Year) / Country</th>
<th>Study design/</th>
<th>AHI Scoring Criteria</th>
<th>OSA Definition</th>
<th>Sample Size (n)</th>
<th>Age (years)</th>
<th>Male (%)</th>
<th>Mean BMI (kg/m²)</th>
<th>Mean AHI (SD)</th>
<th>Definition of Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavie et al (2004) + Israel (109)</td>
<td>Case-Control</td>
<td>PSG with no clear oxygen desaturation cutoff</td>
<td>RDI &gt;10 + at least one symptom (EDS, chronic fatigue, restless sleep) was considered OSA +.</td>
<td>59</td>
<td>58.5</td>
<td>83</td>
<td>30.6</td>
<td>31.3 (18.5)</td>
<td>OSA + CVD +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55</td>
<td>46.8</td>
<td>85.5</td>
<td>28.4</td>
<td>26.9 (13.8)</td>
<td>OSA + CVD -</td>
</tr>
<tr>
<td>Kokturk et al (2005) + Turkey (108)</td>
<td>Case-Control</td>
<td>PSG 3%</td>
<td>AHI ≥ 5 was considered OSA +.</td>
<td>38</td>
<td>44.5</td>
<td>100</td>
<td>31.7</td>
<td>32.9 (28.7)</td>
<td>OSA+ CVD+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56</td>
<td>42.9</td>
<td>100</td>
<td>32.3</td>
<td>44.9 (41.5)</td>
<td>OSA + CVD -</td>
</tr>
<tr>
<td>Mohsenin and Urbano (2011) + USA (107)</td>
<td>Cross-Sectional</td>
<td>PSG 4%</td>
<td>AHI ≥ 30 was considered OSA+.</td>
<td>11</td>
<td>48</td>
<td>91</td>
<td>41</td>
<td>81 (11)</td>
<td>OSA + Hypertension+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td>45</td>
<td>91</td>
<td>40</td>
<td>76 (9)</td>
<td>OSA + Hypertension-</td>
</tr>
<tr>
<td>Sui and Gao et al (2013) + China (104)</td>
<td>Case-Control</td>
<td>PSG with no clear oxygen desaturation cutoff</td>
<td>AHI ≥ 5 was considered OSA +.</td>
<td>134</td>
<td>57</td>
<td>74</td>
<td>26.56</td>
<td>Median (IQR): 24 (17 - 32)</td>
<td>OSA + Coronary Artery Disease+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>112</td>
<td>56</td>
<td>71</td>
<td>25.9</td>
<td>Median (IQR): 23.5 (17 - 30)</td>
<td>OSA + Coronary Artery Disease-</td>
</tr>
<tr>
<td>Testelmans et al (2013) France (111)</td>
<td>Case-Control</td>
<td>PSG with no clear oxygen desaturation cutoff</td>
<td>AHI ≥ 15 were considered OSA +.</td>
<td>15</td>
<td>53</td>
<td>93</td>
<td>25</td>
<td>36 (19)</td>
<td>OSA + CVD +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>52</td>
<td>80</td>
<td>25</td>
<td>38 (15)</td>
<td>OSA + CVD -</td>
</tr>
<tr>
<td>Johansson et al (2015) + Sweden (112)</td>
<td>Case-Control</td>
<td>Type III AASM Portable Monitor 4%</td>
<td>Sleep disordered breathing characterized by ODI and AHI.</td>
<td>119</td>
<td>79</td>
<td>67</td>
<td>27.7</td>
<td>Median (IQR): 8.8 (2.8-18)</td>
<td>OSA + CVD +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>221</td>
<td>78</td>
<td>40</td>
<td>27.4</td>
<td>Median (IQR): 4.4 (1.8-11.6)</td>
<td>OSA + CVD -</td>
</tr>
</tbody>
</table>

### Cardiovascular Outcomes

- **OSA + CVD +**
- **OSA + CVD -**
- **OSA + Hypertension+**
- **OSA + Hypertension-**
- **OSA + Coronary Artery Disease+**
- **OSA + Coronary Artery Disease-**
- **CVD +** = Either Hypertension or IHD, or history of MI or stroke.
- **Hypertension +** = BP ≥ 149/90 mmHg
- **CAD +** = >50% of stenosis in at least one major coronary artery in visual analysis of angiographic results.
- **Hypertension** = BP > 140/90 mmHg
- **CAD +** = Patients admitted with acute coronary syndrome or cerebrovascular ischemic accident.
- **CVD +** = Either ischemic heart disease (history of angina pectoris/myocardial infarction), heart failure (LVEF < 50% from echocardiography) or TIA/stroke.

### Abbreviations
- **AHI**: Apnea hypopnea index
- **BMI**: Body mass index
- **CAD**: Coronary artery disease
- **CVD**: Cardiovascular disease
- **EDS**: Excessive daytime sleepiness
- **IHD**: Ischemic heart disease
- **IQR**: Interquartile range
- **LVEF**: Left ventricle ejection fraction
- **MD**: Myocardial infarction
- **ODI**: Oxygen desaturation index
- **OSA**: Obstructive sleep apnea
- **PSG**: Polysomnography
- **RDI**: Respiratory disturbance index
- **SD**: Standard deviation
- **TIA**: Transient ischemic attack
- **USA**: United States

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Lavie et al (2004), Israel (109) +
Kokturk et al (2005), Turkey (108) +
Mohsenin and Urbano (2011), USA (107) +
Sui and Gao et al (2013), China (104) +
Testelmans et al (2013), France (111) +
Johansson et al (2015), Sweden (112) +

+ Cardiovascular Outcomes; AHI Scoring Criteria: refers to threshold values for desaturations to score hypopneas; Abbreviations: AASM: American academy of sleep medicine; AHI: Apnea hypopnea index; BMI: Body mass index; CAD: Coronary artery disease; CVD: Cardiovascular disease; EDS: Excessive daytime sleepiness; IHD: Ischemic heart disease; IQR: Interquartile range; LVEF: Left ventricle ejection fraction; MI: Myocardial infarction; ODI: Oxygen desaturation index; OSA: Obstructive sleep apnea; PSG: Polysomnography; RDI: Respiratory disturbance index; SD: Standard deviation; TIA: Transient ischemic attack.
<table>
<thead>
<tr>
<th>Author (Year) / Country</th>
<th>Study design/ Scoring Criteria</th>
<th>OSA Definition</th>
<th>Sample Size</th>
<th>Age (years)</th>
<th>Male %</th>
<th>Mean BMI (kg/m²)</th>
<th>Mean AHI (SD)</th>
<th>Definition of Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiina et al (2006)*+ Japan (106)</td>
<td>Cross-Sectional PSG 4%</td>
<td>AHI ≥ 15 was considered OSA+</td>
<td>41</td>
<td>51</td>
<td>95</td>
<td>30.8</td>
<td>51.9 (3.3)</td>
<td>OSA + MetS+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53</td>
<td>52</td>
<td>92</td>
<td>26.1</td>
<td>43.6 (2.5)</td>
<td>OSA + MetS-</td>
</tr>
<tr>
<td>Cherneva et al (2013) ++ Bulgaria (113)</td>
<td>Cross-sectional PSG 3%</td>
<td>AHI 5-30 events/h = mild to moderate and AHI &gt; 30 severe.</td>
<td>23</td>
<td>57</td>
<td>74</td>
<td>43</td>
<td>58.8 (34.2)</td>
<td>OSA + T2DM +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>42.6</td>
<td>100</td>
<td>43</td>
<td>62.3 (30.6)</td>
<td>OSA + T2DM -</td>
</tr>
<tr>
<td>Sun et al (2015) ++ China(105)</td>
<td>Cross-sectional PSG with no clear oxygen desaturation cutoff</td>
<td>AHI &gt; 5 was considered OSA +.</td>
<td>234</td>
<td>55</td>
<td>NR</td>
<td>26</td>
<td>NR</td>
<td>OSA + T2DM +</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>198</td>
<td>54</td>
<td>NR</td>
<td>25</td>
<td>NR</td>
<td>OSA + T2DM -</td>
</tr>
</tbody>
</table>

++ Metabolic Disorders, AHI Scoring Criteria: refers to threshold values for desaturations to score hypopneas; Abbreviations: AHI: Apnea hypopnea index; BMI: Body mass index; BP: Blood pressure; MetS: Metabolic syndrome; NR: Not reported; OSA: Obstructive sleep apnea; PSG: Polysomnography; SD: Standard deviation; T2DM: Type 2 diabetes mellitus.

Table 2.2 – Characteristics of metabolic studies
<table>
<thead>
<tr>
<th>Author (Year) / Country</th>
<th>Study design/ AHI Scoring Criteria</th>
<th>OSA Definition</th>
<th>Sample Size</th>
<th>Age (years)</th>
<th>Male %</th>
<th>Mean BMI (kg/m²)</th>
<th>Mean AHI (SD)</th>
<th>Definition of Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohnlein et al (2009)</td>
<td>Cross-sectional PSG 4%.</td>
<td>AHI&gt;10 was considered OSA +</td>
<td>10 NR NR NR</td>
<td>10 NR NR NR</td>
<td>25</td>
<td></td>
<td></td>
<td>ESRD + = Patients on maintenance hemodialysis treatment</td>
</tr>
</tbody>
</table>

+++ Other Comorbidities. AHI Scoring Criteria: refers to threshold values for desaturations to score hypopneas; Abbreviations: AHI: Apnea hypopnea index; BMI: Body mass index; ESRD: End stage renal disease; NR: Not reported; OSA: Obstructive sleep apnea; PSG: Polysomnography; SD: Standard deviation;

Table 2.3 – Characteristics of renal disease studies
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Authors</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Adj</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>Kokturk et al (2005)(108)</td>
<td>4.7</td>
<td>8.0</td>
<td>2.4</td>
<td>5.1</td>
<td>↓</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Testelmans et al (2013)(111)</td>
<td>7*</td>
<td>2.6 - 9.6</td>
<td>0.7</td>
<td>0.4 - 1.2</td>
<td>↓</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Johansson et al (2015)(112)</td>
<td>2.7</td>
<td>1.2 - 4.7</td>
<td>2.2</td>
<td>1.1 - 4.8</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>YKL-40 (ng/ml)</td>
<td>Sui and Gao et al (2013)(104)</td>
<td>136.1*</td>
<td>107.2 - 157.2</td>
<td>115.2</td>
<td>87.43 - 143.13</td>
<td>↓</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>Testelmans et al (2013)(111)</td>
<td>17.8*</td>
<td>6.9 - 14.17</td>
<td>5.9</td>
<td>4 - 7.9</td>
<td>↓</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RANTES (ng/ml)</td>
<td>Testelmans et al (2013)(111)</td>
<td>48.1*</td>
<td>37.6 - 65.1</td>
<td>35.3</td>
<td>28.2 - 44.1</td>
<td>↓</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>Testelmans et al (2013)(111)</td>
<td>2.5*</td>
<td>2 - 3.1</td>
<td>1.6</td>
<td>1-2.1</td>
<td>↓</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>YKL-40 (ng/ml)</td>
<td>Sui and Gao et al (2013)(104)</td>
<td>136.1*</td>
<td>107.2 - 157.2</td>
<td>115.2</td>
<td>87.43 - 143.13</td>
<td>↓</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxidative Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>Lavie et al (2004)(109)</td>
<td>18.6</td>
<td>7.3</td>
<td>17.2</td>
<td>6.3</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>PD (nmol/ml)</td>
<td>Lavie et al (2004)(109)</td>
<td>906.5</td>
<td>132.1</td>
<td>901.2</td>
<td>103.9</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>PON1 (U.min/ml)</td>
<td>Lavie et al (2004)(109)</td>
<td>79.5</td>
<td>13.6</td>
<td>86.7</td>
<td>17.6</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Adhesion Molecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sICAM (ng/ml)</td>
<td>Testelmans et al (2013)(111)</td>
<td>219*</td>
<td>208 - 252</td>
<td>185</td>
<td>169 - 204</td>
<td>↓</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vascular Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>Lavie et al (2004)(109)</td>
<td>1</td>
<td>0.4</td>
<td>0.9</td>
<td>0.1</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Endothelin – 1 (pg/ml)</td>
<td>Mohsenin and Urbano (2011)(107)</td>
<td>0.7</td>
<td>0.1</td>
<td>1.4</td>
<td>0.3</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>Mohsenin and Urbano (2011)(107)</td>
<td>90</td>
<td>4.6</td>
<td>74</td>
<td>4.4</td>
<td>↓</td>
<td>0.018</td>
</tr>
<tr>
<td>sEng (ng/ml)</td>
<td>Mohsenin and Urbano (2011)(107)</td>
<td>4.9</td>
<td>0.3</td>
<td>3.5</td>
<td>0.4</td>
<td>↓</td>
<td>0.016</td>
</tr>
<tr>
<td>Lipids/Lipoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>Lavie et al (2004)(109)</td>
<td>5.1</td>
<td>1</td>
<td>5.3</td>
<td>0.9</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sui and Gao et al (2013)(104)</td>
<td>5.4</td>
<td>1.3</td>
<td>5.2</td>
<td>1.03</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>Lavie et al (2004)(109)</td>
<td>1.2</td>
<td>0.3</td>
<td>1.1</td>
<td>0.2</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sui and Gao et al (2013)(104)</td>
<td>1.9</td>
<td>0.6</td>
<td>1.8</td>
<td>0.52</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>Lavie et al (2004)(109)</td>
<td>3.1</td>
<td>0.8</td>
<td>3.3</td>
<td>0.84</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sui and Gao et al (2013)(104)</td>
<td>3.7</td>
<td>1.0</td>
<td>3.3</td>
<td>0.95</td>
<td>↓</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>Lavie et al (2004)(109)</td>
<td>1.9</td>
<td>1</td>
<td>2.0</td>
<td>0.85</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>Lavie et al (2004)(109)</td>
<td>6</td>
<td>1.4</td>
<td>5.5</td>
<td>0.7</td>
<td>↓</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Sui and Gao et al (2013)(104)</td>
<td>1.9</td>
<td>0.6</td>
<td>1.8</td>
<td>0.52</td>
<td>↓</td>
<td>NS</td>
</tr>
<tr>
<td>Cathecolamines</td>
<td>Mohsenin and Urbano (2011)(107)</td>
<td>840</td>
<td>92</td>
<td>790</td>
<td>110</td>
<td>†</td>
<td>NS</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------</td>
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<td>----</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>Mohsenin and Urbano (2011)(107)</td>
<td>38</td>
<td>5.9</td>
<td>54</td>
<td>11</td>
<td>†</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Cardiovascular Outcomes**: *Median and Interquartile range; †: Unadjusted analysis; Abbreviations: Adj: Statistical adjustment; CRP: C-reactive protein; CVD: Cardiovascular disease; HDL: High density lipoprotein; IL-1Ra: Interleukin 1 receptor antagonist; IL-8: Interleukin-8; LDL: Low-density lipoprotein; NS: Not significant (p>0.05); PD: Peroxides; PON-1: Paraoxonase-1; RANTES: Regulated on Activation Normal T Cell Expressed and Secreted; sICAM: soluble intercellular adhesion molecule; SD: Standard deviation; sEng: Soluble endoglin; sFlt-1: Soluble fms-like tyrosine kinase-1; TBARS: Thiobarbituric reactive substances; TNF-α: Tumor necrosis factor alpha; NOTE: Significant association between biomarkers and outcomes are in bold.

Table 2.4 – Characteristics of cardiovascular markers
<table>
<thead>
<tr>
<th>Characteristics of metabolic markers</th>
<th>Authors</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Adj</th>
<th>Significance (p-value)</th>
</tr>
</thead>
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<tr>
<td><strong>Biomarkers</strong></td>
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<td></td>
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<tr>
<td><strong>Inflammation</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>Shiina et al (2006)(106)</td>
<td>1.8</td>
<td>0.2</td>
<td>1.2</td>
<td>0.1</td>
<td></td>
<td>NS</td>
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<tr>
<td></td>
<td>Sun et al (2015)(105)</td>
<td>2.6</td>
<td>1.7-4.7</td>
<td>2.2</td>
<td>1.2-3.5</td>
<td></td>
<td>0.017</td>
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<tr>
<td>YKL-40 (ng/ml)</td>
<td>Sun et al (2015)(105)</td>
<td>205*</td>
<td>146-272</td>
<td>135.7</td>
<td>114-163</td>
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<td>&lt;0.001</td>
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<tr>
<td><strong>Vascular Proteins</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1C</td>
<td>Cherneva et al (2013)(113)</td>
<td>6.7</td>
<td>1.1</td>
<td>5.6</td>
<td>0.44</td>
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<td>Resistin (ng/ml)</td>
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<td>3.23</td>
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<td>0.043</td>
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<td><strong>Lipids/Lipoproteins</strong></td>
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<td></td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>Shiina et al (2006)(106)</td>
<td>5.6</td>
<td>0.2</td>
<td>5.5</td>
<td>0.1</td>
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<td>NS</td>
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<tr>
<td></td>
<td>Cherneva et al (2013)(113)</td>
<td>5.2</td>
<td>1.0</td>
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<td>HDL (mmol/l)</td>
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<td>1.1</td>
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<td></td>
<td>Cherneva et al (2013)(113)</td>
<td>1.3</td>
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<td>0.26</td>
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<td>Sun et al (2015)(105)</td>
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<td>1.3</td>
<td>0.3</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>Cherneva et al (2013)(113)</td>
<td>2.9</td>
<td>0.07</td>
<td>3.2</td>
<td>1.03</td>
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<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sun et al (2015)(105)</td>
<td>3.5</td>
<td>1.1</td>
<td>3.4</td>
<td>1.02</td>
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<td>NS</td>
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<td>Triglycerides (mmol/l)</td>
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<td>2.6</td>
<td>0.2</td>
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<td></td>
<td>Cherneva et al (2013)(113)</td>
<td>1.7</td>
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<td></td>
<td>Sun et al (2015)(105)</td>
<td>1.9</td>
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<td>Fatty acids (mmol/l)</td>
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<td>0.3</td>
<td>0.15</td>
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<td><strong>Glucose (mmol/l)</strong></td>
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<td>Shiina et al (2006)(106)</td>
<td>6.1</td>
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<td>Cherneva et al (2013)(113)</td>
<td>7.0</td>
<td>1.3</td>
<td>4.9</td>
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<td>NR</td>
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<td><strong>Insulin (mU/l)</strong></td>
<td>Cherneva et al (2013)(113)</td>
<td>18.9</td>
<td>16.6</td>
<td>22.6</td>
<td>12.4</td>
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<td>NS</td>
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**Metabolic Disorders: Metabolic Syndrome**  *(Shiina 2006) and T2DM (Cherneva 2013 and Sun 2015).*  *Median and Interquartile range; **Metabolic syndrome characterized by HDL (mmol/l) <1.036 (male), <1.295 (female); Triglycerides ≥ 1.695 mmol/l; BP ≥130/85 mmHg or BP drugs; Fasting glucose ≥ 6.105 mmol/l BMI ≥ 27.5; ↑: Unadjusted analysis ; ↑↑ Adjusted for age, gender, smoking status, total cholesterol, antihypertensive drugs, statins, BMI and mean blood pressure; ↑↑↑↑ Adjusted for Age, BMI and HOMA-IR. Abbreviations: Adj: Statistical adjustment; BMI: Body mass index; CRP: C-reactive protein; HbA1c: Hemoglobin A1c; HDL: High density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: Low-density lipoprotein; NR: Not reported; NS: Not significant (p>0.05); SD: Standard deviation; T2DM: Type 2 Diabetes Mellitus; NOTE: Significant associations are in bold.

Table 2.5 – Characteristics of metabolic markers
### Table 2.6 – Characteristics of renal disease markers

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Authors</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Adj</th>
<th>Significance (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>Kohnlein et al (2009)(110)</td>
<td>ESRD+</td>
<td>11.6</td>
<td>0.02</td>
<td>5.1</td>
<td>4.9</td>
<td>&lt;0.01</td>
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<tr>
<td>cTnT (ug/L)</td>
<td>Kohnlein et al (2009)(110)</td>
<td>ESRD+</td>
<td>0.38</td>
<td>0.3</td>
<td>0.01</td>
<td>NR</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Other Comorbidities:** ★: Unadjusted analysis; **Abbreviations:** Adj: Statistical adjustment; CRP: C-reactive protein; cTnT: Cardiac troponin T; ESRD: End stage renal disease; NR: Not reported; SD: Standard deviation; NOTE: Significant associations between biomarkers and outcomes are in bold.

### Quality Assessment (The Newcastle-Ottawa Scale – NOS for Case Control Studies)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Selection (★★★★)</th>
<th>Comparability (★★)</th>
<th>Outcome/Exposure (★★★★)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavie et al 2004 (109)</td>
<td>★★</td>
<td>★</td>
<td>★★★</td>
</tr>
<tr>
<td>Kortuk et al 2005 (108)</td>
<td>★</td>
<td>★★</td>
<td>★</td>
</tr>
<tr>
<td>Shiina et al 2006 (106)</td>
<td>★★</td>
<td>★★</td>
<td>★</td>
</tr>
<tr>
<td>Kohnlein et al 2009 (110)</td>
<td>★★</td>
<td>★</td>
<td>★★</td>
</tr>
<tr>
<td>Mohesin and Urbano et al 2011 (107)</td>
<td>★★★</td>
<td>★</td>
<td>★★★</td>
</tr>
<tr>
<td>Sui and Gao et al 2013 (104)</td>
<td>★★</td>
<td>★</td>
<td>★★★</td>
</tr>
<tr>
<td>Testelmans et al 2013 (111)</td>
<td>★★</td>
<td>★★</td>
<td>★★★</td>
</tr>
<tr>
<td>Johansson et al 2015 (112)</td>
<td>★★★</td>
<td>★</td>
<td>★★</td>
</tr>
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<td>Sun et al 2015 (105)</td>
<td>★★</td>
<td>★★</td>
<td>★★</td>
</tr>
<tr>
<td>Cherneva et al 2013 (113)</td>
<td>★★</td>
<td>★</td>
<td>★★</td>
</tr>
</tbody>
</table>

Star ratings were given based on manual for scoring the Newcastle-Ottawa Quality Assessment Scale. A study can be awarded a maximum one star for each item within selection and exposure categories, and a maximum of two stars can be given for comparability. Selection: a star is awarded if case is defined with independent validation, if cases are representative, if there are community controls and if controls have no history of disease. Comparability: a star is awarded if study controlled for confounding at the design or analysis stage, an extra star if controlled for any additional factor. Exposure: a star is awarded if exposure is ascertained by records or blinded structured interviews, another star if cases and controls had the same method of ascertainment, and a final star if the non-response rate is the same for both groups.

Table 2.7 – Quality Assessment
2.5 Discussion

The search for biomarkers in patients with OSA has been emphasized recently[84]. Most efforts have been directed towards biomarkers that could be used to screen for OSA[103] [116]; however, the identification of markers that could predict adverse clinical outcomes is also of fundamental importance. The recent negative results from the SAVE trial[79], where CPAP prescription did not prevent cardiovascular events in unselected patients with OSA, support the initiative to develop novel strategies to treat patients with OSA to prevent future complications. The identification of robust circulating biomarkers that would help to risk stratify patients could help substantially in this regard. For example, we would envision that an OSA patient at high risk of subsequent myocardial infarction may have more aggressive treatment of OSA and cardiovascular risk factors, especially given the improved outcomes in patients in the SAVE study adherent to CPAP therapy. In addition, in the future, elevation of certain biomarkers may help to target therapy more precisely. For example, though speculative, patients with higher levels of oxidative stress may benefit with antioxidant therapy, while anti-inflammatory agents may be more beneficial in patients with higher degrees of systemic inflammation. In this review, we have summarized the current knowledge of circulating biomarkers associated with cardiometabolic outcomes in patients with OSA.

2.5.1 Cardiovascular Outcomes

From our review, markers of inflammation may be a promising biomarker in this regard. In general, circulating levels of these biomarkers are elevated in OSA patients and concomitant CVD. Inflammatory processes play a fundamental role in the development of CVD. The oxidative and inflammatory events at the endothelium level (triggered by risk factors, such as smoking, obesity and hypertension) contribute to fatty-fibrotic vascular lesions. The persistence of such
plaques can cause physical trauma and further propagate the inflammatory process[117]. In non-OSA cohorts, substantial data have demonstrated the independent predictive value of CRP, a marker of systemic inflammation, in predicting cardiovascular death, such that CRP levels are used to risk stratify the use of HMG CoA reductase inhibitors[118]. For example, data from the Framingham Heart Study shows that patients with elevated CRP levels (above 3mg/l) have a significantly increased hazard rate (95% CI: 1.16 – 2.15) for cardiovascular events, after adjusting for age, sex, systolic blood pressure, anti-hypertensive therapy, cholesterol, smoking and diabetes[119]. A recent study also showed that the administration of monoclonal antibodies to interleukin-1-B reduces rates of CV events in high risk patients, supporting the pivotal role of inflammation in the pathogenesis of CVD[65].

Furthermore, animals exposed to intermittent hypoxia consistently develop elevated circulating levels of inflammatory biomarkers. The inflammation is likely caused by intermittent hypoxia and re-oxygenation, resulting in oxidative stress, and activation of NFkB pathways. Biomarker levels that are elevated in animal models include IL-1β, IL-6, TNF-α, protein carbonyls, 8-hydroxyguanosine, and CRP[78,120]. Interestingly, the impact of CPAP therapy on levels of inflammatory markers in OSA patients is inconsistent, with some randomized controlled trials showing little benefit of treatment in this regard[87]. We suspect that this may reflect differences in adherence with CPAP therapy rather than the fact that OSA per se does not contribute to systemic inflammation.

Adhesion molecules may also be promising biomarkers in the risk stratification of OSA patients. Adhesion molecules including E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1) modulate the binding of leukocytes to vascular endothelium. They are present in atherosclerotic plaques and contribute to disease progression. In
prospective non-OSA cohorts, circulating levels of these molecules are associated with incident cardiovascular disease. [10-13]. To illustrate, one study demonstrated that circulating levels of ICAM-1 were independently associated with incident CVD and carotid artery atherosclerosis (odds ratios of 5.53 and 2.64, respectively)[10]. Furthermore, patients with OSA have increased serum levels of adhesion molecules [14-16]. In our review, we identified one small study that examined the association between ICAM-1 levels and coronary/cerebrovascular ischemic events[111]. Although this study showed a significant difference in sICAM levels between OSA patients with and without these events, larger studies with a broader array of these molecules are clearly needed.

Circulating angiogenic proteins were investigated in a relatively small sample (n=11) of severe OSA patients (AHI >30)[19]. Soluble fms-like tyrokinase-1 (sFlt-1) and soluble endoglin (sEng) were elevated in the hypertensive group, compared to the normotensive group. Interestingly, both of these molecules are well-known markers for preeclampsia in women, which is also considered to be a disease of endothelial dysfunction. Consequently, they may also reflect potentially useful molecules of CVD risk in patients with OSA.

Oxidative stress also plays a key role in the development of CVD. Reactive oxygen species (ROS) are chemically reactive molecules such as superoxide, hydroxyl radical, and peroxides characterized by an unpaired electron in their outer atomic shell. Under conditions of stress, levels of ROS can increase dramatically, and can overwhelm the antioxidant capacity for detoxification leading to a state of oxidative stress. These ROS can damage a variety of cellular molecules including DNA, RNA, and proteins, which in turn can cause membrane damage, cell death, apoptosis, and activation of inflammation. Oxidative stress is recognized increasingly as a fundamental contributor to the pathogenesis of CVD; indeed, ROS play a role in mediating the
adverse effects of many CV risk factors including diabetes, obesity, smoking, and air pollution. Ischemia reperfusion injury, which is the tissue damage that results due to reestablishment of oxygen supply to ischemic tissue, is also thought to be largely mediated by oxidative stress[86]. The similarity between ischemic/reperfusion injury and intermittent hypoxia leads to a unifying paradigm linking oxidative stress to CVD and OSA[75]. Indeed, many animal studies have shown robust activation of oxidative stress when they are exposed to intermittent hypoxia as reflected by increased levels of 8-isoprostane, protein carbonyls, and other markers[74][121].

However, the studies we identified in our review did not demonstrate increased oxidative stress markers in OSA patients with CVD. It must be noted, that we only found two studies in this regard and the sample sizes were small. In addition, only four markers were tested and the temporal relationship between oxidative stress and clinical symptoms of OSA and CVD was also unclear. Given the theoretical links between OSA, oxidative stress, and CVD, future studies of oxidative stress biomarkers should be considered despite these negative studies.

2.5.2 Metabolic Disorders

Intermittent hypoxia and sleep fragmentation are linked to metabolic dysfunction in OSA. Increased sympathetic activation, oxidative stress, systemic inflammation, and alteration of adipokines can predispose OSA patients to hypertension, insulin resistance and β-cell dysfunction. These conditions can lead to type 2 diabetes mellitus, potentially causing vascular complications in OSA patients. Indeed, OSA is an independent risk factor for T2DM. Evidence from prospective cohort studies indicate a relative risk of 1.35 with a 95% confidence interval from 1.24 to 1.47[122]. Additionally, CPAP treatment seems to improve glycemic control and insulin resistance[123], especially if done in a well-controlled, supervised settings[88]. A bidirectional relationship between OSA and T2DM has been suggested, since diabetes can affect
respiratory control, leading to sleep-disordered breathing[122]. In our review, inflammatory and vascular proteins (resistin and endoglin) were significantly associated with the presence of diabetes and metabolic syndrome in OSA patients. However, it must be noted that these studies were not prospective in nature, and we cannot exclude the possibility that the metabolic comorbidities caused elevations in these markers as opposed to the other way around.

2.5.3 Renal Outcomes

It well recognized that renal failure can contribute to OSA, and that more frequent dialysis and ultrafiltration can improve OSA severity in them[124,125]. However, it is becoming increasingly recognized that OSA may contribute to kidney injury through a variety of mechanisms including oxidative stress, sympathetic activation, and renin-angiotensin-aldosterone system activation[126,127]. In our review, we identified one cross sectional study that showed significant increases in levels of C-reactive protein and troponin levels in OSA patients with end-stage renal disease[110]. However, prospective studies are required to determine if other markers are associated with risk of accelerated renal dysfunction.

An ideal prognostic OSA biomarker should be disease-specific, treatment-sensitive, exist in a causal pathway, and predict improvements in outcome[128]. Previous reviews focused on identifying unique OSA signatures that highlighted inflammatory, metabolic, and oxidative stress molecules as potential markers[84], however prognostic biomarkers haven’t been systematically explored[103][129]. Our review identified that inflammatory markers (CRP, YKL-40), vascular proteins (resistin, endoglin) and adhesion molecules (ICAM) are biologically linked with OSA and potential cardiometabolic consequences and thus may be useful prognostic markers in patients with OSA. Also, the limited number of studies on oxidative stress markers and catecholamines justify future investigations to clarify their potential role in risk stratifying individuals with OSA.
This is the first report attempting to identify prognostic markers for cardiometabolic complications in the OSA population. A number of limitations of our analysis need to be acknowledged. First, there was significant heterogeneity among the studies that limited the possibility of pooling the data using meta-analysis. Second, the surprising lack of prospective studies makes interpretation of prognostic markers difficult. Specifically, it is possible that the cardiometabolic comorbidities may have at least partially caused the elevation in markers, which would limit their utility as potential prognostic variables. Third, in our review we focused on circulating markers and did not include studies of other biologic fluids (e.g. saliva, urine). Because of the frequency, ease, consistency, and availability of blood collection in clinical laboratories, we believed that blood tests would be more useful as a future clinical test which is the reason, we focused on this. Finally, there is a risk of publication bias, in that negative studies may be less likely to be published.

2.6 Conclusions

In this comprehensive systematic review, we identified ten cross-sectional studies that examined the potential utility of prognostic biomarkers in patients with OSA. In general, inflammatory markers, vascular proteins, and adhesion molecules seem to be associated with adverse cardiometabolic outcomes. Although oxidative stress and catecholamine levels were not predictive of cardiometabolic disease, the number of studies were limited. We did not find any prospective studies. Based on this review, oxidative stress and cellular adhesion molecules might be promising candidates to be further studied in large samples, which led to our two subsequent studies.


3.1 Summary

Oxidative stress (OS) drives cardiometabolic diseases. Intermittent hypoxia consistently increases oxidative stress markers. Obstructive sleep apnea (OSA) patients experience intermittent hypoxia and an increased rate of cardiovascular disease; however, the impact of OSA on OS markers is not clear. The objective was to assess relationships between OSA severity and biomarker levels. Patients with suspected OSA referred for a polysomnogram (PSG) provided fasting blood sample. Plasma levels of 8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG) and superoxide dismutase (SOD) were measured. The relationship between OSA and OS was assessed both before and after controlling for confounders (age, sex, smoking history, history of cardiovascular disease, ethnicity, diabetes, statin usage, body mass index-BMI). 402 patients were studied (68% male, mean age +/- SD =50.8 ± 11.8yrs, apnea-hypopnea index (AHI)=22.2±21.6 events/hour, BMI=31.62±6.49Kg/m². In a multivariable regression, AHI significantly predicted 8-isoprostane levels (p=0.0008) together with age and statin usage; AHI was not a predictor of 8-OHdG or SOD. Female sex (p<0.0001) and no previous history of cardiovascular disease (p=0.002) were associated with increased antioxidant capacity. Circulating 8-isoprostane levels may be a promising biomarker of the severity of oxidative stress.
in OSA patients. Prospective studies are needed to determine whether this biomarker is associated with long-term cardiometabolic complications in OSA.

3.2 Introduction

On the previous study we identified a substantial gap in the literature. We identified that oxidative stress markers and adhesion molecules are promising candidates to identify cardiometabolic complications in OSA patients, however the number of studies were limited, and the majority failed to control for potential confounding factors. With that in mind, we investigated the relationship between OSA and well-known markers of oxidative stress in this chapter.

Obstructive sleep apnea (OSA) is the most common respiratory sleep disorder, with an estimated 425 million middle aged adults world-wide having moderate to severe disease[37][37]. OSA is characterized by recurrent collapse of the upper airway leading to intermittent hypoxia and sleep fragmentation. Patients with OSA are at increased risk of developing cardiometabolic diseases including cardiovascular disease (CVD), atrial fibrillation, renal disease, hypertension, diabetes, stroke, and metabolic syndrome[126,130–132]. For more information, see section 1.2 and 1.3 of chapter 1.

Oxidative stress is a likely contributor to OSA related pathologies[133]. Reactive oxygen species (ROS) are chemically reactive molecules produced in the normal metabolism of oxygen. Under stress conditions, ROS levels can increase dramatically, overwhelming antioxidant capacity and therefore leading to a state of oxidative stress (OS). ROS can damage cellular molecules which can cause DNA modification, cell death, apoptosis, and inflammation[134–136]. In addition, oxidative stress is recognized increasingly as a fundamental contributor of CVD; indeed, ROS play a role in mediating the adverse effects of many CV risk factors including
diabetes, obesity, smoking, and air pollution[134]. Moreover, markers of oxidative stress such as 8-isoprostane levels are an independent risk factor for coronary heart disease[137].

In OSA, analogous to ischemic/reperfusion injury, intermittent hypoxia and consequent reoxygenation can increase the production of ROS[86]. In this regard, rodents exposed to chronic intermittent hypoxia, a validated animal model of OSA, have increased OS biomarkers[138]. Furthermore, studies in OSA patients suggest a potential relationship between many OS biomarkers and OSA, including markers of lipid peroxidation, antioxidant capacity, and DNA oxidation[76]. However, these studies had relatively small numbers of patients (usually <100) without the ability to control adequately for confounders, and larger studies are thus needed[93,139].

The objective of this study was to assess circulating levels of three oxidative stress markers in a large cohort of patients with suspected OSA. Each of these markers measures a different component of OS; specifically, 8-isoprostane is a marker of lipid peroxidation, 8-hydroxydeoxyguanosine (8-OHdG) is a marker of RNA/DNA oxidation, and superoxide dismutase (SOD) is a protective antioxidant enzyme. Because our goal was to represent the overall oxidative status of the patients in this study, we chose two well-known pro-oxidant markers (8-isoprostanе and 8-OHdG) and an antioxidant marker (SOD).

3.3 Materials and Methods

3.3.1 Sample and Laboratory Analysis

This study was approved by the University of British Columbia Research Ethics Board (H13-00346) and Vancouver Coastal Health Research Institutes (V11-80199). Adults (≥19 years old) referred for suspected OSA to the University of British Columbia Hospital Sleep Disorder Laboratory for inpatient polysomnography (PSG) were recruited. Patients were recruited from

PSG was performed using conventional instrumentation and scored according to the recommendations of the American Academy of Sleep Medicine (AASM)[140]. PSG recordings include electroencephalography (EEG) channels, electro-oculograms (left and right), submental electromyograms (EMG), and bilateral tibialis anterior EMG, airflow using nasal pressure and oral thermistor, respiratory efforts using inductance plethysmography belts placed around the chest and abdomen, and oxygen saturation ($SaO_2$) with finger pulse oximetry. PSGs were scored by experienced registered polysomnographic technologists blinded to laboratory results. An obstructive apnea was defined as a decrease in respiratory airflow $\geq$90% for $\geq$10 s with continued respiratory efforts; an obstructive hypopnea is defined as a decrease in respiratory airflow of $\geq$30% for $\geq$10 s followed by a decrease in $SaO_2$ of $\geq$3% or an arousal. Frequency of apneas and hypopneas was used to calculate the apnea hypopnea index (AHI) per hour of sleep time.

Patients were diagnosed as having OSA based on an AHI of $\geq$5 events/hour. An AHI between 5 and 15 events/hour was considered mild OSA, 15-30 events/hour was considered moderate and above 30 events/hour was considered severe.

Consenting patients (appendix B1) completed a questionnaire (appendix B2) about their family and medical histories, sleep habits and symptoms, mood disorders, alcohol use, smoking status, presence of diabetes (types I and II) and sleepiness on the night of their PSG. History of previous cardiovascular disease (CVD) was determined based on previous diagnosis of hypertension, myocardial infarction, cardiac arrhythmias, angina, and congestive heart failure.

Fasting blood (15 ml) was collected by venipuncture on the morning after PSG (appendix B3), and plasma was stored in a -80°C freezer. ELISA and colorimetric assays (Cellbiolabs, CA,
USA) were used to test sample levels of 8-isoprostone, 8-hydroxydeoxyguanosine (8-OHdG) and superoxide dismutase (SOD). The samples were analyzed in March and April of 2019.

3.3.2 Statistical Analysis

Statistical Analysis Software (SAS version 9.4, SAS Institute Inc, USA) was used to determine descriptive statistics on patient demographics/characteristics and levels of oxidative stress markers. We used Pearson’s correlation to assess the relationship between each of the markers and the markers with relevant confounders (continuous variables). To assess the relationship between categorical variables and oxidative stress markers a Student T-test was used. Variables investigated for potential confounding were BMI, severity of sleep apnea (AHI), age, sex, smoking status, previous heart disease, diabetes, statins usage, ethnicity (Caucasians and non-Caucasians). Variables with a p-value of less than 0.2 were included in multivariable linear regression models (in addition to age and sex).

3.4 Results

A total of 402 patients were included in the study; baseline characteristics are shown in table 3.1. Most of the patients were Caucasian and the majority were male (68%), with a mean age of 50.8 yrs. In general, they had moderate OSA (mean AHI was 22.2/hr) and were obese (mean BMI 31.6 kg/m²).
Results of the univariate analyses are shown in Table 3.2. In univariate analyses, increased AHI and decreased age were significantly associated with elevated 8-isoprostane levels; increased BMI tended to be associated with increased levels (p=0.07) while statin use tended to decrease levels (p=0.17). 8-OhDG levels were only associated with sex (p=0.017) with a greater level in males. Increased SOD activity was significantly associated with increasing BMI and female sex, with a trend for increasing AHI (p=0.08); presence of heart disease was associated with a decreased level (p=0.09). A significant negative relationship was found between 8-isoprostane and SOD activity (r=-0.31, p<0.0001) but not between 8-isoprostane and 8-OhDG, or between SOD and 8-OhDG (p>0.2).
Table 3.2 – Univariate Analysis

Based on a threshold p-value <0.2, we constructed multivariable models including these variables, age and sex (table 3.3). Significant independent predictors of increased 8-isoprostane levels included increasing AHI, reduced age, and non-use of statins. AHI was not an independent predictor of the other two markers. SOD activity was significantly increased in females, while BMI and the presence of heart disease decreased levels. Percent below 90% of oxygen saturation was initially included in the model for isoprostane; however, due to the high correlation with AHI and no additional effect on the model we excluded this from the final model (although when it was included with AHI, AHI still remained an independent predictor p=0.0013 while saturation was not).
<table>
<thead>
<tr>
<th></th>
<th>8 – Isoprostane (pg/ml)</th>
<th>8-OhdG (ng/ml)</th>
<th>SOD Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>p-value</td>
</tr>
<tr>
<td>Age</td>
<td>60.32</td>
<td>17.49</td>
<td>0.0006</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>132.27</td>
<td>431.21</td>
<td>0.7592</td>
</tr>
<tr>
<td>BMI</td>
<td>37.74</td>
<td>31.98</td>
<td>0.2388</td>
</tr>
<tr>
<td>AHI</td>
<td>32.13</td>
<td>9.492</td>
<td>0.0008</td>
</tr>
<tr>
<td>Statin Usage</td>
<td>-1172.65</td>
<td>515.17</td>
<td>0.0234</td>
</tr>
<tr>
<td>Heart Disease*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: AHI: Apnea-Hypopnea Index; BMI: Body mass index; OHdG: Hydroxydeoxyguanosine; SOD: Superoxide dismutase; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure. – variables not included

Table 3.3 - Multiple Linear Regression Models

3.5 Discussion

We investigated markers of lipid peroxidation (8-isoprostane), DNA degradation (8-OHdG) and antioxidant capacity (SOD) in a large cohort of patients with suspected OSA. OSA severity was independently associated with circulating 8-isoprostane levels even after controlling for relevant confounders such as age, sex, BMI, and statin usage. However, AHI was not independently associated with the other biomarkers of OS we measured. Female sex, and no previous history of cardiovascular disease were associated with increased SOD activity.

Animal studies consistently demonstrate that intermittent hypoxia increases a broad range of OS biomarkers[78]. For example we showed that exposure of mice to 8 weeks of intermittent hypoxia (fraction of FiO₂ reduced to 6%, 60/hr during the day for 8 weeks) increased circulating levels of isoprostane[133], while another showed increased levels 8-OHdG levels with intermittent hypoxia exposure[141]. Our results differ somewhat from these animal investigations. In our study of patients with OSA, only 8-isoprostane levels were associated with AHI while the other biomarkers were not. These differences might be related to several factors including differences in species, duration of hypoxia (weeks vs. years), and the intensities of hypoxia stimuli; the degree of hypoxia is more severe in most animal models than in human OSA. For example, an exposure
to 6% FiO₂ for 30 seconds every minute results in an oxy-hemoglobin saturation of 55-60%[133]. In contrast, the degree of desaturation in patients with OSA tends to be more modest, as in our cohort, even patients with severe OSA only spent 12.29% of the study below 90% on average.

Our study confirms and extends the results of previous investigations in terms of an association between 8-isoprostane levels and OSA. Carpagnano and colleagues reported that 8-isoprostane breath condensate levels in 18 patients with OSA were elevated compared to obese subjects and health controls[142]. A meta-analysis of 222 patients with OSA and 194 controls showed substantial increases in the standardized mean difference (g=1.1) of 8-isoprostane levels[139]. However, this study combined different sources of 8-isoprostane (plasma, exhaled breath condensate, and urine) and the results were not adjusted for known confounding factors. Our study exceeds the sample size of all studies included in this recent meta-analysis by (222 patients with OSA versus 329) and controlled for important confounders.

In our study, severity of OSA was not associated with 8-OHdG. However, a study by Pialoux and colleagues reported increases of 40-46% (p<0.05) in 8-OHdG when subjects were exposed to an intermittent hypoxia protocol[143]. This study only included 10 healthy male subjects, and they were exposed to a fairly significant degree of oxygen desaturation over a short time (4 days) only during the daytime while awake. The applicability of these findings to humans with OSA is thus unclear.

Our study is particularly relevant given the association between 8-isoprostane and cardiovascular disease in non-OSA cohorts[137]; in women, high urinary levels are associated with doubled odds of developing cardiovascular disease[68]. 8-isoprostane is associated with many risk factors for coronary heart disease including obesity and smoking[137]. Also, accepted
markers of cardiovascular disease, such as c-reactive protein (CRP)[144], correlate with 8-isoprostane \( r=0.097, p<0.001 \)[145].

The demonstration of an independent association between OSA severity and isoprostane in our study, together with the animal work showing increased levels of isoprostane with experimentally induced intermittent hypoxia[133] suggests that OSA is a cause of OS. In turn, this OS likely partially drives the greater rates of cardiometabolic complications seen in OSA patients. Although prospective studies need to be done using robust clinical outcomes to verify this, isoprostane might represent a useful biomarker of cardiovascular risk in patients with OSA and could be used to help stratify risk at the time of diagnosis. This is important as currently there is a paucity of studies published using prospective biomarkers in the context of OSA[42]. Furthermore, this biomarker might represent a reasonable intermediate target for reduction by therapies for OSA (e.g. CPAP) or antioxidants.

There are differences in the oxidative stress response in men and women to stimulation by intermittent hypoxia, which is believed to be related to estrogen variations governing ROS production[146]. Our study indicates that sex was independently associated with SOD levels. More specifically, more than half of the subjects in the highest quartile of SOD levels were females (data not shown), suggesting that women in our study cohort had increased antioxidant capacity. Our adjusted (table 3.3) and unadjusted analysis (table 3.2) indicate the significance between female sex and SOD levels. A similar sex difference was also reported by Wang et al[147] in their multivariate regression analysis of SOD levels and documented coronary artery disease in 590 patients. In contrast to our findings, they found that age and smoking status were also associated with SOD levels, while our results indicated that the relationship between age and SOD was not statistically significant \( p=0.079 \), as was the relationship between smoking status and SOD levels
It is essential to highlight that in contrast to our study, the study by Wang et al did not adjust for sleep parameters.

There were many strengths to our study. First, we measured a broad range of OS stress markers in a large cohort. Second, sleep parameters were obtained from inpatient PSG as opposed to questionnaires or home-sleep studies. Third, we were able to control for a number of important confounders including BMI. Fourth, we used circulating levels of markers drawn at the same time of day (morning fasting). However, we also acknowledge a number of limitations to our study. First, the history of cardiovascular disease and smoking was based on self-reported data as opposed to medical chart review. Second, the study was done in one centre in Canada. These results may not be generalizable outside of this population. Third, the degree of desaturation was fairly modest in our study (i.e. on average 4.62% of the study below 90%). It is possible that there may have been a greater impact on biomarkers such as SOD and 8-OHdG if patients with more substantial desaturation were studied.

3.6 Conclusions

OSA severity was an independent predictor of circulating 8-isoprostane levels, a marker of lipid oxidation, in a clinic-based cohort. However, OSA severity was not associated with antioxidant and DNA oxidation markers.

This study supports the findings from our systematic review from the previous chapter, where oxidative stress can be found in OSA patients. Also, we determined that OSA was independently associated with one of the markers of oxidative stress, even after adjustment for confounding factors. One might hypothesize that this marker might predict future CVD in the context of OSA. This hypothesis will be tested in future chapters of this thesis.
Chapter 4: Obstructive Sleep Apnea Severity, Body Mass Index, and Circulating Levels of Cellular Adhesion Molecules.


4.1 Summary

The purpose of this chapter was to investigate the relationship between obstructive sleep apnea (OSA) severity, body mass index (BMI) and circulating levels of inflammatory adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin). A cross-sectional clinical cohort study, on all consecutive adults referred to the University of British Columbia (UBC) Sleep Laboratory for a polysomnogram (PSG) for suspected OSA provided a morning blood sample. Samples were analyzed with multiplex immune-assay (MilliporeSigma, CA) to assess levels of adhesion molecules. 488 patients were studied; the majority were male (68%) with a mean age of 50 yrs, mean AHI of 23 events/hr, and mean BMI of 32 kg/m². In multivariable linear regression models, all three adhesion molecules were significantly associated with BMI (E-selectin p<.0001; ICAM-1 p=0.0007; VCAM-1 p=0.0003). However, only E-selectin was independently associated with AHI (p=0.02); there was no significant interaction between AHI and BMI for E-selectin (p=.33). Although all three adhesion molecules were associated with BMI, only E-selectin was independently associated with OSA severity. Futures studies are needed to determine the clinical significance of the relationship between E-selectin and OSA.
4.2 Introduction

On the previous study we identified that 8-isoprostane was associated with OSA after adjustment for potential confounding factors. Based on the findings of chapters 2 and 3, it remained to be determined if the same would hold true (i.e. similar associations) in regard to cellular adhesion molecules and OSA. In this chapter, we investigated the relationship between OSA and cellular adhesion molecules.

Obstructive sleep apnea (OSA) is the most common respiratory disorder during sleep with an estimated 420 million adults globally with moderate to severe OSA[37]. OSA is characterized by recurrent closing or narrowing of the upper airway, leading to intermittent hypoxia (IH), sleep fragmentation, and arousals. Obesity is strongly associated with the presence of OSA[148]. Furthermore, patients with OSA are at high risk of incident cardiovascular disease (CVD)[149].

Inflammation is being increasingly recognized as a risk factor for CVD[150]. Both OSA and obesity are potential pro-inflammatory states [151]. Animals exposed to intermittent hypoxia, a model of human OSA, experience increased oxidative stress and systemic inflammation[133]. Some studies have also shown increased levels of inflammatory markers in OSA, though separating the effect of obesity from OSA is sometimes challenging[152]. Adipose tissues are metabolically active, producing inflammatory cytokines[153]. Obesity and OSA may also synergistically affect inflammatory markers, at least in terms of levels of interleukin -6 and C-reactive protein[154].

Cellular adhesion molecules (CAMs), such as E-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are a group of molecules involved
in mediating adhesion and migration of leukocytes to the vascular endothelium[155]. CAMs are significantly increased in the presence of endothelial dysfunction (a precursor of CVD)[156]. Obesity induces endothelial dysfunction by directly releasing inflammatory mediators into the circulation, which causes increased production of CAMs[157]. In non-OSA cohorts, increased levels of ICAM-1 and E-selectin increased the odds of incident coronary heart disease by 5.53 (95% CI, 2.51-12.21) and 2.3 (95% CI, 1.14-3.6) respectively[71]. Animal studies consistently show that intermittent hypoxia also causes endothelial dysfunction, upregulating CAM pathways[133]. Studies of CAM in patients with OSA are limited by a small size, [158] and the independent contribution of OSA and obesity in affecting CAM levels is unclear[159–161].

The goal of this study was to assess circulating levels of adhesion molecules in a cohort of patients with suspected OSA studied with polysomnography. More specifically, we investigated the cross-sectional association between circulating levels of CAM (I-CAM-1, V-CAM-1, E-selectin levels) with BMI, and sleep apnea severity as measured by apnea-hypopnea index.

### 4.3 Materials and methods

#### 4.3.1 Study Population

For a description of the population studied in this chapter, please refer to section 3.3 on chapter 3.

#### 4.3.2 Polysomnography

For a description of the population studied in this chapter, please refer to section 3.3 on chapter 3.

#### 4.3.3 Assessment of Adhesion Molecules

Fasting blood (15 ml) was collected in the morning after PSG, and serum/plasma was stored in a -80°C freezer. Milliplex MAP Human Cardiovascular Disease Panel 1 (HCVD1-67
AK) multiplex Luminex (EMD Millipore, Etobicoke, ON, Canada) was used to determine the levels of ICAM-1, VCAM-1 and E-selectin. All assays followed the manufacturer’s protocol and were tested in duplicates. The samples were processed in batches.

4.3.4 Statistical Analysis

Descriptive statistics were used to characterize the sample of interest and levels of adhesion molecules. Univariate linear regression was used to assess the unadjusted relationship between the variables of interest and levels of E-selectin, ICAM-1 and VCAM-1. Multivariable linear regression modelling was used to control for potential confounders (age, sex, smoking status and previous heart disease) to assess the independent effect of OSA and BMI on adhesion molecules levels. We chose to control for all variables reported in final models because they were previously associated with adhesion molecules. The variables included in the final multivariable model were age, sex, BMI (as a continuous variable), AHI (as a continuous variable), statin usage, diabetes, ethnicity, previous heart disease and smoking status. A multiplicative interaction was tested on the regression models to assess potential statistical interaction term between BMI and OSA.

A sample size of 197 patients would be adequate to detect a modest (r=0.2) correlation between two variables (e.g. AHI and CAM), and 350 patients would be adequate to detect a very small relationship (r=0.15). As such, our sample size should be sufficient. Analysis was performed using Statistical Analysis Software (SAS, SAS Institute Inc, USA).

4.4 Results

A total of 488 patients were included in the study (Table 4.1). Most of the patients were white (77%) and the majority were male (68%), with a mean age of 50 yrs. Mean AHI was 22.7/hr and mean BMI was 32.0 kg/m². The descriptive statistics suggests a relationship between levels of
obesity (overweight to obesity) and OSA severity, as well as a gradual increase in adhesion molecules levels (Table 4.1). For instance, ICAM-1 levels increased ~6% from normal weight to overweight, and 15% from normal to obese patients. VCAM-1 levels were similar between all classes but were higher in severely obese patients. Finally, E-selectin levels shows the largest increases of 16% from normal weight to overweight, and 19% from normal to obese patients.

Results of the univariate linear regression analyses are shown in Table 4.2. Male sex (p=0.001), AHI (p<.0001), BMI (p<.0001), and smoking (p=0.02) were significantly associated with elevated E-selectin levels. BMI (p=0.0002), diabetes (p=0.02), and smoking (p<.0001) were significantly associated with increased levels of ICAM-1. VCAM-1 was associated with increased age (p=0.0005), BMI (p=0.0004), diabetes (p=0.0006) and previous history of cardiovascular disease (p=0.03). There were significant correlations between levels of adhesion molecules (see Appendix C).

In multivariable linear regression models, all three adhesion molecules were significantly associated with BMI (E-selectin p<.0001; ICAM-1 p=0.0007; VCAM-1 p=0.0003) (Table 4.3). However, only E-selectin was significantly associated with AHI (p=0.02). No significant interaction between AHI and BMI (p=.33) with E-selectin was found. Percentage of time spent below 90% of oxygen saturation during sleep was initially included in the models; however, due to the high correlation with AHI and no additional improvement in the model fit (results are not shown), we excluded this from the final model.
<table>
<thead>
<tr>
<th></th>
<th>Entire Cohort (n=488)</th>
<th>Normal Weight (n=59)</th>
<th>Overweight (n=157)</th>
<th>Obesity Class 1 (n=143)</th>
<th>Obesity Class 2 (n=68)</th>
<th>Obesity Class 3 (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>50 ± 12</td>
<td>48 ± 13</td>
<td>51 ± 12</td>
<td>52 ± 11</td>
<td>50 ± 12</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>331 (68%)</td>
<td>38 (67%)</td>
<td>122 (78%)</td>
<td>100 (70%)</td>
<td>45 (66%)</td>
<td>26 (43%)</td>
</tr>
<tr>
<td>BMI (Kg/m²), mean ± SD</td>
<td>32 ± 7</td>
<td>23 ± 1</td>
<td>27 ± 1</td>
<td>32 ± 1</td>
<td>37 ± 1</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>AHI (events/hr), mean ± SD</td>
<td>22.7 ± 21.7</td>
<td>14.3 ± 13.9</td>
<td>18.9 ± 16.4</td>
<td>25.8 ± 22.2</td>
<td>27.5 ± 24.8</td>
<td>27.5 ± 30.2</td>
</tr>
<tr>
<td>% time below 90% SaO₂, mean ± SD</td>
<td>5 ± 14</td>
<td>1.8 ± 4</td>
<td>2 ± 8</td>
<td>4 ± 10</td>
<td>9 ± 18</td>
<td>15 ± 26</td>
</tr>
<tr>
<td>ESS, mean ± SD</td>
<td>13 ± 6</td>
<td>14 ± 6</td>
<td>12 ± 6</td>
<td>13 ± 6</td>
<td>15 ± 6</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>Heart Disease, n (%) *</td>
<td>98 (20%)</td>
<td>9 (15%)</td>
<td>28 (18%)</td>
<td>31 (22%)</td>
<td>17 (25%)</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Ethnicity (white) n (%)</td>
<td>377 (77%)</td>
<td>37 (63%)</td>
<td>124 (78%)</td>
<td>111 (77%)</td>
<td>55 (81%)</td>
<td>50 (82%)</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>38 (8%)</td>
<td>4 (7%)</td>
<td>7 (4%)</td>
<td>10 (7%)</td>
<td>9 (13%)</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>44 (9%)</td>
<td>2 (4%)</td>
<td>10 (6%)</td>
<td>15 (10%)</td>
<td>9 (13%)</td>
<td>8 (13%)</td>
</tr>
<tr>
<td>Statins users, n (%)</td>
<td>96 (20%)</td>
<td>11 (19%)</td>
<td>19 (12%)</td>
<td>33 (23%)</td>
<td>15 (22%)</td>
<td>18 (30%)</td>
</tr>
</tbody>
</table>

**Adhesion Molecules**

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1 (ng/ml), mean ± SD</th>
<th>VCAM-1 (ng/ml), mean ± SD</th>
<th>E-Selectin (ng/ml), mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73 ± 35</td>
<td>925 ± 219</td>
<td>47 ± 20</td>
</tr>
<tr>
<td></td>
<td>65 ± 31</td>
<td>912 ± 237</td>
<td>36 ± 18</td>
</tr>
<tr>
<td></td>
<td>69 ± 41</td>
<td>909 ± 214</td>
<td>43 ± 19</td>
</tr>
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<td></td>
<td>73 ± 27</td>
<td>895 ± 190</td>
<td>51 ± 18</td>
</tr>
<tr>
<td></td>
<td>84 ± 34</td>
<td>936 ± 219</td>
<td>50 ± 18</td>
</tr>
<tr>
<td></td>
<td>81 ± 37</td>
<td>1038 ± 246</td>
<td>58 ± 26</td>
</tr>
</tbody>
</table>

**Abbreviations**: AHI: Apnea-Hypopnea Index; BMI: Body mass index; ESS: Epworth Sleep Scale; ICAM-1: Intercellular adhesion molecule-1; n: number of subjects; SaO₂: Oxygen Saturation; SD: Standard deviation; VCAM-1: Vascular Cell Adhesion Molecule-1; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure. **Definitions**: Normal weight: BMI 18.5-24.9; Overweight: BMI 25-29.9; Obesity Class 1: BMI 30-34.9; Obesity Class II: BMI 35-39.9; Obesity Class III: BMI ≥ 40;

**Table 4.1 – Patient Characteristics**
### Table 4.2 - Effect of Age, Sex, BMI, AHI, Statin usage, Diabetes, Ethnicity, Previous Heart Disease and Smoking status on E-selectin, ICAM-1 and VCAM-1 Estimated by using Univariate Linear Regression Models.

<table>
<thead>
<tr>
<th></th>
<th>E-selectin</th>
<th></th>
<th>ICAM-1</th>
<th></th>
<th>VCAM-1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated Difference in E-selectin (ng/ml), 95% CI, p-value, Adjusted R-squared</td>
<td>Estimated Difference in ICAM-1 (ng/ml), 95% CI, p-value, Adjusted R-squared</td>
<td>Estimated Difference in VCAM-1 (ng/ml), 95% CI, p-value, Adjusted R-squared</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Univariate Linear Regression</strong></td>
<td>Estimate</td>
<td>95% CI</td>
<td>p-value</td>
<td>Adj. R-squared</td>
<td>Estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age (per 1-year increase)</td>
<td>-0.11</td>
<td>-0.26</td>
<td>0.04</td>
<td>0.15</td>
<td>0.002</td>
<td>-0.06</td>
</tr>
<tr>
<td>Sex (males vs females)</td>
<td>6.2</td>
<td>2.37</td>
<td>10.04</td>
<td><strong>0.001</strong></td>
<td>0.01</td>
<td>6.35</td>
</tr>
<tr>
<td>BMI (per one-unit increase)</td>
<td>0.83</td>
<td>0.58</td>
<td>1.08</td>
<td><strong>&lt;0.001</strong></td>
<td>0.07</td>
<td>0.83</td>
</tr>
<tr>
<td>AHI (per one-unit increase)</td>
<td>0.17</td>
<td>0.09</td>
<td>0.25</td>
<td><strong>&lt;0.001</strong></td>
<td>0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>Statin Usage (users vs. not user)</td>
<td>1.85</td>
<td>-2.70</td>
<td>6.41</td>
<td>0.42</td>
<td>-0.0007</td>
<td>2.14</td>
</tr>
<tr>
<td>Diabetes (with vs. without)</td>
<td>6.57</td>
<td>-0.11</td>
<td>13.26</td>
<td>0.06</td>
<td>0.005</td>
<td>13.22</td>
</tr>
<tr>
<td>Ethnicity (White vs. not white)</td>
<td>-2.88</td>
<td>-7.17</td>
<td>1.41</td>
<td>0.18</td>
<td>0.001</td>
<td>-3.82</td>
</tr>
<tr>
<td>Previous Heart Disease* (with vs. without)</td>
<td>-0.41</td>
<td>-4.88</td>
<td>4.05</td>
<td>0.85</td>
<td>-0.002</td>
<td>2.96</td>
</tr>
<tr>
<td>Smoking Status** (current vs not current)</td>
<td>7.30</td>
<td>0.99</td>
<td>13.61</td>
<td><strong>0.02</strong></td>
<td>0.008</td>
<td>22.72</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; BMI: Body mass index; CI: Confidence Interval; ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular Cell Adhesion Molecule-1; ; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure; **Smoking status compared currently smokers versus not currently smokers; ex-smokers and never smokers are combined. *Each exposure was considered separately in the statistical model.
<table>
<thead>
<tr>
<th></th>
<th>E-selectin</th>
<th>ICAM-1</th>
<th>VCAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted Estimated Difference in E-selectin (ng/ml), 95% CI, p-value</td>
<td>Adjusted Estimated Difference in ICAM-1 (ng/ml), 95% CI, p-value</td>
<td>Adjusted Estimated Difference in VCAM-1 (ng/ml), 95% CI, p-value</td>
</tr>
<tr>
<td><strong>Multivariable Linear Regression</strong> (model included all variables listed on the table*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (per 1-year increase)</td>
<td>-0.11</td>
<td>-0.27</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex (males vs females)</td>
<td>7.82</td>
<td>4.0</td>
<td>11.65</td>
</tr>
<tr>
<td>BMI (per one-unit increase)</td>
<td>0.85</td>
<td>0.59</td>
<td>1.11</td>
</tr>
<tr>
<td>AHI (per one-unit increase)</td>
<td>0.09</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Statin Usage (users vs. not user)</td>
<td>-0.16</td>
<td>-4.89</td>
<td>4.56</td>
</tr>
<tr>
<td>Diabetes (with vs. without)</td>
<td>4.27</td>
<td>-2.26</td>
<td>10.81</td>
</tr>
<tr>
<td>Ethnicity (White vs. not white)</td>
<td>-3.6</td>
<td>-7.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Previous Heart Disease* (with vs. without)</td>
<td>-0.08</td>
<td>-4.53</td>
<td>4.35</td>
</tr>
<tr>
<td>Smoking Status** (current vs not current)</td>
<td>4.76</td>
<td>-1.26</td>
<td>10.78</td>
</tr>
<tr>
<td><strong>Adjusted R-squared</strong></td>
<td>0.13</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Abbreviations: AHI: Apnea-Hypopnea Index; BMI: Body mass index; CI: Wald Confidence Interval; ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular Cell Adhesion Molecule-1; Heart disease included: Hypertension, Myocardial Infarction, Cardiac Arrhythmias, Angina, and Congestive Heart Failure; **Smoking status compared currently smokers versus not currently smokers; ex-smokers and never smokers are combined. *Model: confounder evaluated were age, sex, BMI, AHI, Statin Usage, Diabetes, Ethnicity, Previous Heart Disease and Smoking status.

Table 4.3 - Effect of Age, Sex, BMI, AHI, Statin usage, Diabetes, Ethnicity, Previous Heart Disease and Smoking status on E-selectin, ICAM-1 and VCAM-1 Estimated by using Multivariable Linear Regression Models.
4.5 Discussion

We investigated whether circulating levels of cellular adhesion molecules (ICAM-1, VCAM-1 and E-selectin) were associated with BMI or OSA severity in a large clinical cohort of patients with suspected OSA. BMI was independently associated with all three adhesion molecules. However, only E-selectin was associated with OSA severity. This is the largest study to date to exploring the association between circulating adhesion molecules and OSA. The degree whether the relationship between E-selectin and OSA reaches clinical significance remains to be determined in future studies.

Our study is consistent with other reports that have shown a relationship between adhesion molecules and obesity. Adipose tissue releases inflammatory adipokines, causing oxidative stress and endothelial dysfunction. Upregulation of endothelial tissues (i.e. activation when responding to a stimulus, such as increased shear stress in arterial walls or presence of inflammatory cytokines) causes an increase release of adhesion molecules (E-selectin, ICAM-1, VCAM-1)[153,157]. In a large non-OSA cohort (n=664), E-selectin, VCAM-1 and ICAM-1 were independently associated with measures of obesity (body mass index and waist-hip ratio), even after controlling for age, sex, ethnicity, smoking, blood pressure, statins and insulin[155]. Ferri et al also showed a close relationship between obesity and adhesion molecules. ICAM-1, VCAM-1 and E-selectin were significantly higher in obese patients in comparison to non-obese, and significantly reduced after a 12 week weight loss program[153]. A meta-analysis of OSA cohorts showed similar findings to our studies, where in the presence of obesity and sleep apnea, obesity was a stronger predictor for increased circulating adhesion molecules[161]. For instance, in multivariable adjusted models, AHI was independently associated with VCAM-1, but BMI was independently associated ICAM-1 and VCAM-1.
Our study extends the results of other investigators with respect to adhesion molecules and OSA. We previously reported that VCAM-1 levels were independently associated with time spent below an oxyhemoglobin saturation of 90%[162]. This study is an enlargement of that previous cohort, including three hundred and twelve more patients. El-Solh and colleagues reported that levels of ICAM-1, VCAM-1 and E-selectin were all increased in 15 subjects with OSA compared to matched controls without OSA[160]. Another study of 68 patients showed a significant difference in adhesion molecules and selectins when comparing severe desaturation patients vs mild to moderated[159]. The data concerning adhesion molecule levels and OSA have been summarized by a meta-analysis by Nadeem et al[161]. They showed that, ICAM-1, VCAM-1 and selectin levels were significantly higher in OSA patients in comparison to controls. The pooled mean difference was 2.93, 2.08 and 1.45 (p<0.0001) respectively. Similarly, our study also shows a significant independent association between E-selectin and AHI. Our results with respect to E-selectin are consistent with previous studies that have shown levels of other adhesion molecules (i.e. P-selectin, L-selectin) are also elevated in patients with OSA[163,164]. We used AHI as a measure of the burden of OSA. AHI is highly correlated with levels of oxygen desaturation, therefore previous reports that used desaturation in their results could be compared to our findings using AHI.

In contrast to these previous reports, in our study ICAM-1 and VCAM-1 were not significantly associated with OSA severity after controlling for confounders. Of note, our study far exceeds the sample size of all previously cited papers, which might account for the difference in results.

Given the links between OSA, obesity, CVD, and adhesion molecules, we could speculate that these molecules may be useful in helping to risk stratify patients with OSA. That
is, elevated levels of these markers (particularly E-selectin) may help predict which patients are at particularly high risk of future CVD. If future studies are able to demonstrate the utility of these markers in this regard, they may be used to help personalize OSA management by enabling a group to be targeted for more aggressive management of CV risk factors and OSA[42]. Of note, previous research from our group showed that C-reactive protein in OSA patients may also help predict cardiovascular outcomes[165], suggesting that the concept of using molecular inflammatory markers to risk stratify OSA for CVD may have potential. That is, inflammatory markers might be useful in advancing the concept of “biochemical phenotypes” in OSA, which may complement symptom (e.g., excessive sleepiness) and physiologic phenotypes (e.g. severe OSA by polysomnography)[41].

Our study has many strengths. First, sleep variables were obtained from inpatient polysomnography as opposed to many other studies that utilize home-sleep studies or questionnaires. Second, levels of adhesion molecules were obtained from the same time of the day for all patients (morning fasting). Third, we collected data on important clinical confounders that could be significantly associated with levels of adhesion molecules.

However, we acknowledge a number of limitations. First, many of the confounding factors were collected based on self-reported data as opposed to medical-chart reviews. Second, this represents a clinic-based rather than community-based cohort. As such, there may be selection bias in that individuals who are more symptomatic and/or with more severe OSA are represented compared to community-based cohorts. However, these are the patients that clinicians assess and require management decisions. We also do not have follow up data to determine if patients with OSA and high levels of E-selectin developed CVD at a later time. Additionally, we didn’t explore the potential effects that specific variables of sleep fragmentation
could have in the levels of adhesion molecules. We used the AHI to assess the burden of the sleep disorder. Future studies should explore if variables such as total sleep time, sleep efficiency or arousal index are also associated with levels of E-selectin in suspected OSA patients. Finally, we acknowledge that AHI is usually correlated with BMI and this could influence the effect estimates in the multivariable regression models. However, this is a common problem inherent to the sleep medicine literature.

4.6 Conclusions

Body mass index is associated with levels of E-selectin, ICAM-1 and VCAM-1. E-selectin was independently associated with OSA severity.

In this chapter, we found similar results to chapter 3, where one of the markers was associated with OSA after controlling for confounding. This also supports the findings from chapter 2, where a substantial gap in the literature is present by lack of properly adjusted analysis in large samples. Overall, out of the six markers studied (8-isoprostane, 8-OHdG, SOD, ICAM-1, VCAM-1, and E-selectin); one oxidative stress marker (8-isoprostane) and one cellular adhesion molecule (E-selectin) were independently associated with OSA. This provided us with enough evidence to go ahead and hypothesize if these molecules could indeed predict risk of incident CVD in suspected OSA patients (chapters 5 and 6).
Chapter 5: Circulating Levels of Cell Adhesion Molecules and Future Risk of Cardiovascular Events in Obstructive Sleep Apnea

5.1 Summary

Obstructive sleep apnea (OSA) patients are at increased risk of cardiovascular disease (CVD). Cell adhesion molecules (CAM) are increased in OSA and CAM are also implicated in the development of CVD. Do CAM (ICAM-1, VCAM-1 and E-selectin) have prognostic value in identifying risk of adverse cardiovascular events in OSA? Patients with suspected OSA referred for a polysomnogram (PSG) provided a fasting blood sample. Plasma levels of ICAM-1, VCAM-1 and E-selectin were determined by multiplex Luminex Assay (Milliporesigma ON, Canada). Cardiovascular events were determined by deterministic linkage to provincial health databases. 418 patients were included in the analysis. They were mostly male (68.2%) with a mean age of 50.7 yrs, median apnea-hypopnea index of 16.5 events/hour, and mean BMI of 31.7 kg/m2. A total of 55 cardiovascular events occurred in 8-yrs of follow up, where 36 were first events. Higher levels of ICAM-1 were independently associated with developing a first event (OR=4.12 95% CI 1.47-11.55, 2nd and 3rd tertiles vs. 1st tertile), including in patients with OSA (OR=3.7 95% CI 1.29-10.54). E-selectin was significantly associated with cardiovascular events in patients with moderate to severe OSA (OR = 4.07 95% CI 1.06 – 15.61, 2nd and 3rd tertiles vs. 1st tertile) but not in patients without moderate to severe OSA (OR=0.60 95% CI 0.15-2.31), p-value for interaction = 0.04. In a suspected OSA cohort, patients with higher levels of ICAM-1 (>816 ng/ml) were significantly more likely to experience a cardiovascular event within 8 years after PSG. In moderate to severe OSA patients, a higher E-selectin (>36.4 ng/ml) was significantly associated with cardiovascular events. ICAM-1 and E-selectin could be useful
biomarkers to identify OSA patients at increased risk of future CV complications and help direct personalized care.

5.2 Introduction

In the previous chapter we saw that E-selectin was associated with OSA in a multivariable analysis. This showed that cellular adhesion molecules are promising markers in OSA populations. In this chapter, we tested to see if cellular adhesion molecules are useful to predict CVD risk in suspected OSA patients.

Obstructive Sleep Apnea (OSA) is the most common respiratory sleep disorder with close to half a billion people having moderate to severe disease globally[37]. Untreated OSA is associated with a significantly increased risk of cardiovascular disease (CVD) including stroke and myocardial infarction[73]; however, identifying patients with a particularly high risk has been challenging as standard metrics of OSA severity (such as the apnea hypopnea index) are not particularly discriminative. The ability to identify such a high-risk group could help direct more aggressive treatment of OSA and other CV risk factors (personalized or precision care) or facilitate patient selection for recruitment into clinical trials of CV prevention. Recent efforts have been made to utilize novel parameters to help identify a group at high risk of CVD[166]; these have included using symptom clusters [41], PSG clusters[167], comorbidities,[168] and desaturation parameters[169] to help identify such patients.

Circulating levels of inflammatory markers may be a potentially useful method to risk stratify OSA patients[165]. Inflammation plays a pivotal role in the initiation and progression of CVD[170]. In addition, OSA is characterized by intermittent hypoxia and oxidative stress, which in turn activates systemic inflammation [20]; both animals exposed to intermittent hypoxia and patients with OSA have increased levels of inflammatory markers including C-reactive protein
(CRP), interleukins, and cellular adhesion molecules[171–173]. This inflammation is likely a contributing factor to premature CVD in OSA patients.

Cell adhesion molecules (CAMs) are biomarkers of inflammation that might be particularly helpful in this regard. CAMs, such as endothelial selectin (E-selectin), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) increase binding and recruitment of white blood cells to the endothelium [158], contribute to atherosclerotic plaque development, and are linked to the occurrence of CVD[71,174,175]. Given that OSA can also increase CAM, we hypothesized that circulating CAM levels would be associated with future risk of CV events in OSA. The hope is that these molecules could be used to establish a ‘biochemical phenotype’, and eventually help to risk stratify OSA patients in terms of CVD risk.

5.3 Study Design and Methods

5.3.1 Study Design, Setting and Participants

This cohort has been used and previously described[176]. Briefly, consenting adults (≥19 years old) referred for suspected OSA to the University of British Columbia Hospital Sleep Disorder Laboratory for inpatient polysomnography (PSG) were recruited from 2003 to 2008. Patients that were unable to speak English and being treated for OSA were excluded. On the night of their PSG, patients completed a detailed questionnaire about their medical history, sleep symptoms and habits. The patients included in this study are a subset of the same cohort of patients from chapters 3 and 4.

Apneas and hypopneas were scored according to the recommendations of the American Academy of Sleep Medicine (AASM), where hypopneas were defined according to a reduction in airflow with either a 3% desaturation or an arousal from sleep[140]. Patients were diagnosed as
having OSA based on an AHI of ≥5 events/hour. An AHI between 5 and 15 events/hour was considered mild, ≥15-30 events/hour was considered moderate and ≥30 events/hour was considered severe OSA[140].

This study was approved by the University of British Columbia Research Ethics Board (H13-00346) and Vancouver Coastal Health Research Institutes (V11-80199).

5.3.2 Laboratory Analysis

Fasting blood (15 ml) was collected on the morning after PSG, centrifuged, and stored in a -80°C freezer. Endothelial selectin (E-selectin), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) were measured using Milliplex Map Human Cardiovascular Disease Panel 1 multiplex Luminex assay (Milliporesigma ON, Canada) from frozen plasma samples. Manufacturer’s protocol was followed, and all measurements done in duplicates.

5.3.3 Ascertainment of Cardiovascular Events

The major outcome of interest was a composite of incident cardiovascular and cerebrovascular events which included cardiovascular death, hospitalization for cardiovascular conditions, stroke and cardiac procedures (percutaneous coronary intervention-PCI, coronary artery bypass graft- CABG); follow-up time was 8 years from PSG date for all patients. The outcomes were obtained by linking our cohort to provincial health databases as has been done in previous studies[165]. The codes indicating deaths from cardiovascular-related causes are summarized in supplemental table 7.2 (appendix D); hospitalizations, procedures and events codes and definitions are in supplemental table 7.3 (appendix D). These events were identified by deterministic linkage of consenting patients to different provincial health databases through Population Data BC (PopdataBC). For more details on the provincial databases used and
Comorbidities and potential confounding were determined based on self-reports from
health questionnaires. History of previous cardiovascular disease (CVD) was determined based on
previous doctors’ diagnoses of hypertension, myocardial infarction, cardiac arrhythmias, angina,
and congestive heart failure. Smoking status (currently smoking versus not currently smoking),
usage of statins, and presence of diabetes were also self-reported.

Only BC residents were included in the cohort. To be considered a BC resident, we required
continuous provincial health registration with no larger than a 93-day (~ 3 month) gap in
registration following PSG date.

5.3.4 Continuous Positive Airway Pressure Adherence

CPAP adherence was determined by chart review. Two independent trained researchers
(BP and AHA) reviewed all charts for objective and subjective data on CPAP adherence. CPAP
providers’ reports and patient reports dictated in the physicians’ notes were used to determine
adherence. Adherence was defined as minimum of 4 hours per night for at least 70% of the
nights[178]. In the absence of objective measures, physician notes indicating a clear positive
response to CPAP prescription and usage were considered as adherent to treatment. Non-
adherence was defined as reported use below 4 hours per night for at least 70% of the nights,
clear intolerance to CPAP, and failed to return for a follow-up consultation after being prescribed
CPAP. The data was plotted and scored independently. Interrater reliability was excellent (kappa
value of 0.99).
5.3.5 Statistical Analysis

Continuous variables that were normally distributed were summarized with mean and standard deviations then tested with t-tests. Continuous variables that were skewed were summarized with medians and IQRs then tested with Mann-Whitney-U tests. Categorical variables were summarized with counts and proportions and compared with Chi-square tests when all expected cell counts were >5 or Fisher’s exact tests otherwise.

Associations of baseline variables and occurrence of cardiovascular events were modelled using logistic regression to estimate odds ratios with 95% confidence intervals; only first events were used in the analysis. We modelled the incidence of events using logistic regression because 89% of the patients had full follow-up time (8 years) and the results were not substantially different when using Cox proportional hazards models. We assessed univariate associations of adhesion molecules and comorbidities with cardiovascular events then constructed final adjusted models. Levels of adhesion molecules were divided in tertiles (as opposed to quartiles) to minimize excessive data segmentation. Also, preliminary analysis dividing the data in quartiles yielded similar results.

Adjusted models included age, sex, AHI, body mass index (BMI), previous heart disease, diabetes, Epworth Sleepiness Scale (ESS), statin usage, CPAP adherence and smoking status (current smokers vs. non-current smokers) as these variables were felt to be important confounders. Due to the high correlation between oxygen desaturation and AHI and the fact that replacement of AHI with desaturation did not change results appreciably, AHI was the variable used in the models as a measure of OSA severity. For CPAP usage adjustment, a three-level categorization was created; patients were classified as prescribed and adherent, prescribed and non-adherent, and not prescribed.
As an exploratory analysis, we determined whether the association between adhesion molecule levels and cardiovascular events differed according to sleep apnea severity. That is, we performed an interaction analysis, using fully adjusted models, based on AHI thresholds of 5 and 15 (i.e. any OSA, and moderate to severe OSA) to determine if odds ratios differed.

We used similar descriptive and inferential statistics to investigate the association between CPAP adherence and cardiovascular events in this cohort. For this analysis, only patients prescribed CPAP were included.

Cell sizes <6 were censored to protect patient privacy and comply with Population Data BC regulations regarding small sizes. Statistical Analysis Software (SAS version 9.4, SAS Institute Inc, USA) was used.

5.4 Results

A total of 1983 patients were recruited in the cohort, of those 488 patients had CAM levels measured. Of these, 70 did not have adequate follow up due to BC non residence or lack of consent for data linkage. The final number of patients recruited for this study was, thus, 418 (table 5.1). Most of the patients were Caucasian (80%) and the majority were male (68.7%), with a mean age of 50.7 years. Median AHI was 16.5/hr and mean BMI was 31.7 kg/m². There was a total of 36 first events included in the analysis (table 5.2). The majority of first events were CV death, myocardial infarction, unstable angina, or percutaneous coronary intervention. Age, male sex, AHI, statin use, and diabetes were associated with higher rates of cardiovascular events table 7.4 (appendix D).

5.4.1 Association of Cardiovascular Events with CAM Levels

The baseline characteristics according to CAM tertiles is shown in table 5.1. For ICAM-1 and E-selectin, BMI significantly increased across the tertiles. Greater AHI was associated with E-
selectin tertiles (p<0.001). Increased subjective sleepiness was associated with higher ICAM-1 tertiles.

For ICAM-1 and E-selectin, rates of first cardiovascular events were similar in the 2nd and 3rd tertiles and greater than in the first. That is, the top two tertiles (2nd and 3rd) of ICAM-1 had event rates of 11.4% and 10.7%, while the first tertile had an event rate of 3.6%. E-selectin 2nd and 3rd tertiles had event rates of 11.4% and 8.6%, respectively, while the 1st tertile of E-selectin had an event rate of 5.7%. For VCAM-1, the rate in the 3rd tertile (12.3%) was greater than the 1st (7.2%) and 2nd tertiles (6.3%).

The associations between CAM levels and cardiovascular events are shown in table 5.3. For ICAM-1, we compared the highest two tertiles with the 1st for the multivariate models. In fully adjusted models, a higher ICAM-1 level was independently associated with cardiovascular events (OR 4.12, 95% CI 1.47 – 11.55, p= 0.007). Although odds ratios were above one, VCAM-1 and E-selectin levels were not significantly associated with the incidence of cardiovascular events.

5.4.2 Interaction with OSA Severity

We compared odds ratios of events according to CAM tertiles by varying degrees of OSA severity (table 5.4). We found that in OSA patients, first cardiovascular event rates were greater in the two higher tertiles of ICAM vs. the 1st tertile (11.8% vs. 3.3%) with an adjusted OR similar to the entire cohort of suspected OSA (adjusted OR=3.7, CI: 1.29-10.54). In patients without OSA and in the lowest tertile of ICAM-1, there were no cardiovascular events (0/17 patients) suggesting that the absence of OSA together with low ICAM levels is indicative of a very low risk group; non-OSA patients in the second and third tertiles of ICAM-1 experienced an event rate of 7.8%. When patients were stratified by the presence or absence of moderate to severe OSA, ICAM-1 was similarly predictive in both groups with no significant interaction (p=0.60).
In contrast, E-selectin appeared to be more predictive in patients with OSA than patients without OSA. Specifically, when only OSA patients were considered, patients in the two higher tertiles had a rate of 1st cardiovascular event of 10.9% compared to 5.4% in the 1st tertile (adjusted OR=2.52, CI: 0.93–7.63). Rates of events in patients without OSA was similar (or less) in the top two vs. lowest tertile (4.8 vs. 7.7%; OR=0.34, 95% CI 0.04–3.0; p-value for interaction = 0.1). In patients with moderate to severe OSA, E-selectin was highly predictive of cardiovascular events, with rates of 14.2% and 4.9% in higher vs. lowest tertiles respectively (OR=4.07, 95% CI: 1.06–15.61) with a significant interaction effect (p=0.04).

5.4.3 CPAP Adherence and Cardiovascular Events

We assessed rates of events in patients prescribed CPAP (N=134, table 7.5, appendix D). This number might be an underestimate of patients prescribed CPAP as patients may have been prescribed by their family doctor, or this data may not have been available in the record. The rate of events was 14.81% and 12.26% in the CPAP non-adherent and CPAP adherent groups, respectively. In fully adjusted models, there was a reduced odds ratio of cardiovascular events similar in magnitude to previous observational studies (OR 0.69, 95% CI 0.17 – 2.80, p=0.60) [73]; however, likely due to small numbers, results were not statistically significant (table 7.6, appendix D).
<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Overall Cohort (n=418)</th>
<th>Tertile 1 (n=139)</th>
<th>Tertile 2 (n=140)</th>
<th>Tertile 3 (n=139)</th>
<th>P-value</th>
<th>Tertile 1 (n=139)</th>
<th>Tertile 2 (n=141)</th>
<th>Tertile 3 (n=138)</th>
<th>P-value</th>
<th>Tertile 1 (n=139)</th>
<th>Tertile 2 (n=140)</th>
<th>Tertile 3 (n=139)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) – mean ± SD</td>
<td>50.7 ± 11.5</td>
<td>52.0 ± 11.3</td>
<td>49.3 ± 11.6</td>
<td>50.9 ± 11.5</td>
<td>0.16</td>
<td>50.0 ± 11.2</td>
<td>52.4 ± 11.7</td>
<td>51.7 ± 12.1</td>
<td>0.30</td>
<td>51.0 ± 11.7</td>
<td>50.9 ± 11.6</td>
<td>50.3 ± 11.3</td>
<td>0.85</td>
</tr>
<tr>
<td>Sex (Male) – n (%)</td>
<td>287 (68.7)</td>
<td>91 (65.9)</td>
<td>97 (68.8)</td>
<td>99 (71.2)</td>
<td>0.64</td>
<td>92 (78.6)</td>
<td>88 (75.2)</td>
<td>61 (52.1)</td>
<td>&lt;0.001</td>
<td>83 (60.1)</td>
<td>96 (68.6)</td>
<td>108 (77.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI (kg/m²) - mean ± SD</td>
<td>31.7 ± 6.5</td>
<td>30.3 ± 6.2</td>
<td>30.8 ± 5.5</td>
<td>33.9 ± 7.1</td>
<td>&lt;0.001</td>
<td>31.2 ± 5.5</td>
<td>31.1 ± 5.7</td>
<td>32.4 ± 7.6</td>
<td>0.21</td>
<td>29.3 ± 5.8</td>
<td>32.4 ± 6.3</td>
<td>33.3 ± 6.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AHI - events/hr – median (IQR)</td>
<td>16.5 (7,30.4)</td>
<td>16 (7,4,30.0)</td>
<td>16.6 (6,9,28.1)</td>
<td>17.8 (7,1,35.4)</td>
<td>0.49</td>
<td>16.6 (8,2,34.8)</td>
<td>18.4 (7,9,30.9)</td>
<td>13.8 (5,4,26.8)</td>
<td>0.08</td>
<td>12.8 (6,24.3)</td>
<td>16 (7,1,30.1)</td>
<td>20.4 (8,2,38.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AHI Category</td>
<td>Other</td>
<td>Asian</td>
<td>Caucasian</td>
<td>Moderate</td>
<td>Severe</td>
<td>0.42</td>
<td>0.0009</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>68 (16.3)</td>
<td>127 (30.4)</td>
<td>114 (27.3)</td>
<td>109 (26.1)</td>
<td></td>
<td>51 (12.8)</td>
<td>14 (11.9)</td>
<td>29 (24.8)</td>
<td></td>
<td>26 (18.8)</td>
<td>22 (15.7)</td>
<td>20 (14.3)</td>
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</tr>
<tr>
<td></td>
<td>17 (12.3)</td>
<td>49 (35.5)</td>
<td>36 (26.1)</td>
<td>36 (26.1)</td>
<td></td>
<td>40 (34.2)</td>
<td>39 (33.1)</td>
<td>51 (26.5)</td>
<td></td>
<td>51 (37)</td>
<td>41 (29.3)</td>
<td>35 (25.0)</td>
<td></td>
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<tr>
<td></td>
<td>27 (19.1)</td>
<td>39 (27.7)</td>
<td>43 (30.5)</td>
<td>32 (22.7)</td>
<td></td>
<td>24 (20.5)</td>
<td>34 (28.8)</td>
<td>37 (31.6)</td>
<td></td>
<td>37 (26.8)</td>
<td>41 (29.3)</td>
<td>36 (25.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 (17.3)</td>
<td>39 (28.1)</td>
<td>55 (25.2)</td>
<td>41 (29.5)</td>
<td></td>
<td>38 (32.5)</td>
<td>31 (26.3)</td>
<td>20 (17.1)</td>
<td></td>
<td>24 (17.4)</td>
<td>36 (25.7)</td>
<td>49 (35.0)</td>
<td></td>
</tr>
<tr>
<td>% Time below 90% SaO₂, median (IQR)</td>
<td>0.32(0.1,2.5)</td>
<td>0.31(0.1,1.7)</td>
<td>0.2 (0,2.3)</td>
<td>0.7 (0,1,3.6)</td>
<td>0.05</td>
<td>0.5 (0,1,2.8)</td>
<td>0.3 (0,1,2.2)</td>
<td>0.3 (0,1,2.5)</td>
<td>0.49</td>
<td>0.2 (0,1,5)</td>
<td>0.4 (0,1,2.4)</td>
<td>0.6 (0,1,3.2)</td>
<td>0.30</td>
</tr>
<tr>
<td>ESS &gt; 11 – n (%)</td>
<td>136 (30.1)</td>
<td>31 (22.3)</td>
<td>44 (31.2)</td>
<td>51 (36.7)</td>
<td>0.03</td>
<td>30 (25.6)</td>
<td>45 (38.1)</td>
<td>28 (23.9)</td>
<td>0.03</td>
<td>32 (23)</td>
<td>44 (31.4)</td>
<td>50 (35.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Heart Disease* - n (%)</td>
<td>90 (21.5)</td>
<td>26 (18.7)</td>
<td>29 (20.6)</td>
<td>35 (25.2)</td>
<td>0.40</td>
<td>32 (27.4)</td>
<td>31 (26.3)</td>
<td>22 (18.8)</td>
<td>0.25</td>
<td>28 (20.1)</td>
<td>29 (20.7)</td>
<td>33 (26.6)</td>
<td>0.76</td>
</tr>
<tr>
<td>Smoking Status* - n (%)</td>
<td>37 (8.8)</td>
<td>9 (6.5)</td>
<td>8 (5.7)</td>
<td>20 (14.4)</td>
<td>0.02</td>
<td>9 (7.7)</td>
<td>10 (8.5)</td>
<td>6 (5.1)</td>
<td>0.58</td>
<td>9 (6.5)</td>
<td>11 (7.9)</td>
<td>17 (12.1)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diabetes - n (%)</td>
<td>32 (7.6)</td>
<td>9 (6.5)</td>
<td>9 (6.4)</td>
<td>14 (10.1)</td>
<td>0.42</td>
<td>13 (11.1)</td>
<td>8 (6.8)</td>
<td>10 (8.5)</td>
<td>0.50</td>
<td>12 (8.6)</td>
<td>&lt; 6</td>
<td>15 (10.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Ethnicity Group</td>
<td>Other</td>
<td>Caucasian</td>
<td>Asian</td>
<td>Other</td>
<td>Statin User</td>
<td>0.06</td>
<td>0.81</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>328 (80)</td>
<td>110 (82.1)</td>
<td>113 (80.7)</td>
<td>105 (77.2)</td>
<td></td>
<td>88 (77.9)</td>
<td>93 (81.6)</td>
<td>96 (84.2)</td>
<td></td>
<td>110 (80.9)</td>
<td>118 (86.8)</td>
<td>100 (72.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (11.9)</td>
<td>11 (7.9)</td>
<td>9 (6.6)</td>
<td>10 (6.5)</td>
<td></td>
<td>13 (11.5)</td>
<td>10 (8.8)</td>
<td>9 (7.9)</td>
<td></td>
<td>12 (8.8)</td>
<td>8 (5.9)</td>
<td>16 (11.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (11.4)</td>
<td>22 (16.2)</td>
<td>10 (6.6)</td>
<td>11 (9.6)</td>
<td></td>
<td>12 (10.6)</td>
<td>9 (7.9)</td>
<td>9 (7.9)</td>
<td></td>
<td>14 (10.3)</td>
<td>10 (7.4)</td>
<td>22 (15.9)</td>
<td></td>
</tr>
<tr>
<td>Adhesion Molecules (ng/ml)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>25 (21.7)</td>
<td>20 (17.5)</td>
<td>25 (22.1)</td>
<td>0.64</td>
<td>23 (16.9)</td>
<td>32 (23.4)</td>
<td>25 (18)</td>
<td>0.35</td>
</tr>
<tr>
<td>Rate of 1st Cardiovascular Event</td>
<td>36 (8)</td>
<td>16 (11.4)</td>
<td>15 (10.7)</td>
<td>10 (7.9)</td>
<td>0.75</td>
<td>8 (60.1)</td>
<td>60.1 (83.4)</td>
<td>83.4 - 352.7</td>
<td></td>
<td>9.7 - 36.4</td>
<td>36.4 - 52.1</td>
<td>52.11 - 220.8</td>
<td>.</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; BMI: Body mass index; ESS: Epworth Sleepiness Scale; ICAM-1: Intercellular adhesion molecule-1; IQR: Interquartile range; SD: Standard deviation; SaO₂: Oxygen Saturation; VCAM-1: Vascular Cell Adhesion Molecule-1; *Heart disease included: Hypertension, Myocardial Infarction, Cardiac Arrhythmias, Angina, and Congestive Heart Failure. *Current Smokers.

**Table 5.1 - Patient Characteristics by Adhesion Molecule Tertiles (n=418)**
<table>
<thead>
<tr>
<th>Outcome</th>
<th>First Event (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Death*</td>
<td>13.9 (CVD)</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>5.6</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>8.3</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>19.4</td>
</tr>
<tr>
<td>Stroke</td>
<td>5.6</td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>13.9</td>
</tr>
<tr>
<td>Coronary Artery Bypass Grafting</td>
<td>2.8</td>
</tr>
<tr>
<td>Percutaneous Coronary Intervention</td>
<td>19.4</td>
</tr>
<tr>
<td>Cardioversion</td>
<td>5.6</td>
</tr>
<tr>
<td>Pacemaker</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*Causes of Death: Hyperlipidemia, myocardial infarction, atherosclerotic heart disease, chronic ischemic heart diseases and myocarditis (appendix D)

Table 5.2 – Percentage of Cardiovascular Events (n=36)
<table>
<thead>
<tr>
<th>ICAM-1</th>
<th>Unadjusted Tertiles</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 vs 1</td>
<td>3.46 (1.23, 9.72)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>3 vs 1</td>
<td>3.24 (1.14, 9.18)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Tertile 2+3 vs 1</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>3.35 (1.27, 8.81)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Fully Adjusted***</td>
<td>4.12 (1.47, 11.55)</td>
<td>0.007</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Unadjusted Tertiles</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>2 vs 1</td>
<td>0.88 (0.34, 2.23)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>3 vs 1</td>
<td>1.81 (0.79, 4.11)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Tertile 3 vs 1+2</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>1.93 (0.96, 3.84)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Fully Adjusted***</td>
<td>1.44 (0.68, 3.04)</td>
<td>0.34</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Unadjusted Tertiles</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>2 vs 1</td>
<td>2.11 (0.87, 5.11)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>3 vs 1</td>
<td>1.54 (0.61, 3.91)</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Tertile 2+3 vs 1</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>1.82 (0.81, 4.12)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Fully Adjusted***</td>
<td>1.85 (0.76, 4.50)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

***Adjusted for Age, Sex, Smoking Status, AHl, BMI, Heart Disease, ESS > 11, Diabetes, CPAP usage, Statin Usage

Table 5.3 - Unadjusted and Adjusted Odds Ratios for Adhesion Molecule Tertiles and Cardiovascular Events
<table>
<thead>
<tr>
<th>With OSA versus Without OSA (AHI &lt;5 versus AHI ≥ 5)</th>
<th>OR (95% CI)</th>
<th>p-value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 (2+3 vs 1)</td>
<td>2+3 vs 1 at AHI &lt;5</td>
<td>Cannot be estimated</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI ≥5</td>
<td>3.7 (1.29, 10.54)</td>
</tr>
<tr>
<td>VCAM-1 (3 vs 1+2)</td>
<td>OR (95% CI)</td>
<td>p-value for interaction</td>
</tr>
<tr>
<td></td>
<td>3 vs 1+2 at AHI &lt;5</td>
<td>5.03 (0.43, 58.4)</td>
</tr>
<tr>
<td></td>
<td>3 vs 1+2 at AHI ≥5</td>
<td>1.23 (0.55, 2.73)</td>
</tr>
<tr>
<td>E-selectin (2+3 vs 1)</td>
<td>OR (95% CI)</td>
<td>p-value for interaction</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI &lt;5</td>
<td>0.34 (0.04, 3.0)</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI ≥5</td>
<td>2.52 (0.93, 6.84)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>With Moderate to Severe OSA versus Without Moderate to Severe OSA AHI &lt;15 versus AHI ≥ 15</th>
<th>OR (95% CI)</th>
<th>p-value for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 (2+3 vs 1)</td>
<td>2+3 vs 1 at AHI &lt;15</td>
<td>6.48 (0.77, 54.86)</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI ≥15</td>
<td>3.41 (1.03, 11.29)</td>
</tr>
<tr>
<td>VCAM-1 (3 vs 1+2)</td>
<td>OR (95% CI)</td>
<td>p-value for interaction</td>
</tr>
<tr>
<td></td>
<td>3 vs 1+2 at AHI &lt;15</td>
<td>2.26 (0.58, 8.82)</td>
</tr>
<tr>
<td></td>
<td>3 vs 1+2 at AHI ≥15</td>
<td>1.28 (0.50, 3.23)</td>
</tr>
<tr>
<td>E-selectin (2+3 vs 1)</td>
<td>OR (95% CI)</td>
<td>p-value for interaction</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI &lt;15</td>
<td>0.60 (0.15, 2.31)</td>
</tr>
<tr>
<td></td>
<td>2+3 vs 1 at AHI ≥15</td>
<td>4.07 (1.06, 15.61)</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; CI: Confidence Intervals; ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1 OR: Odds ratio; OSA: Obstructive Sleep Apnea; Adjusted for age, sex, smoking status, BMI, heart disease, ESS > 11, diabetes, CPAP adherence, and statins.

Table 5.4 - Stratified Odds Ratios for Cardiovascular Events by AHI Levels
5.5 Discussion

In our prospective study, we found that patients in the top two tertiles of ICAM-1 (i.e. above 816 ng/ml) were significantly more likely to suffer a CV event during eight years of follow (OR=4.12, 95% CI 1.47,11.55). This effect was similar in patients with OSA, and in moderate to severe OSA (OR=3.7 and 3.41, respectively). E-selectin (>36.4 ng/ml) was significantly associated with cardiovascular events in patients with moderate to severe OSA (OR= 4.07), but not in patients without OSA. VCAM-1 was not associated with events. This is the first study investigating the prognostic utility of adhesion molecules in a large suspected well-defined OSA population.

OSA and the consequent intermittent hypoxia lead to oxidative stress and activation of pro-inflammatory transcription factors, such as HIF-1α and NFκB, which are responsible for expression of ICAM-1, VCAM-1 and selectins[158]. This concept is supported by data from human and animal studies in OSA[133,164,179–181] including in intervention trials using CPAP[151] showing elevated levels of CAM in OSA patients and reductions with CPAP. Activation of CAM represents a potential pathway by which OSA leads to premature CVD. In addition, variability of CAM levels in OSA patients due to genetic and other factors might explain some of the variability in CV risk associated with OSA. For example, we have recently shown that E-selectin levels are affected not only by OSA severity, but also body mass index and particular genetic polymorphisms (e.g., single nucleotide polymorphism rs579459 of the ABO gene)[176].

Our results with respect to ICAM-1 are consistent with studies in other populations (i.e., that did not assess degree of OSA). In a case control study, Hwang et al compared 204 patients with incident CVD to 316 control subjects and found that patients in the highest quartile of ICAM-1 had a 5.53 higher odds of developing CVD (95% CI, 2.51 – 12.21)[71]. Similarly, Luc et al
compared 317 men to 613 matched controls; adjusted baseline levels of ICAM-1 were associated with an increased relative risk (RR) for myocardial infarction (RR 1.34; 95% CI 1.07-1.67) over 5 years[182]. Our study extends these results to specific OSA populations with detailed PSG information and an 8-year prospective follow-up. Our study shows that ICAM-1 levels in patients with suspected OSA sent to a sleep clinic are predictive of prospective CV events even after controlling for confounders such as OSA severity (AHI). In addition, in the group of patients with low ICAM and without OSA, no cardiovascular events were seen suggesting these are a group who would be considered very low risk.

Our findings with respect to E-selectin are particularly intriguing. In the case control study by Hwang et al (referenced above), high levels of E-selectin (fourth quartile) were not significantly associated with incident coronary heart disease (OR=1.6, 95% CI 0.78 – 3.3) [71]. Although it is likely that some of these patients in the study had OSA, in unselected populations E-selectin does not seem to be a robust marker of incident CV events. These results are consistent with our results in which we found that in the entire cohort, E-selectin was not a significant predictor of cardiovascular events. However, we found that E-selectin was predictive of events in patients with OSA and particularly predictive in patients with moderate to severe OSA, suggesting it is a biomarker particularly relevant and specific to these populations. This concept is consistent with our previous study in which E-selectin was more strongly associated with OSA severity than other adhesion molecules[173].

In contrast, VCAM-1 was not significantly associated with cardiovascular events in the whole cohort (OR=1.44, 95% CI 0.68-3.04, p=0.34) nor in patients with OSA (OR=1.23, 95% CI 0.55-2.73, p-value for interaction=0.28). This is in agreement with the study by Hwang et al, where VCAM-1 was not associated with fatal and non-fatal CVD events (OR=1.01, 95% CI 0.79-
1.30)[71] in an unselected cohort. It could be speculated that the different role of VCAM-1 in the establishment of cardiovascular lesions, differences in surface receptors[183] and the limited number of events seen in our study could explain our null findings.

The identification of ICAM-1 and E-selectin as potential markers for CVD in OSA raises the possibility of pharmacological treatments specifically directed towards these molecules. Recent trials in sickle cell anemia have successfully targeted selectins (using the drug GMI-1070r) to reduce the incidence of vaso-occlusive events[184]. Another drug, Alicaforsen, is a highly selective inhibitor for ICAM-1 that is being investigated in the field of inflammatory bowel disease[185]. Though speculative, these type of drugs might be useful in OSA in terms of CVD prevention, particularly in patients with elevated levels of ICAM-1 and E-selectin[186].

Our study has many strengths. These included the prospective design, use of objective outcomes for cardiovascular events (as opposed to self-reports), collection of inflammatory markers at the same time each day (morning fasting), use of PSG to assess OSA severity, and ability to adjust for a variety of confounders. In addition, this is the first study of CAM and CVD prognosis done in a suspected OSA population, which is of substantial clinical relevance. However, we acknowledge that our study also has a number of limitations. First, we had a relatively small number of first events. Because we used a broad composite outcome, the severity of each individual event or procedure may vary. Also, this was a single center study, which limits the generalizability of these findings. Larger studies are needed to verify these findings and provide more robust information for calculation of sensitivity and specificity of these molecules in risk prediction. Larger cohorts would allow for the incorporation of other variables into predictive models (e.g. symptom clusters[41], comorbidities, advanced physiologic data[187], other biomarkers[165] such as CRP) using machine learning techniques. Moreover,
we measured baseline levels of ICAM-1, VCAM-1 and E-selectin. This measurement at one point in time does not address possible fluctuations of adhesion molecules over time. Also, there is a risk for residual confounding from unmeasured confounders that weren’t included in our data collection and analysis. Finally, the CPAP adherence data was largely based on chart-review as opposed to objective CPAP downloads.

5.6 Conclusions

Suspected OSA patients with elevated ICAM-1 levels are significantly more likely to experience a cardiovascular event in 8 years of follow up. This association remained significant after adjustment for clinically significant confounding factors (age, sex, smoking status, AHI, BMI, previous heart disease, CPAP use, diabetes and statins). There was a potential interaction between OSA severity and levels of E-selectin, in that E-selectin tended to be more predictive of CV events in patients with moderate/severe OSA. These findings support the previous support what was found on the previous chapter, where E-selectin was associated with OSA in a multivariable analysis.

Although there is need for larger validation studies, ICAM-1 and E-selectin could be potentially useful to identify which OSA patients might be at increased risk of future CV events. ICAM-1 and E-selectin might also be possible targets for future pharmacologic interventions in OSA patients.

The next chapter will explore the potential utility of another class of circulating biomarkers (oxidative) that were also previously identified to be associated with OSA in chapter 3 of this thesis.
Chapter 6: Oxidative Stress Markers and Risk of Incident Cardiovascular Events in Obstructive Sleep Apnea Patients.

6.1 Summary

Oxidative stress (OS) and Obstructive sleep apnea (OSA) are both associated with increased rates of cardiovascular disease (CVD). The identification of which OSA patients are more likely to develop CVD remains a challenge. OS markers could be used to risk stratify OSA patients. The purpose of this study was to assess the prognostic utility of levels of OS markers in incident CVD of suspected OSA patients. Plasma levels of 8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG) and superoxide dismutase (SOD) were measured from patients with suspected OSA referred for a polysomnogram (PSG). A composite outcome of cardiovascular events was defined by deterministic linkage with provincial health databases. Logistic regression was used to assess the relationship between levels of OS markers and incident cardiovascular events. 352 patients were included in the analysis. Most were middle aged (mean age 51.4 years old) male (68%) with moderate OSA (median AHI 16). A total of 31 cardiovascular events were registered in an 8-year follow-up. The majority of events were percutaneous coronary interventions, followed by cardiovascular death, unstable angina and myocardial infarction. In fully adjusted models none of oxidative stress markers were associated with incident cardiovascular events (OR 0.98, 95% CI 0.42 – 2.28 for 8-OHdG, second and third tertile versus first; OR 1.23, 95% CI 0.52 – 2.91 for 8-isoprostane, third tertile versus first and second; and OR 0.77 95% CI 0.34 – 1.73 for SOD, second and third tertile vs first). Oxidative stress markers are not associated with risk of cardiovascular events in suspected obstructive sleep apnea patients. The
results of the present study do not support the use of a single measurement of 8-OHdG, 8-isoprostane and superoxide dismutase for prognosis of OSA patients.

6.2 Introduction

Previously (chapter 3), we demonstrated that in a large sample of patients, OSA was a predictor for levels of 8-isoprostane after adjustment for potential confounding factors. Here we tested if oxidative stress markers would be particularly helpful in identifying which patients are at increased risk of CVD.

Obstructive sleep apnea (OSA) is characterized by frequent events of collapse in the airway, with associated intermittent hypoxia, leading to sympathetic activation, systemic inflammation, and oxidative stress[20]. OSA is highly prevalent, affecting almost half a billion adults across the globe[37]. OSA is a risk factor for both fatal and non-fatal cardiovascular disease(CVD)[73]. However, because OSA is a complex condition with multiple presentations[9] it is difficult to identify which OSA patients are at a greater risk of developing CVD. This chapter focused on using circulating biomarkers to risk stratify OSA patients. More specifically, oxidative stress (OS) markers.

Oxidative stress is a condition where reactive species overwhelm the antioxidant capacity of the cell, leading to cellular damage[134–136]. Oxidative stress is pivotal for the development and establishment of cardiovascular disease[68,137].

8-hydroxydeoxyguanosine (8-OHdG), 8-isoprostane and superoxide dismutase (SOD) are widely studied and validated markers across the literature. 8-OHdG is marker of DNA oxidation and have been shown to be of prognostic value in solid tumors[188]. Also, systematic reviews in cardiovascular disease showed that CVD patients have significantly higher 8-OHdG levels[189]. 8-isoprostane is a marker of lipid peroxidation from arachidonic acid and have been associated...
with asthma[190], cystic fibrosis[191], and many other diseases such as diabetes, cancer, chronic obstructive pulmonary disease, and mostly cardiovascular diseases[139]. Also, 8-isoprostane was identified as an independent risk factor for coronary heart disease and correlated with the extent of diseases[137]. Finally, SOD is an antioxidant marker. A reduced antioxidant capacity is not only associated with Alzheimer’s and amyotrophic lateral sclerosis[192], but also cardiovascular diseases[193].

OSA generates oxidative stress by intermittent hypoxia [86]. This has been validated in animal models of OSA, where rodents have increased OS markers when exposed to intermittent hypoxia[138]. Although there is evidence of potential contribution of OSA to cardiovascular disease through oxidation of cell components, the literature is still scarce[42]. We hypothesized that, in a suspected OSA population, oxidative stress markers (8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG), and superoxide dismutase (SOD) can be used for risk stratification of cardiovascular events. The objective of this study was to determine whether levels of 8-isoprostane, 8-OHdG and SOD predict the occurrence of CVD in a cohort of suspected OSA patients.

6.3 Materials and Methods

6.3.1 Study Design, Setting and Participants

Patients referred to the University of British Columbia Hospital for suspected sleep apnea were recruited. Eligible participants filled questionnaires about their health history and donated blood on the morning after PSG. For more detailed information refer to study design and methods on chapter 5(Section 5.3). The patients included in this study are a subset of the same cohort of patients from chapters 3 and 4.
6.3.2 Laboratory Assays

ELISA and colorimetric assays (Cellbiolabs, CA, USA) were used to test sample levels of 8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG) and superoxide dismutase (SOD). All samples were done in duplicates following the manufacturer’s instructions.

6.3.3 Ascertainment of Cardiovascular Events

A composite outcome of incident cardiovascular events, death for cardiovascular cause, and hospitalizations was used as outcome of interest. Comorbidities and medications were determined based on self-reported questionnaires from the night of PSG. CPAP adherence was determined by chart-review from doctor’s notes and CPAP reports. For more detailed information refer to study design and methods on chapter 5 (section 5.3).

6.3.4 Statistical Analysis

Descriptive statistics included mean standard deviations of normally distributed continuous variables, and median and interquartile range of skewed variables. Categorical variables were summarized as counts and proportions. Logistic regression was used to model the incidence of events and variables of interest. Cell sizes <6 were censored to protect patient privacy. The analysis was done using Statistical Analysis Software (SAS, version 9.4, SAS Institute Inc, USA). For more detailed information refer to study design and methods on chapter 5 (section 5.3).

6.4 Results

A total of 352 patients were included in the study; baseline characteristics are shown in table 6.1. Most of the patients were middle aged (51.4 years old), white (80%) males (68%), with moderate OSA (median AHI of 16). There were 31 events registered in the 8-year follow-up after PSG. Patient with events tended to be older, with a significantly higher AHI and making current
use of statins (Table 6.2). The majority of events (table 6.3) were percutaneous coronary interventions (22.6%), followed by cardiovascular death (16.1%), unstable angina (16.1%) and myocardial infarction (12.9%).

Baseline characteristics showed that BMI increases significantly as levels of 8-OHdG and 8-isoprostane increase. Elevated levels of 8-isoprostane were associated with higher AHI, percentage of time below 90% oxygen saturation, and sleepiness. Female sex was associated with high levels of superoxide dismutase. Results of the univariate analyses are show in Table 6.4. In univariate analyses, age, male sex, AHI and statin usage were associated with increased odds of cardiovascular events.

The association between oxidative stress markers and cardiovascular events is shown in table 6.5. For 8-OHdG and SOD we compared the top two tertiles with the 1st for multivariable models. For 8-isoprostane we compared the top tertile to the first and second. In unadjusted models 8-OHdG, 8-isoprostane and SOD were not associated with odds of incident cardiovascular events (OR 1.08, 95% CI 0.49 – 2.37, OR 1.25, 95% CI 0.56 – 2.81, OR 0.67 95% CI 0.31 – 1.41; for 8-OHdG, 8-isoprostane and SOD respectively). The findings remained the same in fully adjusted models (OR 0.98, 95% CI 0.42 – 2.28, OR 1.23, 95% CI 0.52 – 2.91, OR 0.77 95% CI 0.34 – 1.73; for 8-OHdG, 8-isoprostane and SOD respectively).
<table>
<thead>
<tr>
<th>Baseline characteristics (n=351)</th>
<th>Tertile 1 (n=117)</th>
<th>Tertile 2 (n=119)</th>
<th>Tertile 3 (n=116)</th>
<th>P-value</th>
<th>Tertile 1 (n=117)</th>
<th>Tertile 2 (n=119)</th>
<th>Tertile 3 (n=116)</th>
<th>P-value</th>
<th>Tertile 1 (n=118)</th>
<th>Tertile 2 (n=117)</th>
<th>Tertile 3 (n=117)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) - mean ± SD</td>
<td>50.9 ± 11.1</td>
<td>50.8 ± 12.2</td>
<td>52.5 ± 11.7</td>
<td>0.46</td>
<td>51.6 ± 13.9</td>
<td>52.3 ± 10.3</td>
<td>50.6 ± 10.7</td>
<td>0.53</td>
<td>50.0 ± 11.2</td>
<td>52.4 ± 11.7</td>
<td>51.7 ± 12.1</td>
<td>0.30</td>
</tr>
<tr>
<td>Sex (Male) - mean ± SD</td>
<td>74 (63.8)</td>
<td>78 (65.5)</td>
<td>89 (76.1)</td>
<td>0.09</td>
<td>80 (70.2)</td>
<td>75 (64.7)</td>
<td>82 (70.1)</td>
<td>0.59</td>
<td>92 (78.6)</td>
<td>88 (75.2)</td>
<td>61 (52.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²) - mean ± SD</td>
<td>30.8 ± 5.5</td>
<td>31.1 ± 5.9</td>
<td>32.7 ± 7.5</td>
<td>0.04</td>
<td>29.9 ± 6.1</td>
<td>32.2 ± 6.8</td>
<td>32.6 ± 5.9</td>
<td>0.004</td>
<td>31.2 ± 5.6</td>
<td>31.0 ± 5.7</td>
<td>32.4 ± 7.6</td>
<td>0.21</td>
</tr>
<tr>
<td>AHI (events/hr) median (IQR)</td>
<td>16.6 (6-34.4)</td>
<td>15.3 (6.8-27.8)</td>
<td>16.1 (7.9-27.2)</td>
<td>0.88</td>
<td>13.8 (6.9-27.4)</td>
<td>15.1 (7.6-27.1)</td>
<td>20.2 (7.4-34.8)</td>
<td>0.05</td>
<td>16.6 (8.2-34.8)</td>
<td>18.4 (7.9-30.9)</td>
<td>13.8 (5.4-26.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>% Time below 90% SaO₂ - median (IQR)</td>
<td>0.33</td>
<td>0.35</td>
<td>0.009</td>
<td></td>
<td>0.04-1.3</td>
<td>0.04 (0.1-3.2)</td>
<td>0.5 (0.1-4.5)</td>
<td>0.03</td>
<td>0.5 (0.1-2.8)</td>
<td>0.3 (0.1-2.2)</td>
<td>0.3 (0.1-2.5)</td>
<td>0.49</td>
</tr>
<tr>
<td>ESS &gt; 11 - n (%)</td>
<td>35 (29.9)</td>
<td>27 (22.7)</td>
<td>42 (35.9)</td>
<td>0.08</td>
<td>21 (18.3)</td>
<td>34 (29.3)</td>
<td>45 (38.5)</td>
<td>0.003</td>
<td>30 (25.6)</td>
<td>45 (38.1)</td>
<td>28 (23.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Heart Disease* n (%)</td>
<td>25 (21.4)</td>
<td>28 (23.5)</td>
<td>33 (28.2)</td>
<td>0.46</td>
<td>29 (25.2)</td>
<td>25 (21.6)</td>
<td>31 (26.5)</td>
<td>0.66</td>
<td>32 (27.4)</td>
<td>31 (26.3)</td>
<td>22 (18.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Smoking Status* n (%)</td>
<td>7 (6)</td>
<td>10 (8.4)</td>
<td>8 (6.8)</td>
<td>0.76</td>
<td>7 (6.1)</td>
<td>9 (7.8)</td>
<td>9 (7.7)</td>
<td>0.86</td>
<td>9 (7.7)</td>
<td>10 (8.5)</td>
<td>6 (5.1)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diabetes n (%)</td>
<td>14 (12)</td>
<td>6 (5)</td>
<td>11 (9.4)</td>
<td>0.16</td>
<td>7 (6.1)</td>
<td>13 (11.2)</td>
<td>10 (8.5)</td>
<td>0.38</td>
<td>13 (11.1)</td>
<td>8 (6.8)</td>
<td>10 (8.5)</td>
<td>0.50</td>
</tr>
<tr>
<td>Ethnicity Group</td>
<td>&gt;0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>Caucasian n (%)</td>
<td>89 (80.9)</td>
<td>94 (80.3)</td>
<td>94 (81.7)</td>
<td></td>
<td>96 (84.2)</td>
<td>89 (79.5)</td>
<td>88 (79.3)</td>
<td></td>
<td>88 (77.9)</td>
<td>93 (81.6)</td>
<td>96 (84.2)</td>
<td></td>
</tr>
<tr>
<td>Asian n (%)</td>
<td>11 (10.0)</td>
<td>11 (9.4)</td>
<td>10 (8.7)</td>
<td></td>
<td>9 (7.9)</td>
<td>13 (11.6)</td>
<td>9 (8.1)</td>
<td></td>
<td>13 (11.5)</td>
<td>10 (8.8)</td>
<td>9 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Other n (%)</td>
<td>10 (9.1)</td>
<td>12 (10.3)</td>
<td>11 (9.6)</td>
<td></td>
<td>9 (7.9)</td>
<td>10 (8.9)</td>
<td>14 (12.6)</td>
<td></td>
<td>12 (10.6)</td>
<td>11 (9.6)</td>
<td>9 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Statin Usr n (%)</td>
<td>24 (21.8)</td>
<td>20 (16.9)</td>
<td>26 (22.6)</td>
<td>0.51</td>
<td>23 (20.9)</td>
<td>19 (17)</td>
<td>28 (24.1)</td>
<td>0.41</td>
<td>25 (21.7)</td>
<td>20 (17.5)</td>
<td>25 (22.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Oxidative Stress Levels ⊥</td>
<td>3.30 - 12.08</td>
<td>12.08 - 17.75</td>
<td>17.75 - 49.64</td>
<td></td>
<td>59 - 1406</td>
<td>1406 - 2824</td>
<td>2824 - 42749.78</td>
<td></td>
<td>0.39 - 14.48</td>
<td>14.48 - 22.72</td>
<td>22.73 - 47.98</td>
<td></td>
</tr>
<tr>
<td>Rate of 1st Cardiovascular events</td>
<td>13 (11.1)</td>
<td>10 (8.4)</td>
<td>8 (6.8)</td>
<td></td>
<td>10 (8.7)</td>
<td>9 (7.7)</td>
<td>12 (10.3)</td>
<td></td>
<td>9 (7.7)</td>
<td>13 (11.1)</td>
<td>9 (7.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** 8-OHdG: 8-hydroxydeoxyguanosine; AHI: Apnea-Hypopnea Index; BMI: Body mass index; ESS: Epworth Sleepiness Scale; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure. *Current Smokers. ⊥ 8-OHdG unit is ng/ml; 8-isoprostane unit is pg/ml; Superoxide Dismutase unit is % of inhibition.

Table 6.1 – Patient Characteristics by Oxidative Stress Tertiles (n=352)
### Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>No Outcome (n=321)</th>
<th>Outcome (n=31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log8-isoprostane (pg/ml)</td>
<td>7.67 ± 0.86</td>
<td>7.59 ± 0.97</td>
<td>0.78</td>
</tr>
<tr>
<td>Log8-OHdG (ng/ml)</td>
<td>2.66 ± 0.45</td>
<td>2.56 ± 0.48</td>
<td>0.10</td>
</tr>
<tr>
<td>Log Superoxide Dismutase (%)</td>
<td>2.70 ± 0.79</td>
<td>2.69 ± 0.88</td>
<td>0.97</td>
</tr>
<tr>
<td>Age</td>
<td>50.6 ± 11.4</td>
<td>59.9 ± 11.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>213 (66.6)</td>
<td>27 (87.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>31.4 ± 6.5</td>
<td>32.4 ± 5.2</td>
<td>0.70</td>
</tr>
<tr>
<td>AHI (events/hr)</td>
<td>14.9 (6.9 - 28.2)</td>
<td>25.1 (14.1 - 44)</td>
<td>0.02</td>
</tr>
<tr>
<td>AHI Category</td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>Control</td>
<td>56 (17.4)</td>
<td>&lt; 6</td>
<td>.</td>
</tr>
<tr>
<td>Mild OSA</td>
<td>105 (32.7)</td>
<td>&lt; 6</td>
<td>.</td>
</tr>
<tr>
<td>Moderate OSA</td>
<td>84 (26.2)</td>
<td>11 (35.5)</td>
<td>.</td>
</tr>
<tr>
<td>Severe OSA</td>
<td>76 (23.7)</td>
<td>12 (38.7)</td>
<td>.</td>
</tr>
<tr>
<td>% Time below 90% SaO2</td>
<td>0.32 (0.1 - 2.2)</td>
<td>1.7 (0.1 - 4.8)</td>
<td>0.94</td>
</tr>
<tr>
<td>ESS &gt; 11</td>
<td>94 (29.3)</td>
<td>10 (32.3)</td>
<td>0.76</td>
</tr>
<tr>
<td>Heart Disease*</td>
<td>75 (23.4)</td>
<td>11 (35.5)</td>
<td>0.28</td>
</tr>
<tr>
<td>Smoker</td>
<td>21 (6.5)</td>
<td>&lt; 6</td>
<td>0.40</td>
</tr>
<tr>
<td>Diabetes</td>
<td>26 (8.1)</td>
<td>&lt; 6</td>
<td>0.31</td>
</tr>
<tr>
<td>Ethnicity Group</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Caucasian</td>
<td>253 (81.4)</td>
<td>24 (80.0)</td>
<td>.</td>
</tr>
<tr>
<td>Asian</td>
<td>29 (9.3)</td>
<td>&lt; 6</td>
<td>.</td>
</tr>
<tr>
<td>Other</td>
<td>29 (9.3)</td>
<td>&lt; 6</td>
<td>.</td>
</tr>
<tr>
<td>Statin User</td>
<td>59 (18.8)</td>
<td>11 (39.3)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; BMI: Body mass index; ESS: Epworth Sleepiness Scale; *Heart disease included: Hypertension, Myocardial Infarction, Cardiac Arrhythmias, Angina, and Congestive Heart Failure.

**Table 6.2 - Baseline Characteristics by Cardiovascular Events**
<table>
<thead>
<tr>
<th>Outcome</th>
<th>First Event (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular Death*</td>
<td>16.1 (CVD)</td>
</tr>
<tr>
<td>Atrial Fibrillation</td>
<td>3.2</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>9.7</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>12.9</td>
</tr>
<tr>
<td>Stroke</td>
<td>6.5</td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>16.1</td>
</tr>
<tr>
<td>Percutaneous Coronary Intervention</td>
<td>22.6</td>
</tr>
<tr>
<td>Cardioversion</td>
<td>6.5</td>
</tr>
<tr>
<td>Pacemaker</td>
<td>6.5</td>
</tr>
</tbody>
</table>

*Causes of Death: Hyperlipidemia, myocardial infarction, atherosclerotic heart disease, chronic ischemic heart diseases and myocarditis (appendix D)

Table 6.3 – Percentage of Cardiovascular Events

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08 (1.04, 1.13)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>1.02 (0.97, 1.08)</td>
<td>0.41</td>
</tr>
<tr>
<td>Sex*</td>
<td>3.39 (1.16, 9.94)</td>
<td>0.02</td>
</tr>
<tr>
<td>AHI</td>
<td>1.02 (1.00, 1.03)</td>
<td>0.03</td>
</tr>
<tr>
<td>Heart Disease*</td>
<td>1.80 (0.83, 3.93)</td>
<td>0.14</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.18 (0.77, 6.16)</td>
<td>0.14</td>
</tr>
<tr>
<td>Smoking**</td>
<td>2.12 (0.67, 6.61)</td>
<td>0.20</td>
</tr>
<tr>
<td>Statin</td>
<td>2.80 (1.25, 6.28)</td>
<td>0.01</td>
</tr>
<tr>
<td>ESS &gt; 11</td>
<td>1.15 (0.52, 2.53)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; BMI: Body mass index; CI: Confidence Intervals; ESS: Epworth Sleepiness Scale; OR: Odds ratio; HR: Hazard Ratio; *Male versus Female; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure; **Current smokers versus not currently smoking.

Table 6.4 – Univariate Odds Ratio for Cardiovascular Events
### 8-OHdG

<table>
<thead>
<tr>
<th>Unadjusted Tertiles</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vs 1</td>
<td>0.73 (0.31, 1.75)</td>
<td>0.48</td>
</tr>
<tr>
<td>3 vs 1</td>
<td>0.59 (0.24, 1.49)</td>
<td>0.26</td>
</tr>
<tr>
<td>Tertile 2+3 vs 1</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.08 (0.49, 2.37)</td>
<td>0.85</td>
</tr>
<tr>
<td>Fully Adjusted***</td>
<td>0.98 (0.42, 2.28)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

### 8-Isoprostane

<table>
<thead>
<tr>
<th>Unadjusted Tertiles</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vs 1</td>
<td>0.88 (0.34, 2.26)</td>
<td>0.80</td>
</tr>
<tr>
<td>3 vs 1</td>
<td>1.21 (0.50, 2.93)</td>
<td>0.67</td>
</tr>
<tr>
<td>Tertile 3 vs 1+2</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.25 (0.56, 2.81)</td>
<td>0.59</td>
</tr>
<tr>
<td>Fully Adjusted***</td>
<td>1.23 (0.52, 2.91)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

### Superoxide Dismutase

<table>
<thead>
<tr>
<th>Unadjusted Tertiles</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vs 1</td>
<td>1.50 (0.61, 3.66)</td>
<td>0.37</td>
</tr>
<tr>
<td>3 vs 1</td>
<td>1.00 (0.38, 2.62)</td>
<td>0.99</td>
</tr>
<tr>
<td>Tertile 2+3 vs 1</td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.67 (0.31, 1.41)</td>
<td>0.38</td>
</tr>
<tr>
<td>Fully Adjusted***</td>
<td>0.77 (0.34, 1.73)</td>
<td>0.53</td>
</tr>
</tbody>
</table>

***Adjusted for Age, Sex, Smoking Status, AHI, BMI, Heart Disease, ESS > 11, Diabetes

**Table 6.5 – Unadjusted and Adjusted Odds Ratio for Effects of Oxidative Stress Molecules on Cardiovascular Events**

### 6.5 Discussion

We investigated the prognostic utility of oxidative stress markers on cardiovascular events in a suspected OSA population. In our study, the OS biomarkers we investigated (8-isoprostane, 8-ohdg and SOD) were not significantly associated with incident CV events, either in univariate analysis or after adjusting for confounders.

In OSA, most of the studies in oxidative stress markers were limited by either having a small sample size[194], not being adjusted for potential confounders[195], or had inconclusive results[196]. We recently investigated plasma levels of 8-ohdg, 8-isoprostane and SOD in 400 patients and found that OSA was independently associated with levels of 8-isoprostane[172]. Yamauchi et al showed that oxygen desaturation index (ODI) was a significant predictor for urinary levels of 8-OHdG[197]. Given the link between 8-isoprostane, 8-OHdG and SOD with
CVD, the evidence of an independent role of OSA in increasing levels of oxidative stress and the
evidence that OSA is an independent risk factor for CVD, it seemed logical to test the association
between levels of oxidative stress and CVD in a suspected OSA population. However, we found
that oxidative stress markers provide no prognostic information on the incidence of cardiovascular
events in suspected OSA patients.

In the OSA literature, patient phenotypes have been identified as a useful prognostic tool
in incident cardiovascular diseases. For instance, Mazotti et al, showed that excessive sleepy
patients have increased risk for developing cardiovascular disease[41]. In terms of biochemical
phenotyping, our research group was the first to report that in OSA patients (AHI>5), C-reactive
protein (CRP) levels might be useful to detect incident cardiovascular disease[165].

Using circulating levels of biological markers as a prognostic tool is not an easy task. While
OSA is starting its first steps towards precision medicine, other diseases have been investigating
biomarkers for longer, and very few reached clinical use. Some examples are troponins and CRP
in cardiovascular diseases[198]. However, on a brief search on the medical literature it can be
noticed that although there are many studies linking oxidative stress to almost every disease
possible, no oxidative stress markers are being clinically used for the purposes of diagnosis or
prognosis[199,200]. To reach clinical use researchers must first identify potential biomarker
candidates, replicate the findings in different cohorts, optimize its assay, validate it clinically and
demonstrate a significant benefit from using the marker during patient care[201]. This study
contributes to the body of the literature in OSA posing the question that perhaps oxidative stress
markers might not be worth investing into further testing and other markers should be considered.

Our findings are in agreement with other negative findings in similar outcomes but
different populations and different markers. Oxidative stress markers provided no useful
information to risk stratify type 2 diabetes mellitus patients in terms of major adverse cardiovascular events[202]. Considering that 73% of type 2 diabetic patients have OSA[203] it is possible that the same findings could be applicable to good portion of OSA patients, reiterating that oxidative stress markers might not be good candidates to identify which patients are at greater risk of developing cardiovascular events. Considering that positive studies are three times more likely to be published than negative studies[204], it might also be possible that there are more negative studies using cardiovascular endpoints in other populations but they simply didn’t get published.

Another possible explanation for the negative results could be due a possible lack of power to detect true significance explained by a relatively low rate of event/subject. Data from the SAVE trial indicated an outcome rate of 16% (436/2687)[79]. However, patients on the SAVE trial had previously diagnosed cardiac conditions. In our study, roughly 25% of patients had some kind of pre-existing cardiovascular condition and our overall outcome rate of 8% (31/351). Given the large range on the confidence intervals (8-OHdG: OR 95% CI 0.4-2.2; 8-isoprostane OR 95% CI 0.5 - 2.9; SOD OR 95% CI 0.3 - 1.7) it seems unlikely that doubling the sample size and number of events would significantly impact the results.

We acknowledge a number of limitations to our study. First, patients were recruited from a sleep laboratory based on a hospital, leading to a potential selection bias. The findings on this study might differ if a population-based cohort was used. Second, we measured baseline levels of oxidative stress. Variations over time are not captured and could potentially influence the results. Finally, this is a single-centered study on the west coast of Canada. These results may not be generalizable outside this population. There are many strengths to our study as well. First, we measured well-known oxidative stress markers in a large suspected OSA cohort. To the best of
our knowledge this is the first prospective cohort study using oxidative stress and cardiovascular events in suspected OSA patients. Second, our events were derived from validated databases from hospital discharges, ensuring ascertainment of events. Third, we adjusted for several covariates and risk factors that could possibly influence the results.

6.6 Conclusions

Contrary to our hypothesis, oxidative stress markers were not associated with risk of incident cardiovascular events in suspected obstructive sleep apnea patients. The results of the present study do not support the use baseline levels of 8-OHdG, 8-isoprostane and superoxide dismutase for prognosis of OSA patients.

The next chapter of this thesis will summarize the findings of all studies conducted in this thesis and discuss the findings in the light of the most recent literature.
Chapter 7: Discussion, Future Directions and Conclusions

7.1 Summary of results

This section focuses on the main findings from chapters 2-6. The combined work of this thesis represents an important step towards a better understanding of how clinicians can identify which OSA patients are at greater risk of developing complications associated with OSA.

The grand idea is that circulating biomarkers of inflammation (cellular adhesion molecules) and oxidative stress will help to predict cardiovascular disease in OSA. We designed a series of studies to address this issue and advance the concept.

First, we conducted a rigorous systematic review (chapter 2) exploring which biological markers could be potentially used in the complicated task of risk stratifying OSA patients. We found only ten cross sectional studies and no prospective studies that examined prognostic circulating biomarkers. This review showed a significant gap in the literature. However, based on this review, inflammatory markers and oxidative stress markers might be promising candidates, which led us to our subsequent two studies.

Second, we determined whether a series of oxidative stress markers and inflammatory biomarkers were associated with OSA in cross sectional studies (chapters 3 and 4). We found that out of the six biomarkers studied (three oxidative stress and three adhesion molecules); one marker of OS (8-isoprostane) and one adhesion molecules (E-selectin) were associated with OSA (figure 7.1). We then tested these molecules to see if they actually would predict risk of incident cardiovascular disease.

Consistent with our own findings, E-selectin was indeed a very promising marker in OSA (chapter 5), and ICAM-1 was a predictor for CVD in suspected OSA patients. Contrary to our expectation, isoprostanes weren’t associated with increased risk of incident CVD (figure 7.1).
Figure 7.1 - Schematic representation of the studies and main findings
In conclusion, this thesis provides evidence of an independent effect of OSA on circulating levels of 8-isoprostane and E-selectin. Also, it advances the field of sleep medicine by providing evidence that oxidative stress markers might not be useful to predict which OSA patients will suffer from CVD. Finally, it brings evidence that ICAM-1 and E-selectin can be useful markers to detect which patients are at increased risk of cardiometabolic complications. Further studies are needed to validate these findings, and if verified in other cohorts, may prove to be useful in clinical decision making in the future.

7.2 Implications of the findings

Obstructive sleep apnea (OSA) affects between 700 to 900 million middle aged adults worldwide[37]. Importantly, OSA significantly impacts the health sector, accounting for $12.4 billion per year in the United States. Furthermore, the burden of undiagnosed OSA and associated comorbidities compromises over $150 billion[38]. OSA is an independent risk factor for CVD[73]. One of the biggest challenges with OSA is its heterogeneous presentation. Despite the fact that OSA research evolved and is able to identify subcategories of the pathophysiological processes responsible for the establishment of the disease (endotypes) and its clinical presentation (phenotypes), there is need for better metrics to risk stratify OSA patients for long-term complications[8].

One solution to this clinical problem is the development and testing of circulating biological markers. Such markers should be associated with OSA and its comorbidities, respond to therapeutic interventions and provide a prognostic stratification, guiding future care. In the last few years, there has been a significant increase in publications about biomarkers in OSA[84]. Some candidate markers emerged as promising indicators of OSA. However, none of these studies focused on prognosis[85].
This thesis represents a novel and significant contribution to the OSA field. The systematic review presented on chapter 2 was the first publication in the field that aimed to identify which biological markers was further increased if OSA was concurrent with any of its comorbidities. This project attempted to advance the field by providing potential biomarkers to be further explored depending on the comorbidity of interest. However, it does not mean that these markers reached clinical translation yet. In fact, the “roadmap” for biomarkers discovery and usage is far more complex and requires a scrupulous process from identification to clinical utilization[201]. Not surprisingly, in medicine, very few biomarkers have clinical use. Common examples are HbA1c in diabetes, cardiac troponins in cardiology and creatinine levels in kidney disease.

The common issues why biomarker research has failed for most of the diseases can be explained by limitations on study design, limited sample size, lack of reproducibility, poor choice of biomarker to be investigated and failure to incorporate disease heterogeneity[201]. Interestingly, these challenges were seen in the results of chapter 2. All studies included in the systematic review were cross-sectional. Their sample size was limited, and most of the included publications did not acknowledge the disease complexity and its overlap with other conditions that significantly affect the markers being reported. Additionally, the biomarker literature is greatly affected by publication bias. Some publications mentioned negative results in OSA biomarkers[196], but most of the available data can’t be generalized to other OSA cohorts[205].

Another challenge, and perhaps one of the reasons why OSA haven’t yet passed the initial stages of biomarker discovery, is the lack of specificity of the markers explored so far. The results of chapter 2 showed that potential markers for cardiovascular disease in OSA populations involved inflammatory markers, oxidative stress, adhesion molecules, vascular proteins, lipids,
glucose and catecholamines. Within the inflammatory markers, C-reactive protein, tyrosine-kinase ligands, tumor necrosis factor and interleukins were all significantly higher in patients with a cardiovascular disease and OSA when compared to OSA only. The same was true for intercellular adhesion molecules, endoglin, low density lipoprotein levels, and glucose levels. Metabolic conditions and renal diseases did not differ much in the findings, with CRP being significantly increased when OSA was concurrent with any comorbidity. If one takes CRP as an example it becomes clear why there’s still need for more research. OSA’s sleep fragmentation and intermittent hypoxia induces systemic inflammation by activation of hypoxia-induced factor (HIF-1) and NF-κB[120]. CRP is a classic marker of systemic inflammation, however, is not specific to OSA. In fact, most of the markers identified on the systematic review lack specificity to OSA populations. Systemic inflammation contributes to the pathogenesis of several age-related diseases, including OSA and its associated comorbidities[206]. Ideally, the field should identify a target marker, with population specific reference intervals and validate assays following proper regulatory guidelines[201]. Even though this thesis represents a first step towards the goal of using biomarkers to risk stratify patients, there is a lot to be done.

As part of the work to build evidence around potential markers to be used to risk stratify OSA patients, chapters 3 and 5 aimed to determine if well-known oxidative stress markers could be potentially used in an OSA population. Severity of obstructive sleep apnea was a significant predictor for levels of 8-isoprostane, after adjusting for age, sex, obesity and statin usage. This finding is supported by the current evidence in OSA. The episodes of intermittent hypoxia and repeated arousals from sleep increase oxidative stress by NADPH oxidase and uncoupling of endothelial nitric oxide[78]. In chapter 5, the relationship between oxidative stress markers and cardiovascular events in an OSA cohort was tested. Oxidative stress markers were not associated
with increased odds of a composite cardiovascular outcome. The results seen in chapters 3 and 5 are novel in the OSA literature as very few authors explored the potential use of free-radicals and their clinical utility to determine prognosis of OSA patients. They suggest an independent role of OSA in the rise of oxidative stress (chapter 3), but do not support use of baseline levels to identify which patients will indeed suffer from a clinical endpoint (chapter 5).

Free radicals are involved in many pathogenic processes. This thesis certainly enriches the free-radical paradigm, but yet does not determine where OSA stands in the different scenarios of the paradigm. The free-radical paradigm defines five different possibilities. First, free radicals could be the only cause leading to early stage of disease. Second, free radicals would be one of the many factors contributing to initial stages of disease. Third, free radicals would determine the progression of disease (from early signs to onset). Fourth, free radicals would be responsible for further progression to advanced stages of disease. Lastly, free radicals could be a simple byproduct of the conditions and have no role in the pathogenesis of the disease[207]. It is possible that OSA causes an increase in free radicals enough to be the tipping point between an initial endothelial dysfunction to early stage atherosclerosis; or to take an atherosclerotic plaque to an advanced endothelial lesion. At the same time, it is possible that the increased levels of isoprostanes in OSA are a simple byproduct of the hypoxia insults posed by the disease. Additionally, it might be possible that OSA represents a turning point on the development of comorbidities to some individuals, while for others it has very little contribution. Finally, it can be speculated that OSA’s role in producing free radicals can have a defining role in some comorbidities (such as cardiovascular conditions), while not as much in others (such as metabolic syndrome).
Additionally to chapters 3 and 5, we investigated the evidence around cell adhesion molecules, OSA and cardiovascular disease in chapters 4 and 6. The goal was to determine if OSA had an independent effect on levels of well-known CAMs, such as ICAM-1, VCAM-1 and E-selectin. We saw that after adjustment for confounding factors, OSA remained a predictor for E-selectin levels (chapter 4). The hypothesis tested on chapter 5 was similar to chapter 6. We found that E-selectin seems to be specific to moderate to severe OSA patients. The results from chapter 5 seem promising for future clinical validation, where ICAM-1 (OR 3.7, 95% CI 1.29-10.54) and E-selectin (OR 4.07, 95% CI 1.06 – 15.61) were associated with increased odds of CV events in OSA patients.

7.3 Overall strengths and weaknesses

The research conducted for this thesis had major strengths. The methods used in the literature review to identify the potential markers to be further investigated in the body of the thesis were based on the PRISMA statement. This is particularly important because provides a minimum set of items for reporting and evaluating the studies included in the systematic review. In addition to that, the novelty of the research question that was answered is another strength of the study. Additionally, the diagnosis of OSA was done objectively, using the gold standard testing method, a polysomnography. This is a significant advantage, especially when comparing to studies that classified OSA using questionnaires. Although screening questionnaires have been validated and are clinically used, they lack sensitivity and specificity for mild and moderate cases of OSA. Additionally, most of the studies in biomarkers and obstructive sleep apnea have a relatively small sample size. This thesis used a large sample (n>350) of suspected OSA patients and adjusted for the presence of confounding factors.
Despite the overall strength of the research presented, there are a few weaknesses that should be acknowledged. The samples used to measure oxidative stress and cellular adhesion molecules were stored for many years. However, we doubt this would have been an issue as all samples were kept in -80°C freezers from PSG date, and none of the samples were thawed/refrozen. In addition to that, we took special caution to adjust the results based on the presence of comorbidities. However, these comorbidities were based on patient’s self-reported data. The questionnaire that all patients in the cohort filled at the night of PSG (Appendix B2) included questions that allowed the attempt to justify variations on the results based on medications, smoking or previous conditions. The self-reported nature of this data opens space for the presence of residual confounding. Another limitation is referral bias. The recruited patients were being tested for sleep apnea after being referred from a family doctor or other specialist to rule out or rule in OSA. Another important topic that deserves attention is the effect of duration of pre-existing OSA on the baseline levels of the measured biomarkers and the impact in incidence of events. Based on the evidence reviewed in this thesis it is very likely that sleep apnea is driving the levels of some markers, however, we don’t know for how long our patients had sleep apnea and how severe this exposure was. Community based studies that test healthy individuals through PSG during a lifetime would be an ideal study design to address this limitation of our study. Finally, a limitation was that CPAP adherence data was available mostly through self-reports at the follow-up visits after CPAP prescription. Very few patients had reports of objective adherence, and most of data on adherence had to rely on subjective description by the follow-up physician. This led to a point-estimate of adherence, limiting ability to truly assess the impact of treatment on cardiovascular events.
7.4 Future Directions

The findings from the 5 studies outlined in this thesis represent a significant progress in the development of precision medicine in OSA.

The concept of precision medicine is not new. It evolved over the years and now is widely accepted. “P4 medicine” is one of the concepts in personalized medicine. It stands for personalized, preventative, predictive and participatory. In terms of OSA, personalized can be seen clinically as the clear distinction between subgroups of patients. These subgroups can be classified based on their clinical presentations/symptoms, in different molecular profiles, and pathophysiological characteristics. Predictive is the concept that defines the prognostic determinants of a particular patient. It is the main focus of this thesis and relies on the idea that we can use molecular markers to identify who will be at greater risk of developing undesired consequences of OSA. Preventative relates to strategies that can be done in order to prevent onset of disease. Participatory involves including the patient perception to improve adherence to treatment[40]. In addition to P4, some authors also suggested the use of a clinical fingerprint tool to provide estimations of disease progress and treatment alternatives. The tool is a graphical scheme that includes disease severity, biological activity and impact on the patient domains. It helps clinicians to decide appropriate conducts depending on the traits shown by the patient. Future studies are still needed to validate if this tool provide a more individualized management of OSA patients[166].

Personalized medicine in OSA means that the field should go beyond the old-fashioned simplistic approach of assessing patients solely on AHI. It should also consider information from genomics, physiologic endotypes and phenotypes. Genomics reflect the determinants of an individual and contribute to the anatomical and physiological differences seen in different
people. Next, different individuals will develop OSA and respond differently to treatments depending on its endotypes (anatomical, low muscle responsiveness, low arousal threshold and high loop gain). Finally, data-driven studies have consistently shown three consistent symptom clusters in OSA: first, a group of patients with disturbed sleep; second, a group with minimal symptoms; and last, a group of excessively sleepy patients. Although there is still need for more evidence to understand the relationship between genomics, proteomics, epigenetics, metabolomics, endotypes and phenotypes, it is important to use this knowledge to better interpret “omics” markers in OSA populations.

Future considerations in personalized OSA should cover three main areas: technology for diagnosis and treatment; multi-omics (genomics, proteomics, metabolomics and transcriptomics); and artificial intelligence using big data generated by medical records, biomarkers, and diagnostic and therapeutic devices[166]. The identification and clinical use of biological markers is a challenge in many other diseases. As mentioned before, the path to clinical use is complex and require several different steps. The first step (which OSA has already been through) relies on identifying clinical need supported by extensive economic analysis. This initial phase is called “feasibility”[201]. Second, clinical networks are developed. These networks will collect biological samples from patient cohorts, transitioning to what is called the “content development” phase. The scientific content produced in this thesis belongs to the content development phase. At this stage, the goal is to identify biomarker candidates in specific biological fluids, from well-defined phenotypes (i.e. diagnosed with OSA with or without cardiometabolic conditions). Moving forward, the future steps of the information collected in chapters 3-4 involve replication of previously identified markers (8-isoprostanate in our case) and selection of best candidates. These are the first steps of the validation and qualification phase. As
soon as a biomarker is validated, the assays used to quantify this biological signature need to be validated as well. Upon validation, the assay is tested in potential patients which allows clinical utility to be truly measured. If a clinical benefit is seen and confirmed in multiple investigations the biomarker would be ready to be launched and clinically used[201]. As one can appreciate, OSA is still at its initial stages of biomarker investigation. There is a lot of work to be done, but most certainly the findings from this thesis can help the scientific community to move forward in the extensive path for biomarker development.

Another area of future investigations is “Big Data”. Big data doesn’t apply only for the accumulation of huge amount of information on biomarkers (omics), it also includes the data of CPAP monitoring, social media, registries and cohorts around the world, lifestyle apps, and sleep studies. This information along with health records, anthropometrics, socioeconomic data and geolocation data (air pollution, access to care) provide a wealth of information that allow deep learning through artificial intelligence. Most importantly, most of this data is readily available, and with proper consent, fairly robust population-level studies can be conducted[208]. In regard to omics data (genomics, metabolomics, proteomics, etc.), OSA will benefit remarkably from the development of biobanks. In fact, the data collection approach of this thesis served as grounds to the development of a Canada-wide biobank in OSA. The work here presented complements project 1 of the Canadian Sleep and Circadian Network. Project 1 aims to develop a biobank across Canada and use the scientific information to help to provide a more personalized care to Canadians suffering from OSA and its consequences. With that in mind, future studies should keep collecting data on a large number of individuals with OSA to possibly develop a large enough database where artificial intelligence can be applied. Therefore, getting closer to a clinically useful biosignature.
In addition to big data, the results of this thesis could help to provide information to back up future OSA research in drug development. We provided evidence that OSA is independently associated with increased levels of cellular adhesion molecules in humans. We also showed that CAMs were associated with incident CVD. As discussed above, it is still unknown where the increased inflammation/stress generated by OSA acts. If it’s on the early stages of diseases or perpetuating the insults and establishing advanced diseased. Future research should focus on investigating further what is the role of the increased CAMs brought by OSA in the disease establishment. Also, there is need for randomized controlled trials looking at OSA patients, possible anti-inflammatory therapies and the development of major adverse cardiovascular events.

Finally, OSA is just beginning its first steps towards precision medicine. To achieve a more targeted approach, circulating biomarkers could certainly be helpful. In the common pathway to the clinical use of such markers, OSA is still at the feasibility and content development phase. There is need for more robust studies focusing on identifying potential biomarker candidates. If biobanks are created, and as researchers collaborate, these potential markers could benefit from machine learning tools to help to identify which markers stand out and can be selected for further investigation and potential clinical use. In addition to that, there is need to develop methods to collect large amount of information from OSA patients. The possibilities within Big Data and artificial intelligence can help us to achieve personalized care in OSA much faster. Finally, biomarkers in OSA can be helpful to identify potential drug targets. There is need to investigate the contribution of OSA in the development of comorbidities and whether drugs can be beneficial on reducing the free radical burden in OSA patients.
7.5 Conclusions

Overall, the research conducted in this thesis demonstrated the first steps toward a future precision care approach in OSA. Obstructive Sleep Apnea is an important condition, with a significant public health impact. Data from our systematic review showed potential proteomics and metabolic candidates in OSA. These markers can help to identify patients at greater risk of developing cardiometabolic conditions associated with OSA. Cross-sectional data showed evidence of OSA’s role in increasing levels of well-known oxidative stress markers (i.e. 8-isoprostane) and cell adhesion molecules (i.e. E-selectin). Prospectively, oxidative stress markers were not associated with incident cardiovascular events, while ICAM-1 and E-selectin were.

Identifying patients at greater risk of developing cardiometabolic complications using biomarkers is challenging. Research should focus on expanding efforts to identify potential biomarker candidates, on collaborating to enable use of artificial intelligence and machine learning and identifying potential new therapeutic approaches to OSA patients. Prognostic biomarkers in OSA are far from clinical use but can represent a significant step towards reducing the impact of OSA in our society.
Bibliography


Public Health Agency of Canada. Heart Disease in Canada 2016.


Appendices

Appendix A

Appendices from Chapter 2

A.1 Search Strategy

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A.2 Excluded articles and reasons for exclusion

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<td>5*Obesity not being considered as comorbidity.</td>
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<tr>
<td>Author/Year</td>
<td>Reason for Exclusion</td>
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<tr>
<td>Uysal 2014 [192]</td>
<td><strong>2</strong> Excessive Sleepiness not considered as comorbidity</td>
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<tr>
<td>Andaku 2015 [193]</td>
<td><strong>2</strong> Excessive Sleepiness not considered as comorbidity</td>
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<tr>
<td>Thunstrom 2015 [194]</td>
<td><strong>2</strong> No comparison within OSA group</td>
<td></td>
<td></td>
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<tr>
<td>Jafari and Mohsenin 2016 [195]</td>
<td><strong>2</strong> Endothelial Dysfunctions not being considered comorbidity</td>
<td></td>
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<tr>
<td>Sawatari 2016 [196]</td>
<td><strong>2</strong> Endothelial Dysfunctions not considered as comorbidity</td>
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<tr>
<td>Vicente 2016 [197]</td>
<td><strong>1</strong></td>
<td></td>
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</tr>
<tr>
<td>Kim 2017 [198]</td>
<td><strong>1</strong> Paper states the odds of developing MetS based on baseline levels of CRP and OSA. Does not provide data to compare MetS+ vs. MetS-</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Reasons for exclusion: (1) Prognostic Biomarker linked with comorbidity not present, (2) Other.
A.4 References of excluded papers


molecules with hs-CRP and changes therein after ARB (Valsartan) administration in patients with obstructive sleep apnea syndrome. J Med Invest 53:134–139


135


141


Appendix B

Appendices for the data used in chapters 3 to 6.

B.1 Consent Form

Consent Form

Title of Project: Genetic and Biochemical Markers in Sleep Apnea

Principal Investigator:
Co-Investigator(s):

You are being invited to participate in this study because:

Your physician has sent you for an overnight sleep assessment for the diagnosis of Obstructive Sleep Apnea (OSA). This consent form gives you information about the research study. It is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives, and your doctor if you wish. Please ask the study doctor or study coordinator any questions which you may have about the study. After reading this consent form, if you would like to take part in this study you will be asked to sign this form. Your signature will mean that you have been informed about, and you understand the study, and that you give your permission to take part in this study. You will be given a signed copy of this consent form.

Purpose:
The purpose of the study is to assess whether certain hereditary factors (genes) are associated with OSA. Also, we want to see if certain chemicals in the blood (biochemical mediators) are associated with OSA. Some of these mediators are thought to lead to inflammation in the body and to heart disease. The information collected in this study will enable us to better understand the genetics of OSA and the risk of the chemicals involved, to the general health of OSA patients. This research may ultimately lead to a better diagnosis and a more effective treatment of OSA.

Study Procedures:

If you agree to participate in the research, you will be asked to complete a questionnaire (survey) on the night of your overnight sleep assessment. The survey includes questions about your medical history and would require about 20 minutes to complete. You will also be asked about your parents ethnic heritage. Answering questions is entirely optional.
Either on the night of the sleep assessment or in the morning after, your height, weight, blood pressure, and neck circumference will be measured. Also, in the morning after the sleep assessment, Afterwards, you will be asked to donate approximately 30 mL (two tablespoons) of blood for analysis. In total, it will require about 25 minutes of your time. Your blood sample will be stored and then analyzed, to identify chemicals which are potentially associated with OSA. You will not be contacted again when your blood is analyzed, The blood will not be used for any other purposes, including any commercial uses, and will not be transferred to another institution. Upon completion, patients will be notified of study results by the study coordinator.

The blood will be stored in a freezer at VGH for 20 years. You will have the option to request that your blood sample will be destroyed before the end of 20 years, by contacting the study coordinator at xxx phone number. After 20 years, the blood will be disposed of in the hospital’s hazardous waste container, and destroyed

**Risk and Discomfort:**
There will be some minor discomfort associated with the drawing of blood from an arm vein. The risks associated with blood drawing include pain and bruising at the site of needle insertion. In rare cases, lightheadedness or infection may occur. This procedure is not associated with any long-term pain or complications.

**Benefits:**
There are no known direct benefits to you associated with your participation in this research.

**If you decide not to participate:**
If you decide not to participate in the study, you will receive the regular investigation, treatment and follow-up required for your condition. This will be undertaken by your primary physician in the Sleep Clinic and will include a full overnight sleep study.

**Confidentiality:**
Your confidentiality will be respected. No information that discloses your identity will be released or published. To protect your identity, your personal information, including your date of birth, (some examples are gender, age, details of medical conditions) and other information arising from the study and collected by the investigator will be identified by a number, and kept in a locked filing cabinet. Your name will not appear in any publications or reports produced from this study. Any information resulting from this research study will be kept confidential. Your medical record may, however, be inspected, in the presence of the Investigator or his or her designate, by the UBC CREB or by Health Canada, for the purpose of monitoring the research.
We will keep your data and blood for a total of 20 years. In the future, we may perform further analysis of the blood collected from you including testing for newly discovered genes or biochemical mediators. At any time, your identity will be kept confidential. Your rights to privacy are also protected by the Freedom of Information and Protection of Privacy Act of British Columbia. This act lays down rules for the collection, protection, and retention of your personal information by public bodies such as the University of British Columbia and its affiliated teaching hospitals. Further details about this Act are available upon request. All information collected for the purpose of this study will be kept confidential and secured in a locked cabinet in the Principal Investigator’s research office at UBC Hospital.

Remuneration/Compensation:

There will be no remuneration for the study.

Contact for information about the study:
If you have any questions or desire further information with respect to this study, you may contact Dr. x or one of his associates at phone number. In an emergency, you may also page Dr. x or the physician covering for him 7 days per week, 24 hours per day, by calling phone number.

Conflict of Interest:

The investigator has no conflicts of interest relating to the treatments or procedures involved in this study.

Rights as a research subject:

This study has been approved by University of British Columbia, Clinical Ethics Review Board, an independent ethical review committee that reviewed this study. This Board was established to help protect the rights of research subjects. Any concerns about your rights as a research subject, you may telephone the Research Subject Information Line, Office of Research Services at the University of British Columbia, at phone number.

Consent:

Your participation in this study is entirely voluntary and you may refuse to participate, or withdraw from the study at any time without any consequences or jeopardy to your continuing medical care. Your signature below indicates that you do not waive any of your legal rights by signing this consent form. Your signature indicates that you consent to participate in this study and that you have received a copy of this consent form for your own records.
CONSENT TO PARTICIPATE

I have read and I understand the above description of this research study. I have been informed of the risks involved, and all of my questions have been answered to my satisfaction. Furthermore, I have been assured that a member of the research team will also answer any future questions that I may have. I voluntarily agree to participate in this study. I understand that I will receive a copy of this consent form.

By signing this form I have not waived any of the legal rights which I otherwise would have as a participant in a research study.

I have signed this form before my participation in the study.

SUBJECT

- I agree to take part in this study

Name (please print) ___________________________ Signature ___________________________ Date __________

WITNESS

Name (please print) ___________________________ Signature ___________________________ Date __________

I have explained the nature and purpose of the study to the Subject named above.

INVESTIGATOR or a DESIGNATE

Name (please print) ___________________________ Signature ___________________________ Date __________
B.2 Questionnaires

Vancouver Coastal Health Authority
North Shore/Coast Garibaldi, Vancouver & Richmond

DEMOGRAPHICS:

1. What is your date of birth: \[ \square / \square / \square \] MM/DD/YYYY

2. What is your gender:
   - [ ] Male
   - [ ] Female

3. What is your current Marital status:
   - [ ] never married
   - [ ] married
   - [ ] separated but not divorced
   - [ ] divorced
   - [ ] widowed/widower

4. Does another person regularly share your bed/sleep with you?:
   - [ ] Yes
   - [ ] No

5. What is the highest level of education you have completed?
   - [ ] Less than high school diploma
   - [ ] High school diploma
   - [ ] College or University degree
   - [ ] Masters or above

SLEEP HABITS:

6. After you have turned out the lights to fall asleep, how long does it usually take you to fall asleep?
   - [ ] 0-15 minutes
   - [ ] 16-30 minutes
   - [ ] 31-60 minutes
   - [ ] 61-120 minutes
   - [ ] more than 2 hours

7. On average, how many hours of sleep do you get per night on weekdays?
   - [ ] \( \leq 5 \) hours
   - [ ] 6 hours
   - [ ] 7 hours
   - [ ] 8 hours
   - [ ] 9 hours
   - [ ] 10+ hours

   On weekends?
   - [ ] \( \leq 5 \) hours
   - [ ] 6 hours
   - [ ] 7 hours
   - [ ] 8 hours
   - [ ] 9 hours
   - [ ] 10+ hours

8. On average, how many hours do you spend napping during the daytime on weekdays?
   - [ ] None or nearly none
   - [ ] 1-2 hours
   - [ ] 3-4 hours
   - [ ] 5-6 hours
   - [ ] 7+ hours

   On weekends?
   - [ ] None or nearly none
   - [ ] 1-2 hours
   - [ ] 3-4 hours
   - [ ] 5-6 hours
   - [ ] 7+ hours
9. Over the last 24 months, which of the following, if any, did you have to do to keep from falling asleep at work? Choose all that apply.

☐ I don't have any problems staying awake at work
☐ chew gum or candy
☐ eat
☐ smoke
☐ chew tobacco
☐ drink coffee
☐ drink other caffeinated beverages
☐ listen to radio/music
☐ moves around/keep physically active
☐ other (please describe) [ ]

SLEEP-RELATED SYMPTOMS:

10. Do you have difficulty falling or staying asleep?

☐ never
☐ rarely (less than one night per week)
☐ sometimes (1-2 nights/week)
☐ frequently (3-4 nights/week)
☐ almost always (5-7 nights/week)

11. On average, how many days/night during the last month have you snored or been told you snored?

☐ never
☐ rarely (less than once per week)
☐ sometimes (1-2/week)
☐ frequently (3-4/week)
☐ almost always (5-7/week)
☐ not sure

12. When falling asleep, how often do you have "restless legs" (a feeling of crawling, aching, or inability to keep legs still)?

☐ never
☐ rarely (less than one night per week)
☐ sometimes (1-2 nights/week)
☐ frequently (3-4 nights/week)
☐ almost always (5-7 nights/week)

13. In the last 24 months, have you found that you awaken and briefly cannot move your entire body (i.e., felt paralyzed as opposed to feeling very tired)?

☐ Yes
☐ No

14. In the last 24 months, have you seen or heard things that don’t exist upon waking up or falling asleep?

☐ Yes
☐ No

15. In the last 24 months, have you had sudden muscular weakness (e.g., buckling of your knees) associated with emotion (e.g., while laughing or after hearing a joke)?

☐ Yes
☐ No
DAYTIME ACTIVITY

16. In the last 30 days, how likely are you to doze off or fall asleep in the following situations (in contrast to feeling just tired)? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you.

<table>
<thead>
<tr>
<th>Activity</th>
<th>High Chance</th>
<th>Moderate Chance</th>
<th>Slight Chance</th>
<th>No Chance</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Sitting and reading</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>b. Watching TV</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>c. Sitting inactive in a public place (e.g. theater, Church)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>d. As a passenger in a car for an hour without a break</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>e. Lying down to rest in the afternoon when circumstances permit</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>f. Sitting and talking to someone</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>g. Sitting quietly after lunch without alcohol</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>h. In a car while stopped for a few minutes in traffic</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

HEALTH HABITS:

17. Do you currently smoke cigarettes?
☐ Yes ➝ If Yes, How many per day? ☐ 1-4 ☐ 5-14 ☐ 15-24 ☐ 25-34 ☐ 35-44 ☐ 45+
☐ No

18. Please fill in your average use of the following beverages during the past year.

<table>
<thead>
<tr>
<th>Beverage</th>
<th>never or less than once per month</th>
<th>1-3 per month</th>
<th>1 per week</th>
<th>2-4 per week</th>
<th>5-6 per week</th>
<th>1 per day</th>
<th>2-3 per day</th>
<th>4-5 per day</th>
<th>6-10 per day</th>
<th>11-20 per day</th>
<th>more than 20 per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Cola or other carbonated beverage with caffeine, e.g. Coke, Pepsi, Mountain Dew (consider the serving size as 1 glass, bottle, or can)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>b. Tea or iced tea with caffeine (1 cup or glass), not herbal teas</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>c. Coffee with caffeine (1 cup)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>d. Beer (1gall, bottle, or can)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>e. Wine (4 oz glass)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>f. Liquor, e.g. vodka, gin, etc. (1 drink or shot)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
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</tr>
</tbody>
</table>
OCCUPATIONAL:

19. Which of the following best describes your current occupational status?

☐ employee
☐ self-employed
☐ disabled
☐ retired
☐ unemployed but looking for work
☐ unpaid work at home
☐ full-time student

20. In total over the last 24 months, approximately how many months did you work?  

21. On average, over the last 24 months, during weeks that you worked, approximately how many hours did you work per week?  

22. What was your predominant occupation over the last 24 months (describe)?

23. Please also mark the ONE description that best characterized your occupation over the last 24 months:

**Primary Resources:**

☐ Agriculture  ☐ Forestry
☐ Fishing  ☐ Oil and Gas or Mineral Resources

**Manufacturing:**

☐ Food and Beverage Products  ☐ Petroleum, Coal, Rubber, Plastic, and Chemical Products
☐ Metal and Non-Metallic Mineral Products  ☐ Wood and Paper Products
☐ Other Products:

**Construction:**

☐ General construction  ☐ Road Construction or Maintenance
☐ Heavy Construction (e.g. bridges, overpasses)

**Transportation and Warehousing:**

☐ Warehousing  ☐ Transportation and Related Services

**Trade:**

☐ Retail  ☐ Wholesale

☐ Working for the Federal Government

☐ Military Service

☐ Public Administration excluding working for the federal government  
(e.g. law enforcement, working for the local government)

**Service Sector:**

☐ Accommodation, Food, and Leisure Services  ☐ Healthcare and Social Assistance
☐ Business Services (e.g. accounting, law office)  ☐ Professional, Scientific, and Technical Services
☐ Education  ☐ Utilities
☐ Other Services

**Other:**

☐ Please describe:________________________________________
24. As a percentage, how many of each of the following shifts did you work over the last 24 months (total should equal 100%)

<table>
<thead>
<tr>
<th>Shift Type</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day shift (e.g. 8am -5 pm)</td>
<td></td>
</tr>
<tr>
<td>Evening shift (e.g. 3 pm-11pm)</td>
<td></td>
</tr>
<tr>
<td>Nightshift (e.g. 11 pm to 7 am)</td>
<td></td>
</tr>
</tbody>
</table>

25. Given the hours you work, how much on average would someone employed in a field like yours earn in a year?

- $0-20,000
- 21,000 -40,000
- 41,000-60,000
- 61,000-80,000
- 81,000-100,000
- >100,000

26. Over the last 24 months, did you suffer an occupational injury?

- Yes
- No

If YES, please answer the following cluster of questions.
If NO, please skip to question 27.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. If so, how many injuries did you have?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>b. How many of these, if any, were reported to the Workers Compensation Board?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>c. In how many of these, if any, do you think sleepiness or fatigue in you contributed?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>d. In how many of these, if any, was an emergency room visit required?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>e. Please take a moment and tell us a few words about each incident</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

152
27. Over the last 24 months, did you cause an occupational injury to someone else (do not include accidents described in the above question)?

☐ Yes  ☐ No

If YES, please answer the following cluster of questions.
If NO, please skip to question 28.

a. How many injuries did you cause?

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4+  ☐

b. How many of these, if any, were reported to the Workers Compensation Board?

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4+  ☐

c. In how many of these, if any, do you think sleepiness or fatigue in you contributed?

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4+  ☐

d. In how many of these, if any, was an emergency room visit required?

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4+  ☐

e. Please take a moment and tell us a few words about each incident

MEDICAL HISTORY:

28. What is your height?  ☐ Feet  ☐ inches OR  ☐ cm

29. What is your current weight (to the nearest pound)?  ☐ Pounds OR  ☐ kg

30. When was your weight last measured? Within the last ☐ Months

31. What is your neck circumference (e.g. shirt size) to the nearest inch?

☐ ≤ 11  ☐ 12  ☐ 13  ☐ 14  ☐ 15  ☐ 16  ☐ 17  ☐ 18  ☐ 19  ☐ 20  ☐ 21  ☐ 22  ☐ 22+  ☐ not sure

32. Check the sentence that best describes your situation

☐ I have remained about the same weight (i.e. within 5 pounds) over the last 24 months.

☐ I have gained 5 or more pounds in the last 24 months

Approximately how many pounds did you gain?

☐ 5-10  ☐ 11-15  ☐ 16-20  ☐ 21-25  ☐ >25

☐ I have lost 5 or more pounds in the last 24 months

Approximately how many pounds did you lose?

☐ 5-10  ☐ 11-15  ☐ 16-20  ☐ 21-25  ☐ >25

☐ I am not sure if I have gained or lost weight as I never weigh myself
33. Has a **physician diagnosed** you with any of the following disorders (check all that apply)?

**Psychiatric:**
- ☐ A major mood disorder (e.g. depression)
- ☐ A major anxiety disorder (e.g. panic disorder)

**Sleep Disorders:**
- ☐ Narcolepsy
- ☐ Restless legs syndrome
- ☐ Periodic Limb Movements

**Respiratory:**
- ☐ Asthma
- ☐ Emphysema
- ☐ Chronic Bronchitis

**Cardiovascular:**
- ☐ Hypertension
- ☐ Myocardial infarction (heart attack)
- ☐ Cardiac arrhythmias (irregular heartbeat)
- ☐ Angina
- ☐ Congestive heart failure

**Endocrine:**
- ☐ Diabetes
- ☐ Hypothyroidism (low thyroid level)
- ☐ Polycystic ovarian disease

**Neurology:**
- ☐ Stroke
- ☐ TIA
- ☐ Seizure

34. Have you been previously diagnosed with obstructive sleep apnea by a physician?
- ☐ Yes
- ☐ No

**If YES, please answer the following questions.**
**If NO, please advance to question 35.**

a. Was this diagnosis based on a full sleep study in which you slept in the sleep laboratory overnight?
   - ☐ Yes
   - ☐ No

b. Was this diagnosis based on a home oximetry study (i.e. where you wore a probe on your finger overnight)?
   - ☐ Yes
   - ☐ No

c. If you answered NO to the above two questions, please describe how your physician made the diagnosis
d. What treatment are you currently using for obstructive sleep apnea?
   □ none
   □ Positive airway pressure (e.g. CPAP, BIPAP, smart CPAP)
   □ dental appliance
   □ other (please describe)

35. Have you had surgery specifically to treat your sleep apnea or snoring?
   □ Yes  If YES, please answer the following question:
   □ No  What kind(s) of surgery did you have? (Mark all that apply)
   □ sinus surgery
   □ laser surgery of the palate/throat
   □ somnoplasty (radiofrequency ablation) of the tongue
   □ formal uvulopalatopharyngoplasty (UPPP) under general anesthesia in the operating room
   □ other. Please describe:

Over the last 24 months, on average, how often did you take:

Any type of the following to get to or stay asleep? (i.e. sleeping aids)

36. Prescription medication:
   □ never
   □ very rarely (less than once per month)
   □ rarely (less than one night per week but more than once per month)
   □ sometimes (1-2 nights or days/week)
   □ frequently (3-4 nights or days/week)
   □ almost always (5-7 nights or days/week)
   Name of medication:

37. Non-prescription medication
   □ never
   □ very rarely (less than once per month)
   □ rarely (less than one night per week but more than once per month)
   □ sometimes (1-2 nights or days/week)
   □ frequently (3-4 nights or days/week)
   □ almost always (5-7 nights or days/week)
   Name of medication:
38. Herbal or health food medication
   - never
   - very rarely (less than once per month)
   - rarely (less than one night per week but more than once per month)
   - sometimes (1-2 nights or days/week)
   - frequently (3-4 nights or days/week)
   - almost always (5-7 nights or days/week)
   Name of medication: ______________________________________________________________________

Over the last 24 months, on average how often did you take:

Any type of the following to stay awake? (i.e. stimulants)

39. Prescription medication: (eg. Modafinil, Ritalin)
   - never
   - very rarely (less than once per month)
   - rarely (less than one night per week but more than once per month)
   - sometimes (1-2 nights or days/week)
   - frequently (3-4 nights or days/week)
   - almost always (5-7 nights or days/week)
   Name of medication: ______________________________________________________________________

40. Non-prescription medication (eg. caffeine pills)
   - never
   - very rarely (less than once per month)
   - rarely (less than one night per week but more than once per month)
   - sometimes (1-2 nights or days/week)
   - frequently (3-4 nights or days/week)
   - almost always (5-7 nights or days/week)
   Name of medication: ______________________________________________________________________

Over the last 24 months, on average how often did you take:

Any of the following medications (for any reason)

41. Anti-histamines
   - never
   - very rarely (less than once per month)
   - rarely (less than one night per week but more than once per month)
   - sometimes (1-2 nights or days/week)
   - frequently (3-4 nights or days/week)
   - almost always (5-7 nights or days/week)
42. Antidepressant

☐ never
☐ very rarely (less than once per month)
☐ rarely (less than one night per week but more than once per month)
☐ sometimes (1-2 nights or days/week)
☐ frequently (3-4 nights or days/week)
☐ almost always (5-7 nights or days/week)

43. Other medications: (please list)


Driving Questions:

44. On average over the last 24 months, how many kilometers did you drive per week? [ ] kilometers

45. Over the last 24 months, did you have any motor vehicle accidents or crashes in which you were driving?

☐ Yes ☐ No

If YES, please answer the following cluster of questions.
If NO, please skip to question 47.

a. How many crashes did you have over the last 24 months? 0 1 2 3 4 5+ ☐ ☐ ☐ ☐ ☐ ☐

b. In how many of these, if any, was a police report filed? ☐ ☐ ☐ ☐ ☐ ☐

c. In how many of these, if any, do you think sleepiness or fatigue in you contributed? ☐ ☐ ☐ ☐ ☐ ☐

d. In how many of these, if any, was total property damage greater than $1000? ☐ ☐ ☐ ☐ ☐ ☐

e. In how many of these, if any, were any vehicles involved towed away? ☐ ☐ ☐ ☐ ☐ ☐

f. In how many of these, if any, were emergency room visits required by anyone? ☐ ☐ ☐ ☐ ☐ ☐

g. In how many of these, if any, were fatalities involved? ☐ ☐ ☐ ☐ ☐ ☐

46. Over the last 24 months, did you have any near miss motor vehicle accidents or crashes in which you were driving? (i.e. narrowly avoided property damage or bodily harm)

☐ Yes ☐ No

If so, how many did you have, on average, over this time period?

☐ <1 per month
☐ 1-4 per month
☐ 1-2 times per week
☐ 3-5 times per week
☐ more than 5 times per week but not everyday
☐ everyday or almost everyday
**MOOD QUESTIONS:**

Over the last two weeks, how often have you been bothered by any of the following problems?

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>several days</th>
<th>more than half the days</th>
<th>nearly every day</th>
</tr>
</thead>
<tbody>
<tr>
<td>47. Little interest or pleasure in doing things?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48. Feeling down, depressed, or hopeless?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49. Trouble falling/staying asleep, sleeping too much?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50. Feeling tired or having little energy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51. Poor appetite or overeating?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52. Feeling bad about yourself, or that you are a failure, or have let yourself or your family down?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53. Trouble concentrating on things, such as reading the newspaper or watching TV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54. Moving or speaking so slowly that other people could have noticed. Or the opposite: being so fidgety or restless that you have been moving around more than usual.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55. Thoughts that you would be better off dead or of hurting yourself in some way.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

56. If you have checked off any problem on these questions (47-55), how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

- [ ] Not difficult at all
- [ ] somewhat difficult
- [ ] very difficult
- [ ] extremely difficult

57. **Women Only:** What is your menopausal status:

- [ ] Premenopausal
  - If premenopausal: Are your periods
    - [ ] regular
    - [ ] irregular
  - [ ] Post menopausal (i.e. complete absence of periods)
    - If post menopausal, how long ago did your periods completely stop? [__] Years ago
- [ ] Peri-menopausal
## RESEARCH ANALYSIS REQUISITION

<table>
<thead>
<tr>
<th>STUDY NAME: Genetic and Biochemical Markers in Sleep Apnea</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNEMONIC: GENETICS OF OSA</td>
</tr>
<tr>
<td>RESEARCH #: R170</td>
</tr>
<tr>
<td>STUDY COORDINATOR: Nurit Fox</td>
</tr>
<tr>
<td>TELEPHONE: 67912</td>
</tr>
<tr>
<td>PATIENT NAME:</td>
</tr>
<tr>
<td>Surname         Given         Study Pt. ID</td>
</tr>
<tr>
<td>DAY MONTH YEAR M/F (please circle)</td>
</tr>
<tr>
<td>BIRTH DATE:</td>
</tr>
<tr>
<td>COLLECTION: Date: Time: Collected by,</td>
</tr>
<tr>
<td>CONTAINER: gold top: 1 – 5ml lavender top: 1 – 6ml</td>
</tr>
<tr>
<td>TEST CODE: DSPR5 (phlebotomy and specimen processing for central lab)</td>
</tr>
<tr>
<td>SPECIAL INSTRUCTIONS FOR LABORATORY STAFF – attach aliquot tubes with a small patient label. Accession specimens as follows:</td>
</tr>
<tr>
<td>Gold top centrifuge → serum equally eppendorf tubes &quot;serum&quot; (5) store @-30°C</td>
</tr>
<tr>
<td>Lavender top centrifuge → plasma equally eppendorf tubes &quot;plasma&quot; (5) store @-30°C</td>
</tr>
<tr>
<td>transfer buffy coat (white layer of cells between the plasma and the red cells, ok to include some red cells) store @-30°C</td>
</tr>
<tr>
<td>Tech Initials:</td>
</tr>
</tbody>
</table>
Appendix C

Supplemental table from the paper presented in chapter 4.

<table>
<thead>
<tr>
<th></th>
<th>Correlation Estimate</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 and VCAM-1</td>
<td>0.37</td>
<td>0.29, 0.44</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ICAM-1 and E-selectin</td>
<td>0.43</td>
<td>0.36, 0.50</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>VCAM-1 and E-selectin</td>
<td>0.31</td>
<td>0.23, 0.39</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Table 7.1 – Pearson’s Correlation between Biomarkers of interest
Appendix D

Supplemental material for the paper in chapter 6

The databases used through Population Data BC were Discharge Abstracts (DAD), Cardiac Services of BC (CSBC), Vital Statistics and the Consolidation file. DAD captures administrative, clinical and demographical data from the Ministry of Health on hospital discharges (including deaths, sign-outs and transfers). The ICD-10-CA codes were extracted from DAD. CSBC was used to provide information on PCI and CABG. Vital Statistics collects data on British Columbians birth, adoption, deaths, marriages and divorces. We collected year, month, day of death with underlying cause of death based on ICD-10-CA codes. The Consolidation File includes health insurance information (Ministry of Health Registration and Premium Billing file, Medical Services Plans and postal codes) and it was used to create censoring variables.

All inferences, opinions and conclusions drawn in this paper are those of the authors, and do not reflect the opinions or policies of the Data Stewards(s).

Data extracts were provided by the British Columbia Ministry of Health. These extracts are cited below in compliance with Population Data BC protocols.


### Table 7.2 – CVD Cause of Death Codes

<table>
<thead>
<tr>
<th>ICD10 Code</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>E785</td>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>I219</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>I251</td>
<td>Atherosclerotic Heart Disease</td>
</tr>
<tr>
<td>I259</td>
<td>Chronic Ischemic Heart Disease, Unspecified</td>
</tr>
<tr>
<td>I514</td>
<td>Myocarditis, Unspecified</td>
</tr>
</tbody>
</table>

### Table 7.3 – ICD10/CCI Codes for Endpoints

<table>
<thead>
<tr>
<th>Outcome</th>
<th>ICD10</th>
<th>Diagnosis Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFIB</td>
<td>I48</td>
<td>M</td>
</tr>
<tr>
<td>CHF</td>
<td>I50</td>
<td>M</td>
</tr>
<tr>
<td>MI</td>
<td>I21, I22</td>
<td>Any</td>
</tr>
<tr>
<td>Stroke</td>
<td>I60-164</td>
<td>Any</td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>I200, I249</td>
<td>Any</td>
</tr>
<tr>
<td>VT</td>
<td>I472</td>
<td>Any</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedures</th>
<th>CCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CABG</td>
<td>1IJ76</td>
</tr>
<tr>
<td>PCI</td>
<td>1IJ50, 1IJ57GQ</td>
</tr>
<tr>
<td>Cardioversion</td>
<td>1HZ09</td>
</tr>
<tr>
<td>Defibrillator</td>
<td>1HZ53GRFS, 1HZ53HAFS, 1HZ53LAFS, 1HZ53SYFS</td>
</tr>
<tr>
<td>Pacemaker</td>
<td>1HZ53GRNM, 1HZ53LANM, 1HZ53QANM, 1HZ53GRNK, 1HZ53LANK, 1HZ53QANK, 1HZ53GRNL, 1HZ53LANL, 1HZ53QANL, 1HZ53LAFS, 1HZ53SYFS</td>
</tr>
</tbody>
</table>

Definitions: The events included stroke, myocardial infarction, primary heart failure, acute coronary syndrome, ventricular tachycardia, atrial fibrillation, percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG). Stroke was defined as nontraumatic subarachnoid hemorrhage (I60), nontraumatic intracerebral hemorrhage (I61), cerebral infarction (I63) stroke not specified as hemorrhage or infarction(I64). Transient ischemic attacks (TIA) were not included in the stroke diagnosis. Myocardial infarction was defined as acute myocardial infarction including all transmural sites (I21), and subsequent myocardial infarction (I22). Heart Failure was defined as primary acute decompensated heart failure (I50). Ventricular tachycardia was defined using code ICD-10-CA I47.2. Atrial fibrillation was defined as paroxysmal atrial fibrillation (I48.0). Acute coronary syndrome included unstable angina (I200), unspecified acute ischemic heart disease (I248, I249), angina pectoris with documented spasm (I201), other angina (I208), unspecified angina pectoris (I209), coronary thrombosis not resulting in myocardial infarction (I240), and Dressler’s syndrome (I241). We only included deaths with underlying cardiovascular causes. Death causes included hyperlipidemia (E 785), myocardial infarction (I219), atherosclerotic heart disease (I251), chronic ischemic heart disease (I259), and myocarditis (I514).
### Table 7.4 – Univariate Associations with Cardiovascular Events

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.10 (1.06, 1.14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>1.00 (0.96, 1.06)</td>
<td>0.79</td>
</tr>
<tr>
<td>Sex*</td>
<td>1.78 (0.79, 4.01)</td>
<td>0.15</td>
</tr>
<tr>
<td>AHI</td>
<td>1.01 (0.99, 1.02)</td>
<td>0.10</td>
</tr>
<tr>
<td>Heart Disease*</td>
<td>1.79 (0.86, 3.72)</td>
<td>0.11</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.99 (0.71, 5.51)</td>
<td>0.18</td>
</tr>
<tr>
<td>Smoking**</td>
<td>1.65 (0.60, 4.54)</td>
<td>0.32</td>
</tr>
<tr>
<td>Statin</td>
<td>2.27 (1.08, 4.76)</td>
<td>0.02</td>
</tr>
<tr>
<td>ESS &gt; 11</td>
<td>1.20 (0.59, 2.44)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

**Abbreviations:** AHI: Apnea-Hypopnea Index; BMI: Body mass index; CI: Confidence Intervals; ESS: Epworth Sleepiness Scale; OR: Odds ratio; HR: Hazard Ratio; *Male versus Female; *Heart disease included: Hypertension, Myocardial Infarction; Cardiac Arrhythmias, Angina, and Congestive Heart Failure; **Current smokers versus not currently smoking.

### Table 7.5 – Characteristic by CPAP Adherence

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Not Adherent (n=27)</th>
<th>Adherent (n=107)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.5 ± 10.1</td>
<td>52.8 ± 10.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>17 (63)</td>
<td>81 (76.4)</td>
<td>0.16</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>30.770 ± 5.709</td>
<td>33.189 ± 6.985</td>
<td>0.10</td>
</tr>
<tr>
<td>AHI (events/hr)</td>
<td>13.10 (7.90,25.60)</td>
<td>27.80 (15.60,46.10)</td>
<td>0.02</td>
</tr>
<tr>
<td>AHI Category</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Control</td>
<td>&lt; 6</td>
<td>&lt; 6</td>
<td></td>
</tr>
<tr>
<td>Mild OSA</td>
<td>13 (48.1)</td>
<td>21 (19.6)</td>
<td></td>
</tr>
<tr>
<td>Moderate OSA</td>
<td>7 (25.9)</td>
<td>31 (29)</td>
<td></td>
</tr>
<tr>
<td>Severe OSA</td>
<td>6 (22.2)</td>
<td>50 (46.7)</td>
<td></td>
</tr>
<tr>
<td>AHI Severity</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Non-Severity</td>
<td>21 (77.8)</td>
<td>57 (53.3)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>6 (22.2)</td>
<td>50 (46.7)</td>
<td></td>
</tr>
<tr>
<td>% Time below 90% SaO2</td>
<td>0.141 (0.082,1.624)</td>
<td>1.501 (0.187,7.402)</td>
<td>0.08</td>
</tr>
<tr>
<td>&gt; 5 Minutes below 90% SaO2</td>
<td>7 (25.9)</td>
<td>51 (48.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>ESS</td>
<td>11.0 (6.0,16.0)</td>
<td>9.0 (3.0,14.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>ESS &gt; 11</td>
<td>11 (40.7)</td>
<td>45 (42.1)</td>
<td>0.90</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>7 (25.9)</td>
<td>29 (27.1)</td>
<td>0.90</td>
</tr>
<tr>
<td>Smoker</td>
<td>&lt; 6</td>
<td>12 (11.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Diabetes</td>
<td>&lt; 6</td>
<td>10 (9.3)</td>
<td>0.78</td>
</tr>
<tr>
<td>Ethnicity Group</td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>Caucasian</td>
<td>22 (81.5)</td>
<td>86 (81.1)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>&lt; 6</td>
<td>11 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>&lt; 6</td>
<td>9 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Statin User</td>
<td>&lt; 6</td>
<td>21 (20.6)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td><strong>Unadjusted</strong></td>
<td>0.804 (0.240, 2.695)</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td><strong>Stepwise Adjusted</strong>*</td>
<td>0.871 (0.244, 3.108)</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td><strong>Significance Adjusted</strong></td>
<td>0.774 (0.201, 2.990)</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td><strong>Fully Adjusted</strong>*</td>
<td>0.754 (0.192, 2.970)</td>
<td>0.69</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for Age, Sex, and Smoking Status,
**Adjusted for Age, Sex, Smoking Status, AHI, and BMI
***Adjusted for Age, Sex, Smoking Status, AHI, BMI, Heart Disease, ESS > 11, Diabetes.

Table 7.6 – Unadjusted and Adjusted OR for CPAP on CVD Outcomes