The Impact Of Obstructive Sleep Apnoea On The Mechanisms In The Vessel Wall That Promote Atherosclerosis

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ABSTRACT OF THESIS

Despite use of established therapies, atherosclerotic cardiovascular disease (CVD) morbidity and mortality rates remain unacceptably high, prompting the need to identify additional factors driving residual CVD risk. Obstructive sleep apnoea (OSA) has emerged as a major CVD risk factor, with the majority of deaths in OSA patients being cardiovascular. The studies presented in this thesis investigated relationship between the presence and severity of OSA and the development of atherosclerotic burden in different vascular territories.

A review of the literature was performed, focusing on the prevalence of OSA, its clinical and mechanistic links to atherosclerosis, and results cardiovascular outcome trials of treatment for OSA. This provided a theoretical basis for the studies presented.

A systematic review of high-quality studies catalogued in the Cochrane Library, PubMed, and Embase Library was performed to evaluate the current literature on the impact of the OSA treatment of continuous positive airways pressure (CPAP) therapy on the markers of subclinical atherosclerosis carotid intimal thickening (CIMT) as measured by ultrasound, arterial stiffness, measured by pulse wave velocity (PWV), and endothelial function as measured by flow-mediated dilation (FMD). Treatment with CPAP in patients with OSA has a favourable effect on measures of subclinical atherosclerosis.

The relationship between symptoms suggestive of OSA and global and focal coronary artery disease (CAD) severity was investigated. In the cath lab setting, increased risk of OSA, as measured by a sleep questionnaire validated for use in primary care, did not associate with CAD severity.

Angiogenic function and gene expression of vascular inflammatory and angiogenic markers were measured to investigate the relationship between symptoms suggestive of OSA and coronary artery stenosis severity, angiogenic function, and vascular inflammation *in vitro*. Serum was added to tumour necrosis factor (TNF)-stimulated human umbilical vein

endothelial cells (HUVECs) in culture. Angiogenesis capacity of treated HUVECs was assessed using the Matrigel tubulogenesis assay. Patients at high OSA risk demonstrated differences in angiogenic potential, but not in atherosclerotic disease burden or vascular inflammation.

The relationship between epicardial adipose tissue (EAT), a metabolically active fat depot, and OSA severity with EAT volume, EAT density and body mass index (BMI) were investigated. Participants underwent clinically indicated cardiac computed tomography (CT) and overnight polysomnography (PSG). EAT volume and coronary plaque volume were quantified on coronary computed tomography angiography (CTCA). EAT volume was observed to be associated with OSA severity, independent of BMI.

The impact of OSA on changes in coronary atherosclerotic plaque was examined in short-term and longer-term treatment investigations for CAD as measured by intravascular ultrasound (IVUS). OSA was found to be associated with a greater increase in atheroma volume compared to those without OSA after short-term treatment for an acute coronary syndrome (ACS) event, while patients with OSA had a greater decrease in atheroma volume compared to those without OSA after optimal treatment for CAD.

The studies presented in this thesis demonstrate that the vessel wall is impacted by exposure to OSA. These findings provide a rationale for screening and treating patients for OSA to beneficially impact the progression of atherosclerosis.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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ABSTRACTS AND PRIZES

Abstracts

- **Andrews J**, Shishikura D, Honda S, Nguyen T, Janssan A, DiGiovanni G, Kim S, Keyserling C, Dasseux JL, and Nicholls SJ. *Obstructive Sleep Apnoea Associates with Short-Term Progression of Coronary Atherosclerosis in Patients with Acute Coronary Syndrome: Insights from the CARAT Study.* Presented at the 2017 Annual Scientific Sessions of the Australian Atherosclerosis Society.

- **Andrews J**, Nerlekar N, Gupta V, Mo L, Cameron JD, Seneviratne S, Lin A, Yuvara J, Thomas G, Hamilton GS, Nicholls SJ, Wong DT. *Relationship Between Epicardial Fat Volume and Density with Obstructive Sleep Apnoea and Coronary Plaque Burden.* Presented at the 2019 Annual Scientific Sessions of American Heart Association.

Prizes

- Ahrens Heart Health Researcher Award Recipient – 2017

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LIST OF ABBREVIATIONS

AASM	American Academy of Sleep Medicine
AMI	Acute Myocardial Infarction
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ACE	Angiotensin-Converting Enzyme
ACS	Acute Coronary Syndrome
AF	Atrial Fibrillation
AHI	Apnoea-Hypopnoea Index
ApoA-I	Apolipoprotein A I
ApoB	Apolipoprotein B
ARB	Angiotensin Receptor Blocker
AS	Arterial Stiffness
BMI	Body Mass Index
BP	Blood Pressure
BQ	Berlin Questionnaire
CAC	Coronary Artery Calcium
CAD	Coronary Artery Disease
CARAT	CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial
cDNA	Complimentary Deoxyribonucleic Acid
CI	Confidence Interval
CIMT	Carotid Intima-Medial Thickness
cm	Centimetre
CPAP	Continuous Positive Airways Pressure
CRP	C-Reactive Protein
CT	Computed Tomography
CTA	Tomographic Angiogram
CTCA	Coronary Computed Tomography Angiography
CT-LeSc	Computed Tomography Leaman Score
CVD	Cardiovascular Disease
EAT	Epicardial Adipose Tissue
EBM-2	Endothelium Based Media-2

ECG	Electrocardiogram
EEG	Electroencephalogram
EEM	External Elastic Membrane
EMG	Electromyogram
EOG	Electrooculogram
EPC	Endothelial Progenitor Cells
ESS	Epworth Sleepiness Scale
FBS	Foetal Bovine Serum
FMD	Flow-Mediated Dilatation
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
HbA_{1c}	Glycated Hemoglobin
HDL	High Density Lipoprotein
HIF	Hypoxia-Inducible Factor
hs-CRP	High Sensitivity C-reactive Protein
HU	Hounsfield Unit
HUVECs	Human Umbilical Vein Endothelial Cells
ICAM-1	Intercellular Adhesion Molecule 1
IH	Intermittent Hypoxia
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-8	Interleukin 8
IMT	Intima-Medial Thickness
IQR	Interquartile Range
IVUS	Intravascular Ultrasound
kg	Kilograms
Kg/m^2	Kilograms Per Metre Squared
LAD	Left Anterior Artery
LCx	Left Circumflex
LDL	Low Density Lipoprotein
Lp (a)	Lipoprotein (a)
LV	Left Ventricle
MACCE	Major Adverse Cardiac or Cerebrovascular Events

MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial Infarction
MCP-1	Monocyte Chemoattractant Protein-1
mg/L	milligrams per litre
mm	millimetres
mm Hg	millimetres of mercury
mmol/L	millimoles per litre
MMPs	Matrix Metalloproteinases
m/s	Millimetres Per Second
NHMRC	National Health and Medical Research Council
NIH	National Institutes of Health
NO	Nitric Oxide
NFκB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
ODI	Oxygen Desaturation Index
OR	Odds Ratio
OSA	Obstructive Sleep Apnoea
PAV	Percent Atheroma Volume
PBS	Phosphate Buffered Saline
PCI	Percutaneous Coronary Intervention
PCR	Polymerase Chain Reaction
PCSK9	Proprotein Convertase Subtilisin Kexin Type 9
PDA	Posterior Descending Artery
PR	Prevalence Ratio
PRISMA	Preferred Reporting Items For Systematic Reviews And Meta-Analyses
PSG	Polysomnography
PWV	Pulse Wave Velocity
RCA	Right Coronary Artery
RDI	Respiratory Disturbance Index
REM	Rapid Eye Movement
RICCADSA	Randomised Intervention With CPAP in Coronary Artery Disease and Sleep Apnoea
RNA	Ribonucleic Acid
RR	Relative Risk

RT-PCR	Reverse Transcription Polymerase Chain Reaction
ROS	Reactive Oxygen Species
SAVE	Sleep Apnoea Cardiovascular Endpoints
SD	Standard Deviation
SDB	Sleep Disordered Breathing
SEM	Standard Error of the Mean
SHHS	Sleep Heart Health Study
STEMI	ST Elevation Myocardial Infarction
T2D	Type 2 Diabetes Mellitus
TAV	Total Atheroma Volume
TC	Total Cholesterol
TCFA	Thin-Cap Fibroatheroma
TG	Triglycerides
TNF-α	Tumor Necrosis Factor α
VCAM-1	Vascular Cellular Adhesion Molecule 1
VEGF	Vascular Endothelial Growth Factor
VH	Virtual Histology
WSCS	Wisconsin Sleep Cohort Study

“Breathing dreams like air”

F. Scott Fitzgerald, The Great Gatsby

Chapter 1:

INTRODUCTION

1.1 Introduction

Coronary artery disease (CAD) continues to be a leading cause of morbidity and mortality in the developed world (1). Adults aged greater than or equal to 40 years have an estimated lifetime prevalence of CAD of 49% in men and 32% in women (2). The prevalence of CAD is projected to increase by approximately 18% by 2030 (1). CAD occurs secondary to atherosclerotic alterations of coronary arteries. Traditional modifiable risk factors for atherosclerosis are dyslipidaemia, hypertension, diabetes, obesity, and smoking. The coexistence of multiple risk factors disproportionately increases the risk of developing CAD. Despite the widespread use of primary and secondary prevention therapies tested in large clinical trials targeted against modifiable CAD risk factors, such as dyslipidaemia (3) and blood pressure (BP) (4), cardiovascular related events continue to occur (5). This prompts the need to expand treatment strategies to target other risk factors, and obstructive sleep apnoea (OSA) is one such risk factor.

OSA is a common condition that affects at least 7% of the general population, and 30-50% of CVD patients (6). OSA risk factors include obesity, increased age, smoking, alcohol consumption, and anomalies of the upper airway (7). Symptoms consist of morning fatigue with or without headache, increased daytime sleepiness, and frequent snoring (6). Those with sleep-disordered breathing complaints are referred for overnight polysomnography (PSG) for diagnosis. Apnoea is defined as complete cessation of oronasal airflow for at least 10 seconds. Hypopnea requires a drop of greater than or equal to 30% of oronasal airflow from baseline associated with greater than or equal to 4% decrease in oxyhemoglobin saturation; a drop of greater than or equal to 50% of oronasal airflow from baseline and greater than or equal to 3% decrease of oxyhemoglobin saturation; a reduction in airflow as above along with an associated electroencephalographic arousal. The frequency of apnoeas and hypopnoeas per hour of sleep

are captured as the apnoea–hypopnea index (AHI), the measure used to define and stratify the severity of OSA. The range of OSA severity categories have been defined as mild (AHI= 5/hour- 14.9/hour), moderate (AHI= 15/hour- 29.9/hour), and severe AHI= >30/hour) (8). Oxygen desaturation index (ODI) is the hourly average number of desaturation episodes, which are defined as at least 4% decrease in saturation from the average saturation in the preceding 120 seconds, and lasting 10 seconds.

The frequent nocturnal apnoeic episodes experienced by OSA patients over the years cause repetitive periods of hypoxaemia, sleep deprivation, intrathoracic pressure changes and sympathetic activation (Figure 1.1). These stressors have the potential to lead to the development hypertension, arrhythmias, stroke and atherosclerosis (9). Yet, there is still a lack of evidence that supports the true causal relationship between OSA and cardiovascular events, mainly due to cofounders such as obesity.

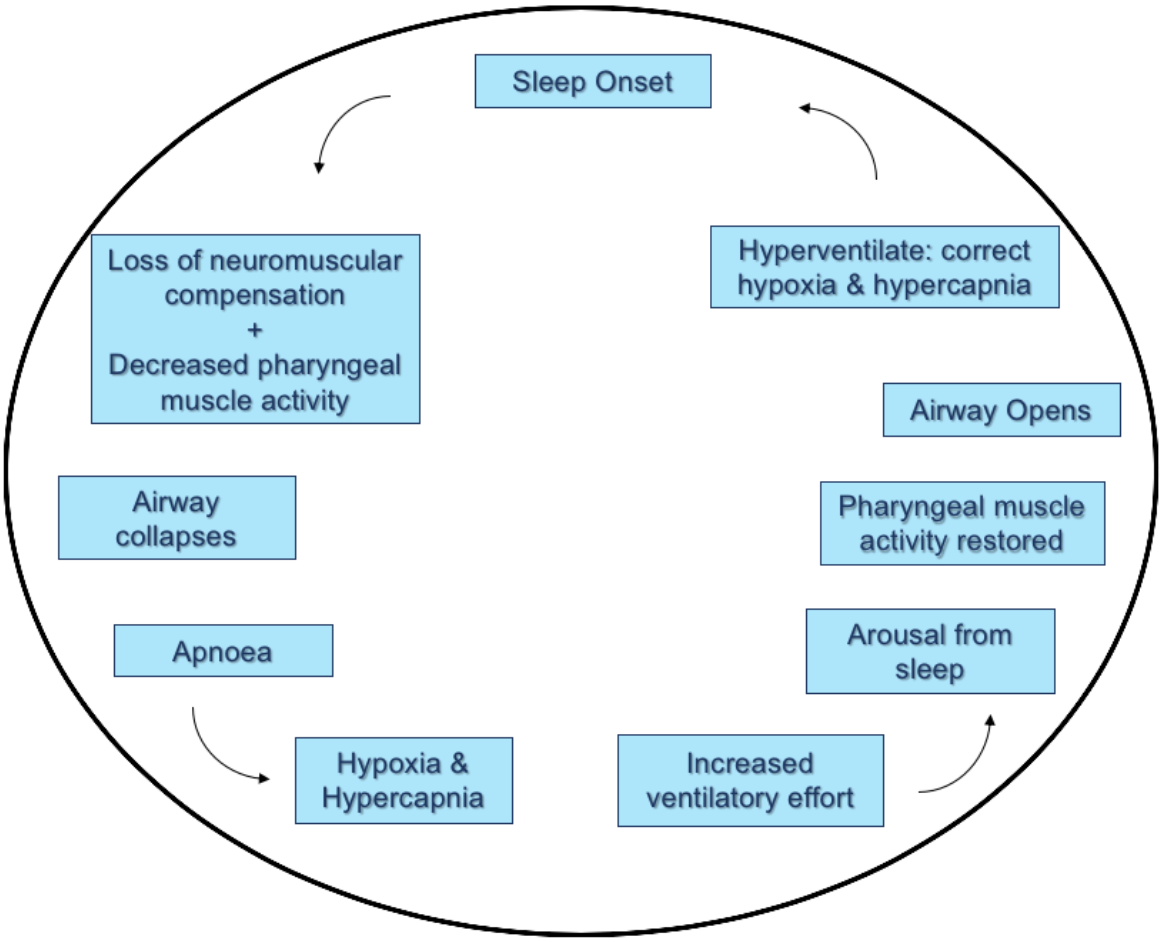


Figure 1.1: Pathophysiology of OSA adapted from Morsey N. E. et al (2019); *Rev Environ Health* (10)

1.2 Pathophysiology of coronary artery disease

Atherosclerosis, the principle cause of CAD, is a chronic disease of the arterial wall that develops over the course of decades. Susceptible sites of the coronary arteries accumulate cholesterol-rich lipids that oxidise and modify provoking an inflammatory response. This response eventually leads to thrombosis or stenosis limiting blood flow, ultimately causing myocardial infarction (MI) (Figure 1.2). What was once thought of as a cholesterol storage disease, atherogenesis is a complex interaction of risk factors including the cells located within the wall of arteries and the blood that they exchange.

Fatty streaks, the first lesions to form, begin to develop during adolescence (11). Low density lipoprotein (LDL) particles leave the blood stream, enter the intima of the arteries, and accumulate over time. The early modifications to the vessel wall originate at the arterial branch points, where adaptive intimal thickening occurs in response to hemodynamic stresses. Inflammation commences as a result of monocytes, lymphocytes, mast cells, and neutrophils accumulating in the arterial wall after both endothelial cells become activated and secrete adhesion molecules, and smooth muscle cells secrete chemokines. Smooth muscle cells located in the intima secrete into the extracellular matrix proteoglycans, collagen, and elastic fibres. Monocytes transform into macrophages upon entry, and take up lipids as multiple small inclusions, and develop into foam cells (Figure 1.2).

Early fibroatheromas form when numerous macrophage foam cells, other activated inflammatory cells, and the other naturally occurring cells accumulate within the artery wall (12). Extracellular proteoglycans, secreted by smooth muscle cells, bind lipids and progressively increase their lipid-binding capacity. Factors then promote the death of macrophages and smooth muscle cells, and the necrotic debris that is created as a result incites further inflammation. These enlarging pools form lipid-rich necrotic cores that dominate the central part of the intima.

As plaques develop, they may grow into adjacent media and adventitia, and distort them. The calibre of the arterial wall may enlarge, and remodel until the plaque occupies approximately 40% of the artery area. Any further plaque enlargement reduces the arterial lumen and may become hemodynamically significant. New vaso vasorum invade and occupy the diseased intima. These vessels of endothelium are fragile due to the lack of support from pericytes, and may leak causing haemorrhage within the arterial wall. These intramural haemorrhages lead to an increase of fibrous tissue.

A thin-cap fibroatheroma (TCFA) develops when proteolytic enzyme activity dissolves fibrous tissue, thinning and weakening sites of the fibrous cap making these lesions vulnerable to rupture (Figure 1.2). A ruptured thin cap exposes the thrombogenic interior arterial wall and produces a thrombus that extends into the arterial lumen causing potentially life-threatening thrombosis. TCFAs and ruptured plaques are distributed in a highly focal pattern and are usually located in the proximal segments of the major coronary arteries (13).

Thin fibrous cap ruptures are often clinically silent, and heal by forming fibrous tissue matrices of cells, collagen fibres, and extracellular space but may rupture again with thrombus formation (12, 14). The rupture, thrombosis, and healing cycle may recur as many as four times at a single site in the arterial wall, resulting in multiple layers of healed tissue. Calcium deposits begin to form within the vessel wall as lesions progress, initially as small aggregates, and later as large nodules. Plaques may rupture into the lumen and expose the nodules, which become sites for thrombosis. Erosion of endothelium underlain by some of the changes described previously or with no underlying histologic abnormality may occur, resulting in thrombosis. The increasing mass of some plaques alone may become sufficient to form significant stenosis that may cause ischemia simply through flow restriction.

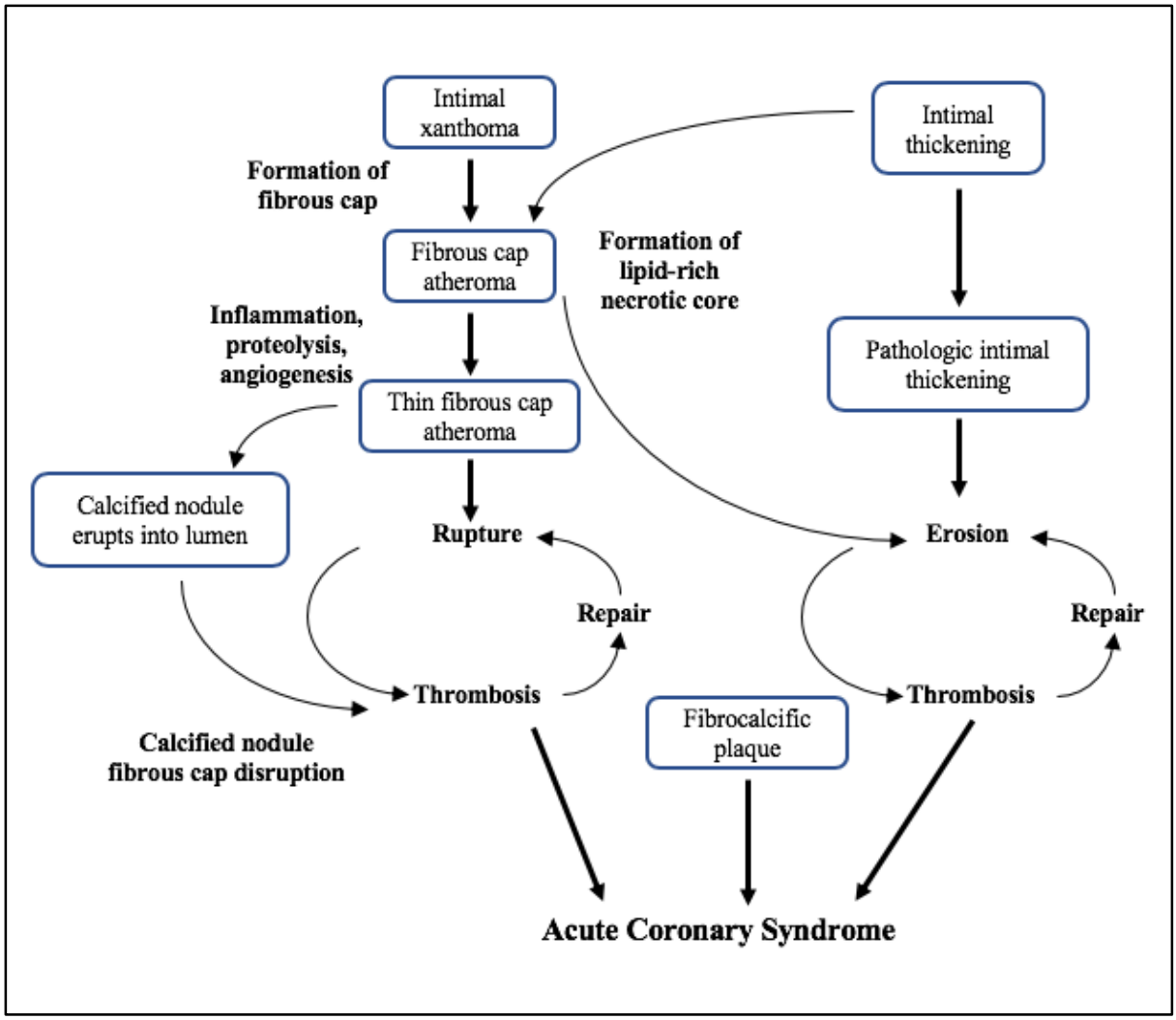


Figure 1.2: Atherosclerosis pathology flow chart adapted from Virmani R, et al (2000); ***Arterioscler Thromb Vasc Biol.*** (14)

1.3 Pathophysiology of obstructive sleep apnoea

The various causes or "phenotypic traits" that contribute to the pathogenesis of OSA are comprised of both anatomical and non-anatomical components (15, 16). The anatomical features are narrow, crowded, and collapsible upper airways. A major driver for pharyngeal narrowing is obesity. Pharyngeal airspace is reduced by increased fat deposition in the soft tissues, tongue, and lateral pharyngeal walls in obese patients with OSA (17, 18). Central adiposity may also contribute to pharyngeal collapsibility via reductions in lung volume and caudal traction mechanisms (19). Pharyngeal airspace may also be affected by the size and shape of craniofacial structures (20). Non-anatomical components include low respiratory arousal threshold, ineffective or sleep induced reductions in pharyngeal dilator muscle activity and unstable ventilatory control (15, 16).

Sleep affects the respiratory system and breathing control in numerous ways. During normal patterns of sleep, there is a decrease in sympathetic nervous activity, blood pressure, and heart rate, while cardiac vagal tone and metabolic rate increase compared to wakefulness (21). However, this pattern is interrupted in individuals with OSA when repeated episodes of intermittent hypoxia (IH) and hypercapnia occur during respiratory efforts to overcome the pharyngeal obstacle. During obstructive apnoea, negative intrathoracic pressure is generated by the inspiratory efforts against the occluded pharynx that leads to increased left ventricular transmural afterload, which increases myocardial oxygen consumption, and impedes stroke volume (22). An arousal from sleep will terminate apnoea, and these arousals allow the upper airway to open and normal ventilation resumes (Figure 1.1). As a result, patients with OSA experience sleep fragmentation, and over time display permanent oscillations in their hemodynamic parameters during the night. IH, intrapleural pressure changes, and arousals, the major contributors to the acute hemodynamic modifications of OSA, all trigger mechanisms

that may result in sympathetic activation and endothelial dysfunction, leading to an increase in arterial stiffness, arterial hypertension, and the development of atherosclerosis.

1.4 Prevalence coronary artery disease in obstructive sleep apnoea cohorts

Studies in the early and mid- 1990s began to describe an increased prevalence of sleep disordered breathing (SDB) in subjects with symptomatic CAD. One study that calculated an apnoea index after overnight PSG in 101 male participants hospitalised with acute MI and 53 asymptomatic controls found that the top quartile was an independent predictor of MI patients (23). An investigation of 142 men that were being evaluated for suspected CAD and compared to 50 age-matched volunteers found that those with CAD had a significantly higher occurrence of SDB as defined by ODI greater than or equal to 5 or AHI greater than or equal to 10 (24). Another study compared 62 individuals that required intensive care admission with unstable angina or MI with age, sex, and BMI matched controls also found a higher prevalence of OSA (odds ratio [OR] 3.0, 95% confidence interval [CI] 1.2-7.5) as defined by respiratory disturbance index (RDI) greater than or equal to 10 in the patients with CAD (25).

More recently, clinic and hospital-based studies have indicated that patients with OSA are more likely to go on to develop CAD. Inadequately treated OSA was associated with an increased likelihood of subsequent symptomatic CAD (OR 4.9; 95% CI 1.8–13.6) after 7 years of follow-up in a study consisting of 182 males free of co-morbidities at baseline that attended a sleep service (26). Further investigation into this cohort that included those with hypertension and diabetes mellitus, found a significantly greater incidence of CAD (relative risk [RR] 4.60; 95% CI 1.83–11.6) in those with OSA compared to those without (27). A large cohort of 1,651 individuals at a single centre compared long term cardiovascular outcomes between patients with a range of SDB and controls found untreated severe OSA was associated with a markedly increased likelihood of both fatal (OR 2.87, 95%CI 1.17–7.51) and non-fatal (OR 3.17, 95%CI 1.12–7.51) CVD (28).

The associations between OSA and CAD have been found to be more modest in community-based studies. The Sleep Heart Health Study (SHHS) enrolled 4,422 participants and found severe OSA predicted an increased risk of developing symptomatic CAD (OR 1.10 [95% CI 1.00, 1.21]), but only in men aged 70 or younger, after a median follow-up of 8.7 years (29). Further analysis of this cohort showed severe OSA to be an overall independent predictor of death, and CAD related death in particular (OR 1.46; 95% CI: 1.14–1.86) (30). The association was strongest in men less than 70 years (OR 2.09; 95% CI: 1.31–3.33). In the Wisconsin Sleep Cohort, those with untreated severe SDB (AHI greater than or equal to 30) had a greater than two-fold risk of incident CAD compared to those with no SDB (19). This finding is a stronger association than that of the SHHS and may be due to the younger population studied.

1.5 Links between obstructive sleep apnoea and atherosclerosis

Studies investigating coronary vasculature have indicated that OSA patients carry a greater subclinical CAD burden than those without OSA. These studies have employed various methods of imaging the arterial wall which has allowed for characterisation of functional and anatomical changes within the vascular tree, and has been increasingly integrated into clinical research programs to evaluate the impact of disease states, such as OSA, on the vasculature.

1.5.1 Pulse wave velocity

Oxygen and nutrients are carried through arteries, and the elastic properties of arteries cause the attenuation of cardiac pulsatility. Over time, the natural ageing process leads to stiffening of the large arteries, in particular the aorta as a result of breaks in elastin fibres, accumulation of collagen, fibrosis, inflammation, medial smooth muscle necrosis, calcifications, and diffusion of macromolecules within the arterial wall. This process is accelerated in the presence of CVD (31). Arterial stiffness (AS) causes a premature return of reflected waves in late systole, increasing central pulse pressure, thus systolic BP. Myocardial

oxygen demand is increased as systolic BP increases the load on the left ventricle. Inflammation, oxidative stress and sympathetic activity, all present in OSA, affect endothelial function (32, 33). Increased oxidative stress, as a result of increased production of reactive oxygen species, is associated with IH (34, 35). Excessive oxidative stress disturbs cellular function, accelerates endothelial dysfunction and increases inflammation, leading to metabolic and cardiovascular complications of OSA (34). Nitric oxide synthase activity is reduced by oxidative stress, and leads to decreased production of nitric oxide, increasing arterial stiffness (36).

Pulse wave velocity (PWV) analysis generates a well-validated assessment of AS. AS does not reflect a stage of the atherosclerotic disease continuum, rather arteriosclerosis. However, PWV is reported to be an important marker for CV events (37), and measuring the vessel PWV has become the gold standard as the non-invasive measure for AS. Increased PWV correlated with measures of OSA severity in several studies. In a study of 42 individuals with and without OSA and no other classical risk factors, both minimum saturation of oxygen and the percentage of time spent with oxygen saturation below 90% significantly correlated with the PWV. Another exploration into the relationship between PWV severity of OSA that included 112 men found significantly higher PWV in those with severe OSA compared to those with no, mild, or moderate OSA (38). Other studies that include other risk factors such as hypertension (39) and metabolic syndrome (40, 41) also report that measures of OSA severity associate with an increase in PWV. An investigation of the influence of OSA and hypertension on PWV in 208 individuals found that there was a significant association with OSA and high PWV, with significance remaining even in normotensive individuals (39). OSA in the presence of metabolic syndrome resulted in significantly higher PWV compared to those is OSA and no metabolic syndrome (40). However, one study of 130 newly diagnosed OSA patients did not show significant differences in carotid-femoral PWV between groups based upon severity of

OSA (42). The fact that the cohort investigated included only patients without clinically diagnosed CVD, and who were also younger and had only mild OSA are possible explanations for the lack of a relationship between AS and OSA.

1.5.2 Flow-mediated dilatation

The endothelium modulates vascular tone by synthesising and releasing several endothelium-derived relaxing factors, such as vasodilator prostaglandins, nitric oxide, and endothelium-dependent hyperpolarisation factors, and endothelium-derived contracting factors (43, 44). A decrease in production or action of these relaxing mediators leads to endothelial dysfunction. Endothelial functions are essential to ensure proper maintenance of vascular homeostasis, and endothelial dysfunction is the basis of CVD associated with pathological conditions toward vasoconstriction, thrombosis, and inflammation (43). Impaired endothelium-dependent vascular relaxation represents a physiologic change of the artery wall preceding plaque formation. IH, intra-pleural pressure swings, and recurrent arousals, the three consequences of OSA, are considered to be the causes of impaired endothelial function (45-47). IH is thought to be the most important factor promoting the production of reactive oxygen species, thus increasing oxidative stress and decreasing nitric oxide (NO) synthetase activity. This leads to NO attenuation, and impaired endothelial function (34, 47).

FMD has been increasingly used as a validated measure of endothelium-dependent vascular relaxation. Studies have consistently demonstrated the inverse relationship found between FMD and OSA severity. A study consisting of 79 minimally symptomatic OSA patients demonstrated that FMD was impaired compared to controls (48). Investigations of FMD impairment have been shown to relate to OSA severity (38, 49). One investigation comprised of 112 men, reported a significant inverse relationship between FMD and AHI, and men with severe OSA had significantly lower FMD compared to controls (38). Another study conducted in cohort of 129 Japanese individuals found significantly impaired FMD in those

with moderate to severe OSA compared to those with mild OSA (49). However, age has been reported to influence the strength of the relationship between FMD and OSA. FMD was reported to associate with OSA status in patients who were younger than 50 (50) and 60 (51) years of age. The association of OSA status with FMD was independent of other risk factors including BMI. However, increased age weakened the association in this cohort (50). These results indicate that testing endothelial dysfunction in younger patients with OSA might be useful to investigate early atherosclerotic changes.

1.5.3 Carotid intima-medial thickness

The dynamic development of atherosclerosis is associated with remodelling of the arterial wall, with the early stages of development lead to arterial wall thickening. The carotid artery wall contains 3 distinct separate layers, the intima, media, and adventitia. Carotid intima-medial thickness (CIMT) may be measured with ultrasound, and an increase in atherosclerotic prone areas is used as an indicator of intimal thickening. CIMT represents an established surrogate marker for atherosclerotic disease as well as an independent predictor of MI and stroke (52). The activation of the sympathetic system as a result of the repetitive apnoeic events accompanied by recurrent arousal, present in the setting of OSA, result in a large transient surge of blood pressure (53). Sharp hemodynamic alterations increase shear stress and impair the vascular structure (54), and promote an inflammatory response. IH activates the nuclear factor kappa B pathway and increases pro-inflammatory cytokines and circulating soluble adhesion molecules (55-57). These intracellular adhesion molecules and cytokines attract leukocytes and monocytes to the endothelial layer, thus promoting atherosclerosis. The vascular consequences of inflammation and endothelial injury induced by the vibrations of snoring are transmitted through soft tissues surrounding the pharynx to the carotid artery wall (58).

The majority of studies have demonstrated a direct relationship between CIMT and OSA severity. Several studies show a correlation between obstruction, as demonstrated by the AHI or the RDI. Investigations of patients with severe OSA have increased CIMT compared to those with less severe symptoms (59, 60), and increased CIMT correlates with increased AHI (60). CIMT has correlated with AHI, and was greater in OSA subjects free of co-morbidities (57). Studies that have investigated the relationship with OSA and CIMT include atherosclerotic plaque formation (61, 62). CIMT correlated with OSA, but the association to the development of atherosclerotic plaque formation was not as strong.

The positive correlation between the degree of CIMT and OSA severity may be a result of the obstructive episodes and hypoxemia present in OSA. The repetitive desaturation re-oxygenation that occurs in OSA generates reactive oxygen radicals that enhance lipid perioxidation leading to vascular endothelium damage (63). The majority of studies show an association between obstruction, as measured by AHI or RDI, and hypoxemia as measured mean nocturnal oxygen less than 92%, or mean nadir oxygen saturation. Yet, hypoxemia has been shown to correlate with CIMT even after adjusting for AHI, suggesting that hypoxemia may be associated with atherosclerosis independent of obstruction (64). The study that did not result in an association between OSA and intima-medial thickness (IMT) was a large cross-sectional subset study of the Sleep Heart Study, that involved more than 1,600 individuals from a community setting, not patients referred to a sleep clinic for investigation. These participants were mainly middle-aged Caucasians with only mild to moderate SDB that were classified as low-risk for CVD (65). This finding highlights that the association between CIMT and OSA is more evident in the more severe cases of OSA.

1.5.4 Coronary artery calcium

Vascular calcification occurs within the medial and intimal layers of the vessel wall. The non-occlusive development of medial calcification occurs along the elastic lamina as the

elastic fibres mineralise and appear to be directly pathogenic by decreasing vascular compliance. The associated rise in high blood pressure is a result of the stiffening of the vessel wall. Intimal calcification forms at the site of an atherosclerotic lesion. The intimal layer of the vessel composed of endothelial cells along with a small amount of subendothelial connective tissue thickens and becomes considerably inflamed where cholesterol is deposited, and cellular necrosis occurs. As atherosclerosis progresses, calcium develops within these lesions. Evidence now supports the active process by which calcium develops within the vessel wall stimulated by inflammatory pathways. The alterations in blood pressure (53), hemodynamics, and increased sheer stress structure as a result of the repetitive apnoeic events and recurrent arousals, present in OSA promote an inflammatory response (54). The activation of the nuclear factor kappa B pathway is promoted by IH and increases pro-inflammatory cytokines and circulating soluble adhesion molecules (55-57). These intracellular adhesion molecules and cytokines attract leukocytes and monocytes to the endothelial layer, further promoting atherosclerotic plaque development, including calcium.

Coronary artery calcium (CAC) assessment has been integrated into CVD risk prediction algorithms as a way of reclassifying individuals previously deemed intermediate risk, and studies utilising CAC scoring have found direct associations with OSA severity. A large subgroup consisting of 1,604 individuals from the Heinz Nixdorf Recall study, a German community based observational study, who underwent home cardiorespiratory sleep studies, found OSA to be prevalent and independently associated with CAC amount in women (OR 0.23, 95% CI 0.04–0.41) and in men less than or equal to 65 years of age (OR 0.25, 95% CI −0.001–0.50) (66). While the Multi-Ethnic Study of Atherosclerosis (MESA), also a large community based study investigated sleep disturbance in 1,465 individuals by home PSG and actigraphy, found the prevalence of CAC to be independently predicted by an AHI greater than or equal to 30, as well as related to sleep fragmentation (prevalence ratio (PR) 1.14; 95% CI

1.02-1.27) and reduced proportions of N3, stage 3, sleep (PR 0.77; 95% CI 0.64-0.92) (67). However, other studies reported that CAC did not independently correlate with OSA after adjusting for the cofounders BMI (multivariate OR 1.16; 95% CI 0.49-2.74)
(68, 69).

1.5.5 Coronary stenosis and plaque burden

A few imaging studies have investigated the relationship between severity of CAD and sleep symptoms. Coronary angiography was conducted on 59 individuals in a Japanese cohort following myocardial infarction or for investigation of angina pectoris, and evaluated the relationship of nocturnal hypoxaemia with coronary atherosclerosis, as measured by the Gensini score. Prevalence of sleep disordered breathing was found in 72.9% of the cohort, and ODI correlated significantly with increased Gensini scores. ODI was also the most significant independent determinant of the Gensini score among the coronary risk factors tested in a multiple regression analysis (70). A retrospective analysis of 81 participants that had undergone multidetector-row helical CT scanning, found that the frequency of noncalcified/mixed plaques was higher in patients with OSA (63%) than in those without OSA (16%). OSA was also associated with more severe stenosis and a greater number of vessels were involved (71). An investigation of patients with stable CAD found the mean total atheroma volume (TAV) as measured by IVUS in the 19 participants to be larger in those with OSA than those without (72). Yet another study of 93 CAD patients found TAV to be significantly greater in those with moderate to severe OSA compared to those with no to mild OSA. However, there were no significant differences in the prevalence of thin cap fibroatheroma in the culprit lesions between those with moderate to severe OSA and no to mild OSA (73).

Many of the studies investigating the relationships between atherosclerosis and subclinical CVD with OSA were cross sectional, therefore causality cannot be determined. A

large number of these studies only consisted of consecutive patients with OSA, but no control group. The lack of prospective data also prohibited investigation into the relationship between atherosclerosis and markers of subclinical CVD with morbidity and mortality. Statin use was also not adjusted for in some of the studies highlighted, and doing so would help illustrate the role of preventive strategies in this population and whether presence of OSA should be an indication for lower treatment thresholds. In future, the roles that systemic inflammation and endothelial dysfunction play in the interactions between OSA and clinical and subclinical atherosclerosis need to be clarified by assessing markers of endothelial dysfunction and systemic inflammation in conjunction with the appropriate imaging modality. Finally, would individuals with OSA derive a greater benefit by screening for subclinical CVD?

1.6 Mechanisms linking obstructive sleep apnoea and atherosclerosis

The mechanisms of OSA associated with contributing to the atherogenesis process are complex as OSA is a heterogeneous disease characterised by multiple mechanisms and complications such as intermittent hypoxemia, hypercapnia, negative intrathoracic pressure increase, and arousal (see Figure 1.3). IH caused by OSA is considered to contribute not only to the cascade of events leading to cardiovascular disease onset but also its progression. Hypercapnia, changes in intrathoracic pressure, and arousals also contribute to cardiovascular disease progression (74-76).

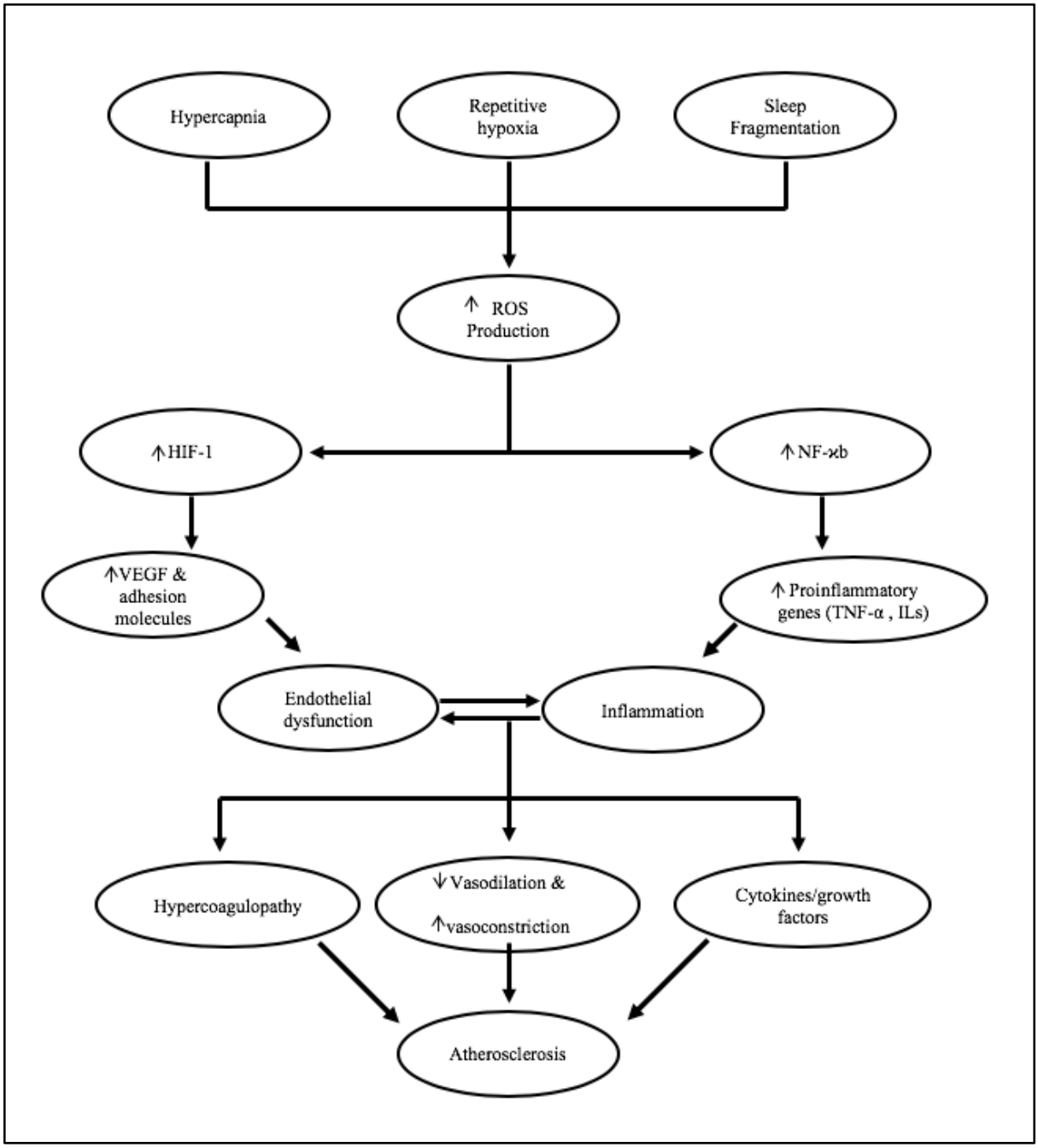


Figure 1.3: Mechanisms in sleep apnoea induced atherosclerosis development

Adapted from Golbidi et al; (2012) *Lung* (77)

HIF-1 = Hypoxia-Inducible Factor-1; ILs = Interleukins; NFκB = Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; ROS = Reactive Oxygen Species; TNF- α = Tumour Necrosis Factor α; VEGF = Vascular Endothelial Growth Factor

1.6.1 Endothelial dysfunction

The vascular endothelium, a thin, single-layer of cells, forms the interface between circulating blood in the lumen and the vessel wall, and regulates vascular tone in response to physical and chemical stimuli. Impaired endothelial function has been shown to be an important early clinical marker for predicting atherosclerosis and future cardiovascular events. Endothelial dysfunction is not only prevalent in OSA (78), via repetitive hypoxia and arousals from sleep, but has also been reported to be associated significantly with the severity of OSA in the absence of coronary artery stenosis (79). Characterised by decreased bioavailability of endothelium-derived NO, endothelial dysfunction is triggered by activation of the sympathetic nervous system (80), systemic inflammation, (81, 82) and oxidative stress (83).

Early studies investigating acetylcholine-induced vasodilatation found a blunted endothelium-dependent response in those with OSA (84, 85). IH has been shown to regulate vasoactive molecules and alter insulin-signaling in vascular endothelial cells (86). A small study of 17 participants reported that obese individuals with no known CVD, and newly diagnosed with OSA had a significantly lower vasodilation in response to acetylcholine compared to the obese control subjects (85). These results have been confirmed by further studies. One such study investigated the effects of nocturnal hypoxaemia on vascular function in 46 OSA patients with and without frequent nocturnal desaturations, and found that endothelial dependent and endothelial independent vasodilatation is impaired in those with OSA and frequent nocturnal desaturations (87). Another study investigating endothelial dysfunction in 40 individuals with and without OSA as measured by FMD found that those with OSA had significantly lower FMD compared to those without OSA, and that AHI was a significant determinant of FMD (88). A study in a large cohort of over 1,000 older adults, 68 years and older, reported that measures of endothelial function were impaired in those with

sleep apnoea. However, the association was weakened in those greater than or equal to 80 years and after adjustment for BMI (89).

1.6.2 Hypertension

A significant number of patients with hypertension develop atherosclerosis which is strongly associated with endothelial dysfunction, a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and portends a potential CV risk (90). There is also a strong relationship between OSA and hypertension that is bidirectional, with a prevalence of OSA in 30-50% of hypertensive individuals (91). Acute increases in blood pressure may also cause upper airway muscle inhibition, along with volume overload and its displacement to the upper body during sleep can lead to pharyngeal oedema (92-94). Repeated periods of desaturation and re-oxygenation of oxyhemoglobin that occur with OSA may account for blood pressure increases (95-97). Renin–angiotensin–aldosterone system activation, triggered by intermittent hypoxemia, is another pathway (98). Obstructive respiratory events typically end with an arousal from sleep which stimulates the sympathetic nervous system, which also results in an increase in blood pressure (95). In an analysis of 709 individual from the Wisconsin Sleep Cohort Study (WSCS) investigating the relationship between SDB and hypertension in a population-based study, there was a 3.2-fold increase in the odds ratio of developing hypertension in those with moderate to severe sleep apnoea after 4 years of follow-up (99). Another large cohort observational study of 1,889 individuals referred to sleep clinics with no history of hypertension, found a 37.3% increase in incident hypertension after a median follow-up of 12.2 years. Those in the study with untreated OSA resulted in an increased risk for developing hypertension (declined CPAP therapy (hazard ratio (HR) 1.96; 95% CI, 1.44–2.66), non-adherent to CPAP therapy (HR 1.78; 95% CI, 1.23–2.58)). Participants with the lowest risk of developing hypertension were those most adherent to treatment with CPAP therapy (HR 0.71; 95% CI 0.53–0.94) (100). However, not all studies

have found such a strong relationship after adjusting for sex, age, and somnolence (101). These results indicate that the risk of incident hypertension in those with SDB is strongest in in those less than 60 years of age (OR 2.24; 95% CI 1.10, 4.54) (102), increased daytime somnolence (OR 2.83; 95% CI 1.33-6.04) (103), and male (OR for hypertension increased across AHI tertiles from 1.0 to 2.1; 95% CI 0.9-4.5 and 1.0 to 3.7; 95% CI 1.7-8.2) (104).

1.6.3 Metabolic abnormalities

There has been an accumulation of evidence showing an association of OSA with glucose and lipid dysfunction, however the mechanisms are complex. OSA may trigger pathological mediating pathways such as sympathetic activation, neurohumoral changes, glucose homoeostasis disruption, inflammation and oxidative stress, through chronic IH (105). According to previous studies, the prevalence of OSA is increased fourfold in patients with obesity. Obesity plays a major part in the development of the metabolic syndrome, which consists of insulin resistance, diabetes or impaired glucose tolerance, hypertension, and dyslipidaemia (106).

1.6.3.1 Glucose metabolism

Type 2 diabetes mellitus (T2D) is a major risk factor affecting CAD, such that 75% of patients with diabetes die as a consequence of CVD, including CAD (107). CAD manifests as a complex disease characterised by small, diffuse, calcified, multivessel disease in those with T2D (108, 109). OSA is associated with increased prevalence of T2D (110) and higher glycated hemoglobin (HbA_{1C})-levels in those without T2D (111) Pancreatic β-cell dysfunction and progressive insulin resistance have been attributed to the inflammatory, sympathoadrenal, and humoral responses generated by the recurrent cycles of hypoxia, reoxygenation, and arousal with sleep fragmentation that characterise OSA (112-115). Numerous studies have investigated the impact of OSA on insulin resistance. AHI and minimum oxygen saturation associate with insulin resistance, independent of BMI (116). The most striking metabolic

consequences of OSA have been found during REM (rapid eye movement) sleep (113, 117). In the WSCS, participants with an AHI greater than or equal to 15 had a 2.3-fold increase (95% CI 1.28-4.11) in prevalence of T2D relative to those with an AHI less than 5, after adjustment for age, sex, and body habitus (110). Another analysis from the SHHS found severity of OSA associated with insulin resistance in 2656 individuals after adjustment for obesity (118). Yet, sleep apnoea was found to be significantly and independently related to incident diabetes (OR 1.78, 95% CI 1.39-2.28) in a cohort of over 47,000 individuals (119). While an historic cohort of almost 9,000 participants reported initial OSA severity predicted subsequent risk for incident diabetes (HR 1.13, 95% CI 1.06-1.20) after 67 months of follow-up (120). Still, risk of diabetes was associated with severe OSA (HR 1.71, 95% CI 1.08–2.71), not mild or moderate OSA, in a cohort of almost 1,500 participants independent of BMI after a median follow-up of 13 years (121). However, not all investigations find an association between OSA and diabetes. One study found glucose to be independently associated with OSA (OR 5.88, 95% CI 1.961–17.63), even after adjustment for BMI, but not insulin resistance (OR 0.54, 95% CI 0.544–1.642) (122). Another study did not find any significant differences in fasting blood sugar (OR 1.21, 95% CI 0.28–5.17) or insulin resistance (OR 2.55, 95% CI 0.69–13.21) in those with OSA compared to the non-apnoeic obese controls; furthermore, obesity was found to be a major determining factor for metabolic abnormalities (BMI (OR) 2.25, 95% CI 1.88–2.71; waist circumference (OR) 1.91, 95% CI 1.52–2.39; waist to hip ratio (OR) 1.91, 95% CI 1.52–2.39) (123).

1.6.3.2 Lipid metabolism

Lipid metabolism deregulation constitutes the pathogenic basis for the development of atherosclerosis and drives a high incidence of cardiovascular-related morbidity and mortality. Some data suggest that dyslipidaemia may be associated with OSA, due to alterations in fundamental biochemical processes, such as IH (124). Several lines of evidence show that OSA

and IH increase lipid delivery from the adipose tissue to the liver through an up-regulation of the sterol regulatory element-binding protein-1 and stearoyl-CoA desaturase-1, during the fasting state, increasing the synthesis of cholesterol esters and triglycerides (125, 126). Oxidative stress can also generate dysfunctional oxidised lipids and reduce the capacity of high-density lipoproteins (HDL) to prevent LDL oxidation (36, 127, 128).

Several studies show there are increase in incidence of dyslipidaemia in subjects with OSA including total cholesterol, LDL, HDL, and triglyceride, few do not, as the main objective was often not the lipid profile (129).One cross-sectional found that while scores for metabolic syndrome significantly increased with OSA severity, there were no differences in lipids in relation to OSA severity (130). The SHHS found that in 4,491 individuals with no CVD at the time of sleep study that serum total cholesterol and triglycerides increased, and HDL-cholesterol levels decreased as OSA severity increased as quantified by RDI. This relationship was stronger in those under 65 years of age (131). However, another analysis that included 886 individuals confirmed that HDL cholesterol and triglyceride levels were also related to the severity of OSA in those over 65 (132). While OSA could worsen the lipid metabolism in non-obese patients, the increase in triglyceride, cholesterol and LDL levels as well as the reduction in HDL levels is more intense in patients with OSA who have a BMI greater than 30 (133). Recently, two studies highlighted the relevance of obesity in the relationship between OSA and lipid abnormalities (133, 134). One investigation found the total cholesterol/HDL-C ratio and TG/HDL-C ratio to be significantly associated with AHI in those with severe OSA after adjustment for BMI (134). The second study found those with OSA had significantly higher levels of triglycerides and total cholesterol, as well as a statistically significant lower level of HDL compared to those without OSA when BMI was less than or equal to 30 kg/m^2. However, these differences were attenuated when BMI was greater than 30 kg/m^2 (133).

While evidence indicates that there appears to be higher degree of diabetes and dyslipidaemia among patients with OSA, the role of OSA in their causality is unclear, as BMI and other cardiovascular confounders attenuate findings in many studies. However, there are positive associations that are once again driven by the level of severity of OSA.

1.6.4 Inflammation

Accumulating evidence supports a central role for inflammation in all phases of atherosclerosis (135, 136). During initial stages of disease, systemic inflammation is initiated in the vascular endothelium in response to such stressors as injury, lipid peroxidation, and infection (137-139) Monocytes infiltrate the endothelium, differentiate into macrophages, ingest oxidised LDL, and become large foam cells, thus promoting atherosclerotic plaque development. Macrophages and foam cells secrete matrix metalloproteinases (MMPs), which assist in the degradation of the extracellular matrix, weakening the fibrous cap, destabilising the plaque that may eventually rupture. Different types of inflammatory reactions are involved in the initiation and progress of atherosclerosis. Inflammatory cells, mainly monocytes, adhere to the endothelium and release inflammatory mediators. The possible mediators may include adhesion molecules such as selectins and ICAM-1 (intercellular adhesion molecule-1), cytokines such as tumour necrosis factor α (TNF-α) and interleukin 1 (IL-1), or chemokines such as monocyte chemoattractant protein-1 (MCP-1) and interleukin 8 (IL-8). OSA is associated with a wide range of pathophysiological features, IH is a key feature in the cardiovascular pathophysiology of the disorder due to the associated intermittent re-oxygenation. Given the close relationship between vascular inflammation and atherosclerosis, circulating biomarkers, such as c-reactive protein (CRP), interleukin 6 (IL-6), or TNF-α, have been investigated for their ability to predict CVD, and have now been included in risk assessments for individuals with OSA.

1.6.4.1 C-reactive protein

CRP, a nonspecific marker of inflammation, is an acute-phase protein and is synthesised in the liver in response to macrophage release of IL-6. CRP is detected at local sites of inflammation or injury. However, the levels of CRP produced in response to vascular inflammation are very low in general. High-sensitivity CRP (hs-CRP) assay methods have been developed to detect small changes in CRP concentrations. Recent studies suggest that CRP is an important risk factor in atherosclerosis and CAD (140-142). CRP plasma levels have been found to be elevated in patients with OSA (143). Several studies have reported that OSA is a potential driver of elevated CRP levels (143-147). In a small study of 42 participants, newly diagnosed patients with OSA had significantly higher plasma CRP levels compared to the age and BMI matched controls, with an independent association between level of CRP and OSA severity (143). A simlar study found elevated CRP levels in those with OSA compared to obese controls, and the main influences were severity of OSA and BMI (144). Another investigation found serum hs-CRP levels to be significantly higher in those with OSA compared to controls (AHI less than 5), and elevated hs-CRP levels were associated with AH independent of BMI (147). Large population studies have speculated that the elevated levels may be due to the presence of traditional CVD risk factors, obesity in particular (148, 149). Further investigations have suggested that obesity rather than OSA is the better predictor of CRP (150-152). The WSCS also failed to detect an association between CRP and OSA independent of BMI after adjustment (153).

1.6.4.2 Interleukin 6

IL-6, a proinflammatory cytokine, has been implicated in the pathogenesis of atherosclerosis (154), and increased levels have been shown to be predictive of CVD risk (155). Raised IL-6 levels are often found to correlate with CRP levels, as it is one of the key regulators of CRP synthesis by the liver (156). A wide variety of cells in the body can release IL-6 and it

has been shown that adipose tissue is responsible for a significant proportion of circulating IL-6 (157). Previous studies have reported that levels of IL-6 are increased in those with OSA compared to controls (144, 158, 159). However, these studies were done in small sample sizes, and the controls were not adequately matched. BMI was found to be the main influence on the elevated levels of IL-6. While a study done in a cohort free of other comorbidities found no difference in IL-6 levels between those with and without OSA (160).

1.6.4.3 Interleukin 8

IL-8 is a multifunctional chemokine that causes neutrophils to leave the bloodstream and travel to atherosclerotic lesions, enhancing oxidative stress (161, 162). Increased risk of developing CVD has been shown in apparently healthy individuals when IL-8 plasma levels are elevated (163). Hypoxia has been reported to induce expression of IL-8 indicating that oxygen desaturation could lead to the upregulation of IL-8 expression (164). Significantly higher levels of IL-8 have been found in individuals with OSA when compared to controls (165).

1.6.4.4 Tumor Necrosis Factor *α*

TNF-α, a pro-inflammatory cytokine, promotes atherosclerosis development by inducing cellular adhesion molecule expression, which mediates leucocyte adhesion to the vascular endothelium (166). A gene polymorphism has been identified to associate with increased TNF-α production and has been reported to be more common in OSA (167). Case-controlled studies investigating the relationship between OSA and TNF-α have found circulating TNF-α levels to be elevated in those with OSA when compared to controls, independent of obesity (158, 168, 169). A prospective study of almost 100 males found TNF-α levels to be higher in those with OSA compared to those without. TNF-α levels were also independently associated with ODI, daytime sleepiness, and cholesterol (168).

Overall, there is a body of evidence suggesting that those with OSA have higher levels of inflammatory markers compared to those without. However, many of the studies investigating the relationship between OSA and inflammation have been cross-sectional, therefore the temporal relationship is unclear. It is also difficult to draw conclusions when there has been so much heterogeneity across the different populations studied. Many of the participants of the study groups were selected based on body weight, BMI, gender, and OSA severity, and may not represent a general population of those with OSA.

1.6.5 Platelet aggregation and blood coagulability

Platelets, the second most numerous population of blood cells, are required to maintain haemostasis and repair of the endothelium, yet play a key role in ACS (acute coronary syndrome) development. When in haemostatic conditions, platelets come in close contact with the endothelium as they are carried by the flow of blood, but do not adhere. In response to an injury to the endothelium, bacterial infection, or alteration to normal blood flow, platelets rapidly decelerate, roll on the injured endothelium, and firmly adhere. Platelets that adhere to the vessel wall at sites of endothelial-cell activation contribute to the development of chronic atherosclerotic lesions, and when these lesions rupture, they trigger the acute onset of arterial thrombosis. Once activated, platelets contribute to atherosclerotic plaque progression by releasing adhesive ligands, such as P-Selectin, that mediate the endothelium-platelet interactions (170). The increases in sympathetic activity present in the OSA setting are thought to be the main driver promoting persistent platelet activity. Repetitive surges of sympathetic neural activity overlap with recurrent arousals from sleep along with increases in concentrations of vasoconstrictive peptide and circulating catecholamines, which directly activate platelets (171). Hypoxia, another key feature of OSA, damages the lining of the endothelium. The circulating platelets that come into contact with the damaged endothelium become activated (172). Platelets have been reported to be excessively activated in patients

with OSA (173). However, obesity has been considered to be the factor responsibly for increased platelet activity (174). Yet, studies investigating the relationship between OSA and platelet activity that have controlled for age, sex, and BMI have found haemoglobin deoxygenation to be the correlating variable to hyperactivity of platelets (175).

1.7 Obstructive sleep apnoea and cardiovascular events

The OSA mediated changes to hemodynamic parameters that have been described previously such as IH, increased sympathetic activity, arousals from sleep result in acute surges in heart rate and blood pressure (176). Oxidative stress promotion which induces endothelial dysfunction, systemic inflammation, and hypercoagulability all lead to the high-risk proatherogenic state that predisposes acute ischemic events. Chronic IH may lead to myocardial ischemia by the activation of hypoxia-inducible factor (HIF)-1α (177). These abnormalities appear more frequently at night, and therefore, may explain the increase in nocturnal CV events (178) including sudden cardiac death (179).

Previous studies have reported more severe CAD in patients with OSA compared to those without. A study investigating tissue perfusion found systolic retrograde flow was higher those with OSA compared to those without (180). OSA has been associated with elevated peak plasma troponin concentration, greater presence of 3 vessel disease, and longer stay in coronary care unit after PCI (percutaneous coronary intervention) (181). OSA may also inhibit the recovery of left ventricular function in patients with acute myocardial infarction (AMI) as patients without OSA reported greater improvement in left ventricular (LV) function 3 weeks post PCI compared to those with OSA (182). Greater AHI has also been associated with lager infarct size, reduced myocardial salvage, and lower ventricular ejection fraction on CMR 3 months post PCI (183). The presence of SDB in patients with AMI has also been found to impact enlargement of the right heart as right atrial diastolic area increased more in patients with SDB than those without 12 weeks after AMI (184).

While studies have shown that OSA is associated with more severe CAD, there is also evidence to support a relationship of poor long-term outcomes in patients with OSA compared to those without OSA. One study found those with severe OSA had a significantly higher incidence of major adverse events compared patients with none to moderate OSA 18 months after AMI (185). Another study found the presence of SDB was a significant predictor of major adverse cardiac or cerebrovascular events (MACCE) (HR 2.28, 95% CI 1.06–4.92), however there were no significant differences in recurrent MI and mortality in patients with and without SDB (186). Yet, a trend towards an increased risk of hospital admissions for heart failure (HR 2.50, 95% CI 0.71-8.77) was observed in SDB patients within a follow-up period of 68 months (187). Studies have also reported an association with OSA and an increase in relative risk of MACCE (188-191). Outcomes from a cohort of 1,311 patients reported the crude incidence of MACCE was higher in those with OSA compared to those without OSA, and OSA was also found to be a predictor of MACCE (OR 1.57, 95% CI 1.10-2.24) (192).

Despite the body of evidence highlighting the negative influence of OSA on cardiovascular outcomes, interest in the cardioprotective effects of OSA has begun to grow in recent years. Ischaemic preconditioning may occur in the myocardium in individuals with OSA as a result of repeated exposure to IH, leading to the upregulation of adaptive pathways which may ultimately facilitate myocardial survival during prolonged acute tissue hypoxia (193). Investigations have shown patients with OSA are more likely have developed a collateral coronary circulation than those without OSA (194). The potential of collateral development to provide cardioprotective effects is further supported by the finding that patients with acute MI and SDB have higher levels of circulating endothelial progenitor cells, angiogenic T cells, and vascular endothelial growth factor in monocytes compared with those without SDB (195). Peak cardiac troponin levels during presentations with ACS have been reported to be lower, an indication of smaller infarct size, in patients with significant SDB than those without (196,

197). However, there is no unequivocal evidence to support the concept that OSA alone, with no additional ischemic stimulus caused by a chronic occlusion of a coronary artery promote the development of collaterals protecting MI patients during rupture of an arteriosclerotic plaque and acute ischemia.

1.8 Obstructive sleep apnoea treatment and coronary artery disease

CPAP continues to be the gold standard for OSA treatment which has been shown to improve daytime sleepiness and quality of life in patients with OSA (198). CPAP treatment is delivered via a nasal or oronasal mask, and acts as a pneumatic splint that increases the pharyngeal cross-sectional area and prevents collapse during sleep. Clinical trials utilising CPAP therapy have reported reductions in blood pressure (199, 200), improved endothelial function (201), and increased insulin sensitivity (202). Previous studies have also shown that the circulating inflammatory markers that are elevated in those with OSA have been reduced by CPAP therapy, such as TNF-α (169), IL-8 (168), and CAM (203). However, less definitive results have been reported when exploring of the impact CPAP has on CRP levels (144, 152). Yet, CPAP has also significantly improved platelet response to therapy in patients with severe OSA (204).

Small single centre observational clinical studies reported encouraging results early on in the benefits of CPAP therapy to patients with CAD (205, 206). The trend in CAD benefit continued with larger studies. A study that included over 1600 men, with endpoints of fatal and non-fatal cardiovascular events, showed treatment with CPAP significantly reduced cardiovascular risk in patients with severe OSAH after 10 years of follow-up (28). While another that included 449 participants found that treatment CPAP therapy was associated with a cardiovascular risk reduction of 64%, and an independent predictor of events (HR 0.36, 95% CI 0.21-0.62) after a median follow-up period of 72 months (207). These promising results led

investigators to perform randomised controlled trials to determine the effectiveness of CPAP therapy on reducing cardiovascular events rates.

Treatment with CPAP compared to usual care in a multicentre study conducted in Spain that included 723 participants with OSA and no prior cardiovascular disease showed no difference in composite cardiovascular end points over a median of 4 years of follow-up (incidence density ratio 0.83, 95% CI 0.63-1.1) (208). A single-centre study, RICCADSA (Randomized Intervention With CPAP in Coronary Artery Disease and Sleep Apnea) trial, which involved 224 participants with OSA and CAD who had just undergone revascularisation, showed no significant difference in the composite endpoint of repeat revascularisation, MI, stroke, or cardiovascular death between CPAP and untreated OSA patients after a median follow-up of 57 months (HR 0.80, 95% CI 0.46-1.41) (209). Most recently, after a median follow-up of 3.7 years, CPAP treatment did not result in a lower rate of the composite cardiovascular events (HR 1.10, 95% CI 0.91-1.32) in the 2717 participants included in the SAVE (Sleep Apnea Cardiovascular Endpoints) study, conducted in those with coronary or cerebrovascular disease and moderate-to-severe OSA (210).

The disappointing results from these studies have raised many questions as to why greater benefit was not seen in those treated with CPAP compared to usual care. The RICCADSA and SAVE studies reported an average AHI of 29 events per hour, which is considered to be moderate OSA, while the Spanish cohort study recruited non-sleepy OSA patients. Would there have been a greater benefit to treatment with CPAP if the study participants had more severe OSA at baseline? These studies also reported mean CPAP usage of less than 4 hours, leaving a significant degree of OSA untreated. The adjusted analysis from the Spanish cohort and RICCADSA studies reported better outcomes among patients with greater than or equal to 4 hours per night of treatment with CPAP than the patients who either did not receive treatment with CPAP or used CPAP less than 4 hours per night, there was no

significant difference in the number of primary end-point events between the CPAP adherent (CPAP greater than or equal to 4 hours per night) group compared to the usual-care group in the SAVE study. How much CPAP usage is required to see a clinically meaningful benefit, and would sleepy OSA patients have different outcomes compared to those that are non-sleepy? The RICCADSA and SAVE studies were also secondary prevention trials. Was the establish CAD in these cohorts no longer modifiable? The Spanish cohort study was a primary prevention trial and there was still no benefit with CPAP treatment. Does evaluating the atherosclerosis driven events in these studies provide a cohort with substantial and progressive atherosclerosis that would demonstrate enough slowing of progression to translate into potential CVD benefit with CPAP treatment? It is clear that randomised controlled trials with carefully phenotyped cohorts are required to answer the question of whether treating OSA is important in reducing CV risk.

1.9 Hypothesis and aims of research study

CVD remains the primary cause of death worldwide, despite the advances in atherosclerosis prevention and treatment. The use of conventional CVD therapies continues to be suboptimal. However, when used as directed, many events still occur. Accordingly, there is a need to more clearly define the factors driving disease progression and understand how targeting these factors reduces CVD risk to develop more effective, individualised approaches to risk prediction, disease prevention and intervention.

A reasonable body of evidence exists suggesting that OSA is associated with an increased burden of atherosclerosis in patients that present with symptomatic CAD, sleep laboratory cohorts, and possibly community based study populations. However, no studies have been able to systematically investigate the clinical and mechanistic links between OSA and the development of atherosclerosis.

Imaging advances enable characterisation of factors implicated in the pathogenesis of atherosclerosis. This body of work was undertaken to to evaluate the impact of OSA on the development of atherosclerosis, and to examine the relationship between OSA and its severity using a range of imaging based measurements of atherosclerosis to gain insights into the multiple factors and mechanisms in the vessel wall with measures of plaque burden and composition, as well as perivascular measures of inflammation that underscore CV risk.

1.10 Outline of thesis

Chapter 1: Introduction

Chapter 2: The impact of CPAP on measures of subclinical atherosclerosis in patients with obstructive sleep apnoea- A systematic review

Chapter 3: The relationship between symptoms suggestive of obstructive sleep apnoea and severity of coronary artery stenosis

Chapter 4: The relationship between inflammatory and angiogenic factors with symptoms suggestive of obstructive sleep apnoea and severity of coronary artery stenosis

Chapter 5: The relationship between epicardial fat volume and density with obstructive sleep apnoea and coronary plaque burden

Chapter 6: The impact of obstructive sleep apnoea on short term changes in coronary atherosclerotic plaque in patients with acute coronary syndrome

Chapter 7: The impact of obstructive sleep apnoea on changes in coronary plaque volume

Chapter 8: Discussion

Chapter 2:

THE IMPACT OF CPAP ON MEASURES OF SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA - A SYSTEMATIC REVIEW

ABSTRACT

Background: OSA is a chronic and prevalent disease characterised by repetitive episodes of complete or partial airway obstruction. The repetitive periods of hypoxaemia, intrathoracic pressure changes and sympathetic activation may contribute to the progression of atherosclerotic disease. It remains unclear whether treatment with CPAP alters the natural history of disease development.

Methods: High quality evidence from human clinical trials of the effects of CPAP therapy of markers of subclinical atherosclerosis was obtained by including randomised trials and observational studies from the Cochrane Library, PubMed, and Embase with an NHMRC Evidence Level of I, II, or III. Search dates were from database inception until August 2019, with independent record screening by two authors. Studies where CPAP treatment was used as the intervention were included, and its duration was at least 4 weeks. The heterogeneity of the available studies required qualitative rather than quantitative assessments of effect sizes as measured from each modality.

Results: 125 results were obtained with 32 studies eligible for inclusion, comprised of 4 randomised controlled clinical trials, 3 randomised sham-controlled clinical trials, 1 randomised crossover clinical trial, and 24 prospective observational studies. It was concluded that treatment with CPAP therapy improves markers of subclinical atherosclerosis. The favourable effects of CPAP therapy were observed in each of the markers of subclinical atherosclerosis as measured by CIMT, PWV, and FMD.

Conclusions: This systematic review of the literature found that improvements in the early stages of atherosclerosis were observed with treatment with CPAP in patients with OSA. This finding provides insight into the promotion of early interventional strategies to alter the natural history of the development of atherosclerosis.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

2.1 Introduction

2.1.1 Introduction and rationale for systematic review

OSA is a chronic disease, characterised by repetitive episodes of complete or partial airway obstruction, with symptoms of snoring, apnoea and daytime somnolence. The repetitive periods of hypoxaemia, sleep deprivation, intrathoracic pressure changes and sympathetic activation experienced by patients with moderate to severe OSA lead to an increased risk of CVD and CV death (29, 211, 212). Investigations of early development of atherosclerotic burden have utilised various methods of imaging the arterial wall to allow for the characterisation of functional and anatomical changes within the vascular tree. The combined thickness of the innermost 2 layers of the carotid artery, referred to as CIMT, has been established as a sensitive and reproducible marker for early and subclinical atherosclerosis (213), and studies have shown that CIMT is predictor of cardiovascular events (214-216). Endothelial dysfunction precedes overt CVD as cardiovascular risk factors induce cell injury and dysfunction (217). Investigations of endothelial function have been performed using different techniques. FMD based on the release of nitric oxide in response to endothelial shear stress is currently the best validated technique endothelial function (218). Arterial stiffness has been shown to be a marker for the early stages of vascular aging (219), and has also been reported to be significantly associated with an increased risk of cardiovascular events (220). PWV is recognised as the gold standard for assessing arterial stuffiness (31).

The current gold standard of treatment for OSA is CPAP, which increases airflow, decreasing the number of upper airway collapses and improves the effective sleep time. Small studies in OSA patients treated with CPAP have demonstrated favourable improvements in blood pressure and metabolic risk factors, as well as inflammatory and oxidative biomarkers associated with atherosclerosis (221-223). Promising results from these targeted analyses led to large studies investigating the impact of CPAP therapy on cardiovascular events (208-210).

These studies failed to show reductions in CV event rates in those treated with CPAP compared to usual care. These studies reported negative results for a number of reasons, and one possibility is the timing of intervention in the disease process. Early detection of atherosclerotic changes in patients with OSA may impact risk stratification and subsequent risk factor reduction. The effects of CPAP therapy in patients with OSA on the subclinical development of atherosclerosis have been investigated, however, these effects have not been investigated systematically.

2.1.2 Objectives

This systematic review aims to incorporate high quality evidence from human clinical trials investigating the impact of the OSA treatment of CPAP therapy on measures of subclinical CVD, through accessing only high-quality clinical trial databases. Subclinical CVD was assessed by carotid intima-media thickness, flow-mediated dilation, and pulse wave velocity.

2.2 Review Protocol

This study was performed based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (224).

2.2.1 Sources

A comprehensive search of the medical literature was performed from database inception until August 2019 using the Cochrane Library, PubMed, Embase to obtain only the highest quality clinical trials.

2.2.2 Search strategy

The following search string was used for the Cochrane Library search:

"MeSH descriptor: [Sleep Apnea, Obstructive] explode all trees"

AND

"MeSH descriptor: [Continuous Positive Airway Pressure] explode all trees"

AND

("MeSH descriptor: [Coronary Artery Calcium] explode all trees" OR "MeSH descriptor: [Carotid Intima-Media Thickness] explode all trees" OR "MeSH descriptor: [Vascular Stiffness] explode all trees" OR "MeSH descriptor: [Vascular Resistance] explode all trees" OR "MeSH descriptor: [Pulse Wave Analysis] explode all trees" OR "MeSH descriptor: [Ankle Brachial Index] explode all trees")

The following search string was used for the PubMed search:

"Sleep Apnea, Obstructive" [Mesh] AND "Sleep Apnea Syndromes" [Mesh]

AND

"Continuous Positive Airway Pressure" [Mesh]

AND

("coronary artery calcium" OR "Carotid Intima-Media Thickness" [Mesh] OR "flow mediated dilation" OR "peripheral arterial tone" OR "Pulse Wave Analysis" [Mesh] OR "Ankle Brachial Index" [Mesh])

The following search string was used for the Embase search:

('obstructive sleep apnea hypopnea syndrome'/exp OR 'obstructive sleep apnea hypopnea syndrome' OR (obstructive AND ('sleep'/exp OR sleep) AND 'apnea'/exp OR apnea) AND ('hypopnea'/exp OR hypopnea) AND ('syndrome'/exp OR syndrome))

AND

'positive end expiratory pressure'/exp

AND

('coronary artery calcium score'/exp OR 'arterial wall thickness'/exp OR 'flow-mediated dilation test'/exp OR (peripheral AND arterial AND ('tone'/exp OR tone)) OR "pulse wave'/exp OR ankle brachial index'/exp)

2.2.3 Eligibility criteria

Only studies that measured the prespecified Studied had to be high quality (NHMRC Evidence level I -III) (225) prospective observational or randomised studies. Only full text manuscripts written in English were included. Studies where CPAP treatment was used as the intervention were included, and its duration was at least 4 weeks, as the acute effects of CPAP were not evaluated. Results of studies investigating the effects of CPAP therapy on endothelium-dependent vascular relaxation were reported as percentage FMD. Results of studies investigating the effects of CPAP therapy on CIMT had to contain values corresponding to CIMT as recorded by ultrasound. Results of studies investigating the effects of CPAP therapy on arterial stiffness as measured by PWV were selected. The studies selected must have been performed on adult humans. Studies that did not meet the above criteria were excluded.

2.2.4 Study selection

The titles, abstracts and keywords of every record were retrieved and separately screened by two authors (JA and DJS) to find potentially relevant studies for the full review. Any discrepancies were resolved by discussion. Full text articles were retrieved if records indicated that eligibility criteria were likely to be met. Duplicate records were excluded.

2.2.5 Data collection process

Data were extracted from the studies independently by JA and DJS. Disagreements were resolved by discussion. The data extracted were study type, study design, study quality, sample size, OSA definition, CPAP qualification, control groups, CPAP treatment duration, the surrogate marker of atherosclerotic disease measured, along with method of measurement, lastly, results of changes in measured levels with exposure to CPAP. When studies included more than one follow-up time point, the results from the maximum duration of CPAP exposure

were included. When studies included other interventions on top of CPAP therapy, the results from the CPAP only arm were reported.

2.2.6 Quality of studies

The quality of each study was appraised, and the level of evidence was graded from I to IV based on the NHMRC Evidence Hierarchy (225) for human studies.

2.2.7 Summary measures

The changes in CIMT were expressed as millimetre (mm) change, while changes in PWV were expressed as meters per second change (m/s), and changes in FMD were expressed as percentage (%) change.

2.2.8 Synthesis of results

Studies of treatment with CPAP therapy are heterogeneous, with different study designs, sample sizes, OSA definitions, CPAP qualifications, and duration of treatment. Therefore, an overall effect of CPAP on any individual measure of subclinical atherosclerosis was assessed qualitatively, as it was not possible to accurately do so quantitatively.

2.3 Results

2.3.1 Study characteristics and selection

The Cochrane Library, PubMed, Embase searches yielded 125 results. Four randomised controlled clinical trials, 3 randomised sham-controlled clinical trials, 1 randomised crossover clinical trial, and 24 were prospective observational studies were eligible for inclusion in this systematic review. Reasons for study exclusion are listed below in Table 2.1.

Table 2.1: Reasons for exclusion of studies from systematic review

Reason for study exclusion	No. of studies
Duplicates	30
Results do not include treatment effect of CPAP therapy	10
Results do not include predefined measures of subclinical CVD	23
Results include other intervention in addition to CPAP therapy	2
Study does not include sufficient CPAP therapy treatment period	2
Results are not clearly or suitably reported	3
Full text article not available	2
Article not written in English	2
Unsuitable study design	10
Article type not a clinical trial	9
Total	**93**

2.3.2 Quality of studies

All studies had an NHMRC evidence level of I-III.

2.3.3 Synthesis of results

Changes in any of the surrogate markers of atherosclerotic disease after four weeks of exposure to CPAP therapy versus 12 months is not comparable. Effect sizes and the homogeneity of studies were also not comparable. On each graph, studies with a statistically significant overall effect are presented in blue. Studies are graphed together in the same units. A 95% confidence interval is presented when reported, or when it can be calculated from the original manuscript (226).

2.3.3.1 Carotid-intima-media thickness

Seven studies measured the effects of CPAP treatment on CIMT. The studies included were randomised controlled trials and prospective observational studies. The population sizes ranged from 24 participants to 206 participants. The OSA definitions and CPAP qualifications ranged from mild (AHI greater than 5) to severe OSA (AHI greater than 30). The treatment duration ranged from 3 months to 12 months, see Table 2.2. In 3 studies, the net effect of CPAP therapy was a statistically significant decrease in CIMT. No significant change was seen in 4 studies, see Figure 2.1.

Table 2.2: Summary of included studies (CIMT)

Authors	Study Design	Size of Population	Treatment Groups	OSA Definition	CPAP Qualification	Treatment Duration
Ng, S. et al; 2017 (227)	Randomised Controlled Trial	N = 90	**Therapeutic CPAP N = 45** Subtherapeutic CPAP N = 45	AHI ≥ 5	AHI ≥ 5	3 months
Li, C. et al; 2009 (228)	Prospective Observational	N = 72	Healthy controls N = 20 Mild OSAHS N = 16 Moderate OSAHS N = 18 **S**evere OSAHS N = 18 **Moderate to Severe OSAHS CPAP Treatment N = 20**	AHI ≥ 5	Moderate to Severe OSAHS	90 days
Kostopoulos, K. et al; 2016 (229)	Prospective Observational	N = 48	No OSA AHI <5 N = 10 OSA AHI 5-10 N = 10 OSA AHI >15 N = 28 **CPAP compliant N = 25**	AHI ≥ 5	AHI >15	3 months
Kim, J. et al; 2017 (230)	Prospective Observational	N = 206	AHI <10 N = 53 OSA AHI ≥15 N = 206 **CPAP compliant N = 118**	AHI ≥15	AHI >15	4 months
Jin, F. et al; 2017 (231)	Prospective Observational	N = 150	**CPAP Treatment N = 100** Healthy controls N = 50	AHI ≥ 5	AHI ≥ 5	3 months
Hui, D. et al; 2012 (232)	Prospective Observational	N = 50	**CPAP Treatment N = 28** Conservative Treatment N = 22	AHI ≥ 5 + symptoms suggestive of OSA	AHI ≥ 5 + symptoms suggestive of OSA	12 Months
Drager, L. F. et al; 2007 (233)	Randomised Controlled Trial	N = 24	**Severe OSA CPAP treatment N = 12** Severe OSA no treatment N = 12	AHI >30	AHI >30	4 months

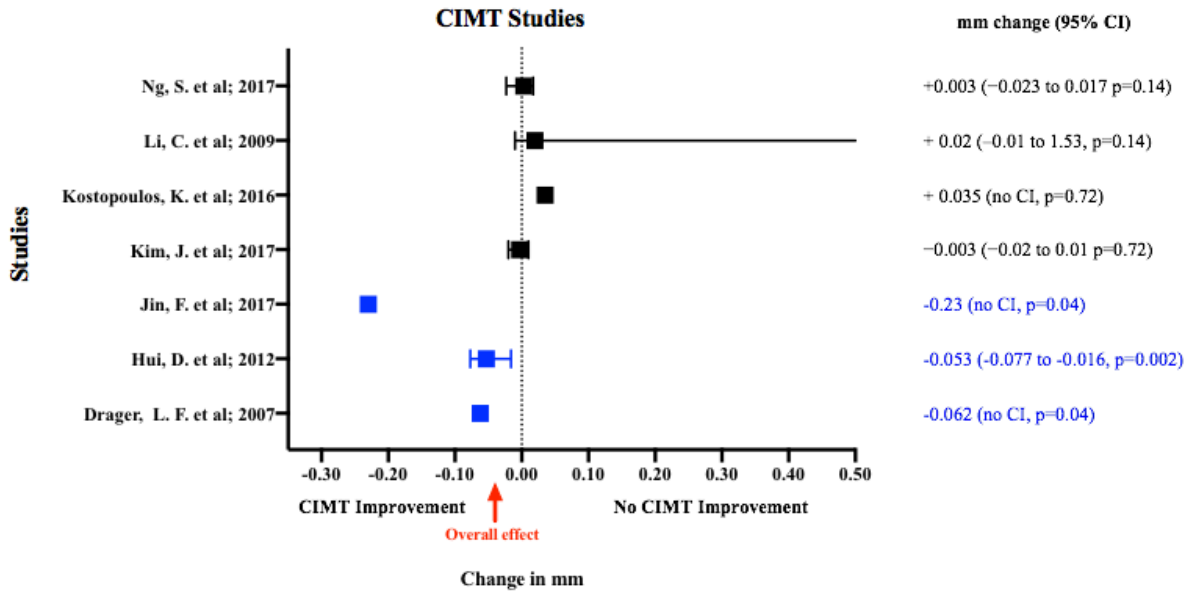


Figure 2.1: Summary of studies of CPAP therapy and CIMT. Studies with statistically significant changes are highlighted in blue. N = 7 studies.

2.3.3.2 Pulse wave velocity

Sixteen studies measured the effects of CPAP treatment on arterial stiffness as measured by PWV. The studies included were randomised controlled trials, randomised crossover and prospective observational studies. The population sizes ranged from 17 participants to 172 participants. The OSA definitions and CPAP qualifications ranged from mild (AHI greater than 5) to severe OSA (AHI greater than 30). The treatment duration ranged from 4 weeks to 6 months, see Table 2.3. In 11 studies, the net effect of CPAP therapy was a statistically significant decrease in PWV. No significant change was seen in 5 studies, see Figure 2.2.

Table 2.3: Summary of included studies (PWV)

Authors	Study Design	Size of Population	Treatment Groups	OSA Definition	CPAP Qualification	Treatment Duration
Shim, C. et al; 2018 (234)	Randomised Sham-Controlled Trial	N = 60	**CPAP treatment N = 28** Sham treatment N = 28	AHI >30	AHI >30	3 months
Shiina, K. et al; 2010 (235)	Prospective Observational	N = 50	**CPAP Treatment N = 50**	AHI ≥ 20	AHI ≥ 20	3 months
Paz y Mar, H. L. et al; 2016 (236)	Randomised Sham-Controlled Trial	N = 149	**CPAP treatment N = 75** Sham treatment N = 74	AHI ≥15	AHI ≥ 15	2 months
Nicholl, D.D. et al; 2018 (237)	Prospective Observational	N = 25	**CPAP Treatment N = 24**	RDI ≥ 15	RDI >15	4 weeks
Mineiro, M.A. et al; 2017 (238)	Prospective Observational	N = 34	**CPAP Treatment N = 34**	AHI ≥15	AHI ≥ 15	4 months
Litvin, A. Y. et al; 2013 (239)	Randomised Crossover	N = 44	**Effective CPAP N = 22** CPAP-Placebo N = 22	AHI >30	AHI >30	mean observation period = 13.2 ± 1.5 weeks
Kuramoto, E. et al; 2009 (240)	Prospective Observational	N = 116	Mild OSAS (AHI < 20) N = 35 Moderate OSAS (AHI ≥ 20 - < 40) N = 35 **S**evere OSAS (AHI ≥ 40) N = 46 **Moderate to Severe OSAS** **CPAP Treatment N = 38**	AHI ≥ 20	AHI ≥ 20	3 months
Kostopoulos, K. et al; 2016 (229)	Prospective Observational	N = 48	No OSA AHI <5 N = 10 OSA AHI 5-10 N = 10 OSA AHI >15 N = 28 **CPAP compliant N = 25**	AHI ≥ 5	AHI >15	3 months
Korcarz, C. et al; 2016 (241)	Prospective Observational	N = 84	**CPAP Treatment N = 74**	AHI ≥ 10	AHI ≥ 10	12 weeks

Kitahara, Y. et al; 2006 (242)	Prospective Observational	N = 17	**CPAP Treatment N = 17**	AHI ≥15	AHI ≥ 15	4 months
Kartali, N. et al; 2014 (243)	Prospective Observational	N = 53	Healthy controls N = 15 Hypertensive + Severe OSA N = 38 **Hypertensive + Severe OSA CPAP Treatment N = 19**	AHI ≥15	Hypertensive + (AHI > 30 OR AHI > 15 + daytime symptoms suggestive of OSA)	3 months
Jones, A. et al; 2013 (244)	Randomised Crossover Trial	N = 54	**CPAP treatment N = 26** Sham treatment N = 21	AHI ≥15	AHI ≥ 15	12 weeks
Jain, S. et al; 2017 (245)	Randomised Controlled Trial	N = 139	Weight loss N = 48 Weight loss + CPAP N = 46 **CPAP Treatment N = 45**	AHI ≥15	AHI ≥ 15	24 weeks
Drager, L. F. et al; 2007 (233)	Randomised Controlled Trial	N = 24	**Severe OSA CPAP treatment N = 12** Severe OSA no treatment N = 12	AHI >30	AHI >30	4 months
Chung, S. et al; 2011 (246)	Prospective Observational	N = 25	**CPAP Treatment N = 25**	AHI ≥15	AHI ≥ 15	mean duration = 138.7± 42.6 days
Buchner, N. J. et al; 2012 (247)	Prospective Observational	N = 172	No OSA N = 55 OSA N = 117 **Effective CPAP N = 49** Ineffective CPAP N = 39	AHI ≥5	AHI ≥ 15	6 months

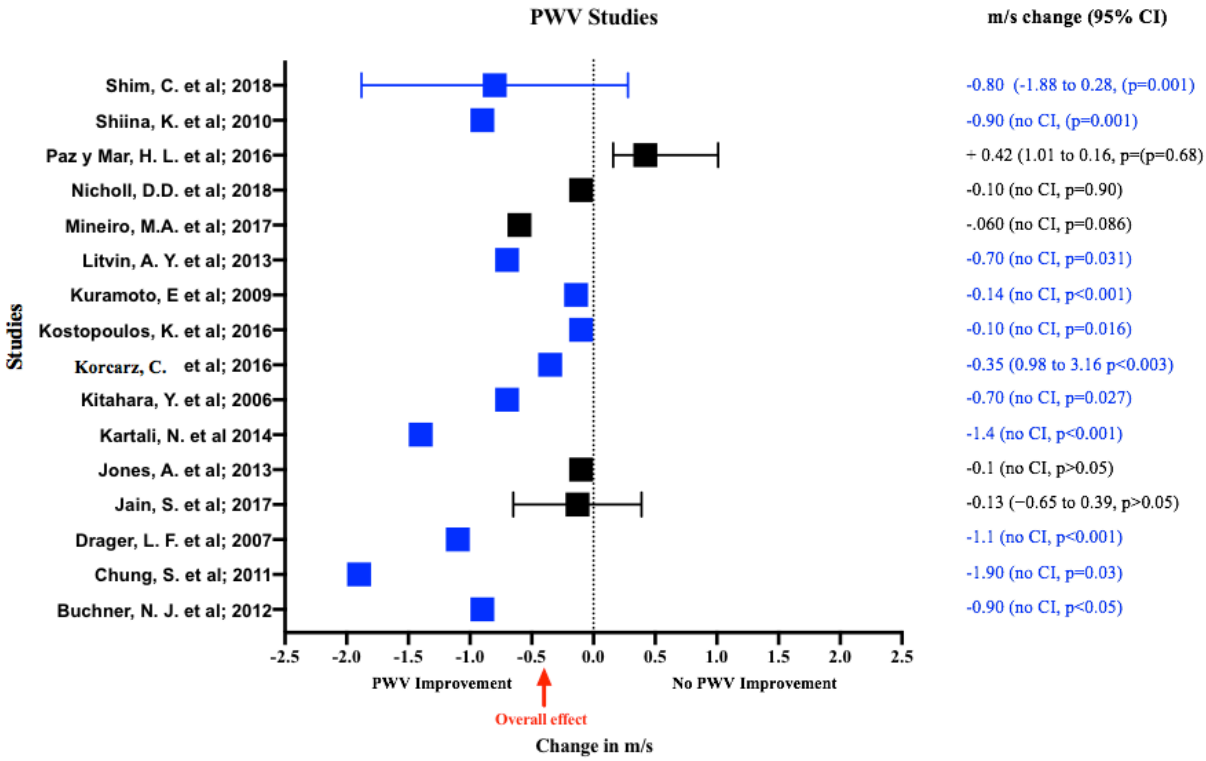


Figure 2.2: Summary of studies of CPAP therapy and PWV. Studies with statistically significant changes are highlighted in blue. N = 16 studies.

2.3.3.3 Flow-mediated dilation

Thirteen studies measured the effects of CPAP treatment on endothelium-dependent vasodilatation as measured by FMD. The studies included were randomised controlled trials, randomised crossover and prospective observational studies. The population sizes ranged from 24 participants to 253 participants. The OSA definitions and CPAP qualifications ranged from mild (AHI greater than 5) to severe OSA (AHI greater than 30). The treatment duration ranged from 4 weeks to 6 months, see Table 2.3. In 12 studies, the net effect of CPAP therapy was a statistically significant increase in FMD. No significant change was seen in 1 study, see Figure 2.3.

Table 2.4: Summary of included studies (FMD)

Authors	Study Design	Size of Population	Treatment Groups	OSA Definition	CPAP Qualification	Treatment Duration
Sardo, L. et al; 2015 (248)	Prospective Observational	N = 35	**CPAP Treatment N = 20** Healthy Controls N = 15	AHI ≥ 5	Symptomatic = AHI ≥ 5 OR Asymptomatic = AHI ≥ 15	4 weeks
Panoutsopoulos, A. et al; 2012 (249)	Prospective Observational	N = 38	**CPAP Treatment N = 20** AHI < 5 N = 18	AHI ≥ 5	AHI ≥ 15	3 months
Oyama, J. et al; 2016 (250)	Prospective Observational	N = 95	**CPAP Treatment N = 29** AHI < 5 N = 18	AHI ≥ 5	AHI > 20	3 months
Nguyen, P.K. et al; 2010 (251)	Randomised Sham-Controlled Trial	N = 35	**CPAP Treatment N = 10** Sham Treatment N = 10	AHI > 15	AHI > 15	3 months
Kostopoulos, K. et al; 2016 (229)	Prospective Observational	N = 48	No OSA AHI <5 N = 10 OSA AHI 5-10 N = 10 OSA AHI >15 N = 28 **CPAP Compliant N = 25**	AHI ≥ 5	AHI >15	3 months
Kohler, M. et al; 2013 (252)	Randomised Controlled Trial	N = 253	**FMD Post-CPAP Treatment N = 64** CPAP Treatment N = 107 Standard of Care N = 101	ODI > 7.5	ODI > 7.5	6 months
Kallianos, A. et al; 2015 (253)	Prospective Observational	N = 40	CPAP Treatment N = 40	AHI ≥ 15	AHI ≥ 15	3 months
Jelic, S. et al; 2008 (81)	Prospective Observational	N = 30	CPAP <4 Hours Daily OR Declined N = 14 **CPAP ≥4 Hours Daily N = 16**	AHI ≥ 5	AHI ≥ 5	4 weeks
El Solh, A. et al;2007 (254)	Prospective Observational	N = 24	**CPAP Treatment N = 14** Healthy controls N = 10	AHI ≥ 5	AHI ≥ 5	8 weeks

Del Ben, M. et al; 2012 (255)	Prospective Observational	N = 138	AHI <5 N = 47 OSA AHI 5-29 N = 61 AHI >30 N = 30 **CPAP Treatment N = 10**	AHI ≥ 5	AHI ≥ 30	6 months
Chung, S. et al; 2011 (246)	Prospective Observational	N = 25	CPAP Treatment N = 25	AHI ≥ 15	AHI ≥ 15	mean duration, 138.7± 42.6 days
Bayram, N. et al; 2009 (256)	Prospective Observational	N = 46 (Male)	**CPAP Treatment N = 29** Healthy Controls N = 17	AHI ≥ 5	AHI ≥ 5	6 months
Bakker, J. et al; 2013 (257)	Prospective Observational	N = 72	**CPAP Treatment N = 15** Bariatric Surgery N = 17	AHI ≥ 10	AHI ≥ 10	6 months

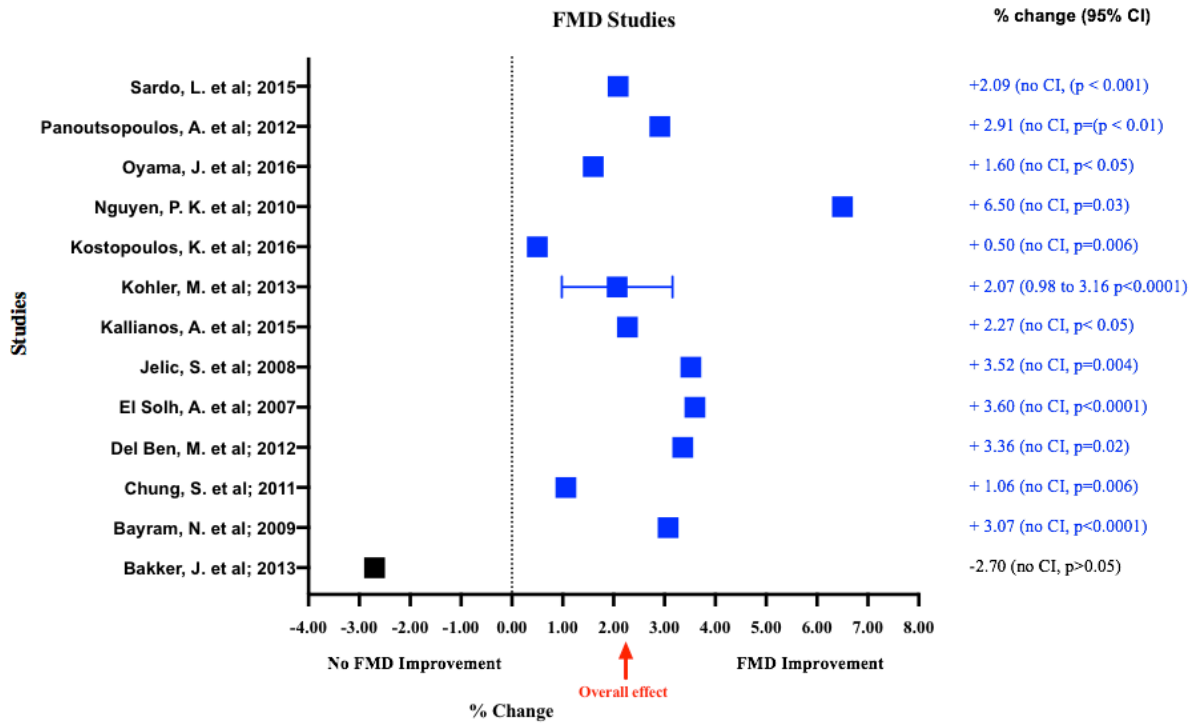


Figure 2.3: Summary of studies of CPAP therapy and FMD. Studies with statistically significant changes are highlighted in blue. N = 13 studies

2.4 Discussion

Patients with OSA have been reported to have alterations in subclinical atherosclerosis. Therefore, this systematic review evaluated the impact of treatment with CPAP therapy on markers of subclinical atherosclerosis in patients with OSA. Overall, treatment with CPAP therapy was shown to improve markers of subclinical atherosclerosis as measured by CIMT, FMD, and PWV.

The development of carotid atherosclerosis observed in patients with OSA is thought to occur as result of IH, recurrent arousals, and inflammation. Vascular inflammation and endothelial injury are possibly induced by the vibrations of snoring transmitted through the soft tissues surrounding the pharynx to the carotid artery wall (58). Several studies have reported increases in CIMT in those with OSA (60). Treatment with CPAP has been shown to reduce circulating inflammatory markers that are associated with the development of OSA (57, 168). Overall, treatment with CPAP resulted in a modest reduction in IMT as 3 of the 7 studies investigating the effects of CPAP treatment on CIMT reported significant reductions in IMT thickening. However, the authors of the studies that did not observe a reduction in IMT concluded that factors such as small sample size, modest CPAP compliance, residual confounders, and short treatment periods may have contributed to negative results.

Arterial stiffness is not a stage in the development of atherosclerotic disease, yet it has been reported to be an important marker for CV events (37). PWV, a marker of aterial stifness has also been shown to be elelvated in those with OSA (38). Blood pressure has been shown to be one of the determinants of arterial stiffness (258, 259), and blood pressure have been improved in those treaeted with CPAP (260). Eleven of the 16 studies exploring the effects of CPAP treatment on PWV reported significant reductions in arterial stiffness. While 4 of the five demonstrated reductions in PWV, they failed to reach significance. Possible reasons

include participants with less severe OSA than other investigations, and lower baseline PWV values.

Endothelial dysfunction is triggered by activation of the sympathetic nervous system (80) systemic inflammation, (81, 82) and oxidative stress (83). Impaired endothelium-dependent vascular relaxation is a prognostic marker of atherosclerosis as it represents a physiologic change of the artery wall preceding plaque formation. FMD has become the validated measure of endothelium-dependent vascular relaxation. FMD has been impaired in minimally symptomatic patients with OSA (48), and has been shown to be inversely related to AHI (38). FMD was improved in 12 of the 13 investigations into the effects of treatment with CPAP. There was a beneficial effect shown in each of the measures of subclinical atherosclerosis included in this review as a result of treatment with CPAP. The treatment period of 3 or 4 months in many instances was enough time to improve oxidative stress and arterial stiffness. However, these findings have yet to be translated into studies that result in reduction in cardiovascular events.

There are limitations to this systematic review that warrant consideration. The studies included were clinic-based and more likely to enrol patients with cardiometabolic comorbidities, and may have biased the true association between OSA and CVD. Coronary artery calcium is also an established marker of subclinical atherosclerosis, and has also been shown to be prevalent in those with OSA (67). However, there were no studies found in the search that included investigations into the effect of treatment with CPAP therapy on coronary calcification. There was heterogeneity among the studies included in regards to the range in sample sizes, definitions of OSA and severity, the amount of compliance and duration of treatment with CPAP therapy. This heterogeneity demonstrates that there have not been enough studies done investigating the impact of CPAP therapy on subclinical atherosclerosis using consistent OSA definitions. More evidence is required to answer the question of whether or

not treatment with CPAP therapy can yield greater benefit in halting the the development of subclinical atherosclerosis.

The studies included in this review investigated the effects of CPAP therapy on the development of subclinical CVD, and improvements were observed in each of the measures of subclinical CVD after a relatively short period of time. Yet, large studies investigating the impact of CPAP therapy on cardiovascular events failed to show reductions in CV event rates in those treated with CPAP compared to usual care (208-210). Possible reasons for the negative results of these studies include insufficient power calculations, inadequate definition of OSA, the varying clinical characteristics of the study populations, and adherence to treatment. The average CPAP usage is reported to be 3.3 hours per night of sleep. Subgroup analyses reported that those exposed to CPAP therapy for an average of greater than four hours per night of sleep derived greater benefit from treatment (210). However, many of the limitations of these studies listed above are also limitations to the studies included in this review. Yet, improvements in the early stages of atherosclerosis were observed. These findings suggest that benefit in the early stages of atherosclerosis may be achieved after a modest form of treatment. Whereas a reduction CV events after treatment with CPAP compared to usual care in a population with developed CVD require much more aggressive treatment, such as better adherence and longer follow-up period.

Chapter 3:

THE RELATIONSHIP BETWEEN SYMPTOMS SUGGESTIVE OF OBSTRUCTIVE SLEEP APNOEA AND SEVERITY OF CORONARY ARTERY STENOSIS

ABSTRACT

Background: OSA is a highly prevalent sleep disorder. However, it remains underdiagnosed and undertreated. Untreated OSA is a known risk factor contributing to CAD.

Methods: A prospective, cross-sectional study was performed in patients undergoing clinically indicated coronary angiography. A medical history was taken and sleep questionnaires were administered prior to catheterisation procedure, and a blood sample was taken. Coronary artery stenosis severity was determined by angiographic Gensini score as mild (less than 10), moderate (10-50), severe (greater than 50), and the maximum stenosis was recorded. An OSA prediction questionnaire, OSA50, was scored as low risk OSA (0-5), high risk OSA (6-10). A daytime sleepiness questionnaire, Epworth Sleepiness Scale (ESS), was scored as sleepier (11-24), less sleepy (0-10).

Results: The overall cohort (n=99) had a mean age of 68.1 years, a median BMI of 30.4 kg/m^2 (IQR (26.35 kg/m^2, 32.81 kg/m^2)), and 23% were female. The median total cholesterol was 3.63 mmol/L (IQR 3.02 mmol/L, 4.95 mmol/L), and the median LDL-C was 1.80 mmol/L (IQR 1.50 mmol/L, 2.70 mmol/L). The median Gensini score was 35.0 (IQR 12.0, 65.5), and the median maximum stenosis was 70% (IQR (10%, 90%)). The median ESS was 4 (IQR (2, 7)), the mean OSA50 score was 5.85 (SD (2.70)), and OSA was previously diagnosed in 16% of participants. There were no significant differences in Gensini score (high risk OSA [median (IQR)] 41.00 (21.00, 66.50) vs low risk OSA [median (IQR)] 31.25 (6.50, 64.75); p=0.21), or maximum stenosis (high risk OSA [median (IQR)] 70 (40, 90) vs low risk OSA [median (IQR)] 60 (10, 95); p=0.31) between those at high risk or low risk of OSA.

Conclusion: While conventional assessments for symptoms suggestive of OSA associate with cardiovascular events, they do not appear to associate with focal or global measures of

obstructive disease as measured by angiography. Accordingly, mechanisms apart from plaque burden maybe more likely to underscore the relationship between OSA and CVD.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

3.1 Introduction

Despite the use of established therapies targeting traditional risk factors, cardiovascular events continue to occur. In the search for alternatives, OSA has emerged as a risk factor to target. OSA is characterised by recurrent episodes of partial or complete airway obstruction resulting in apnoeas or hypopnoeas, and leads to IH and frequent arousals. These breathing disturbances result in an increase in sympathetic activation, alterations in blood pressure, and vascular atherogenic changes. Strong associations have been shown between OSA and hypertension, ischemic heart disease, stroke, arrhythmia, chronic heart failure (60). OSA is accepted as a highly prevalent sleep disorder, and the prevalence of OSA is reported to affect 7% of the western world general population (6). The OSA prevalence found in patients with CAD, metabolic syndrome, and hypertension ranges from 30% to 70% (9, 146, 261).

Incidence of OSA is common in the clinical setting of STEMI (ST elevation myocardial infarction). OSA also negatively impacts coronary plaque burden, microvascular obstruction, and recovery of left ventricular function after percutaneous coronary intervention (PCI) (182). Elevated peak plasma troponin concentration, greater presence of three vessel disease, and longer stay in coronary care unit after PCI have all been associated with the presence of OSA (181). Historically, OSA has been reported to be a predictor of restenosis and target vessel revascularisation (189, 262).

Despite the significant impact of OSA on affected individuals, it is still largely underdiagnosed and undertreated. Untreated OSA is a known risk factor contributing to CVD, and leading to MI (9, 23, 28, 263). Long-term follow-up studies have reported untreated severe obstructive sleep apnoea-hypopnoea significantly increased the risk of fatal and non-fatal CV events compared with healthy participants (28).

PSG is the gold standard for diagnosing sleep apnoea. However, access to sleep clinics to administer the overnight sleep test is limited and expensive. Therefore, OSA screening

questionnaires have been developed and are utilised as screening tools to identify those at risk for OSA in cohorts with cardiovascular diseases.

3.1.1 Aims and rationale of study

The objective of this study was to use the OSA50 questionnaire, a sleep screening questionnaire previously validated in the primary care setting to describe the prevalence of symptoms of OSA (264), to estimate the risk of OSA in patients undergoing angiography, and compare severity of obstructive coronary artery disease in those at high and low risk of OSA.

3.1.2 Hypothesis

The hypothesis of this study was prevalence of symptoms suggestive of OSA in participants would be high among a cohort at least 40 years of age referred for a clinically indicated coronary catheterisation, and the risk of OSA as measured by the OSA50 questionnaire would correlate with global and focal severity of CAD as measured by Gensini score and maximum stenosis of a single lesion, respectively.

3.2 Methods

3.2.1 Study outline

This prospective cross-sectional study recruited 99 participants for participation in the study, and the participants were required to fulfil the eligibility criteria listed below.

Eligibility Criteria

- Males and females age ≥ 40 years of age
- A clinical indication for a coronary angiogram
- Patients with stable ischaemic heart disease (stable angina) or recent acute coronary syndrome (unstable angina, non-ST elevation myocardial infarction, ST-elevation myocardial infarction)
- No condition that in the opinion of the responsible physician or investigator renders the participant unsuitable for the study such as co-morbid disease with severe

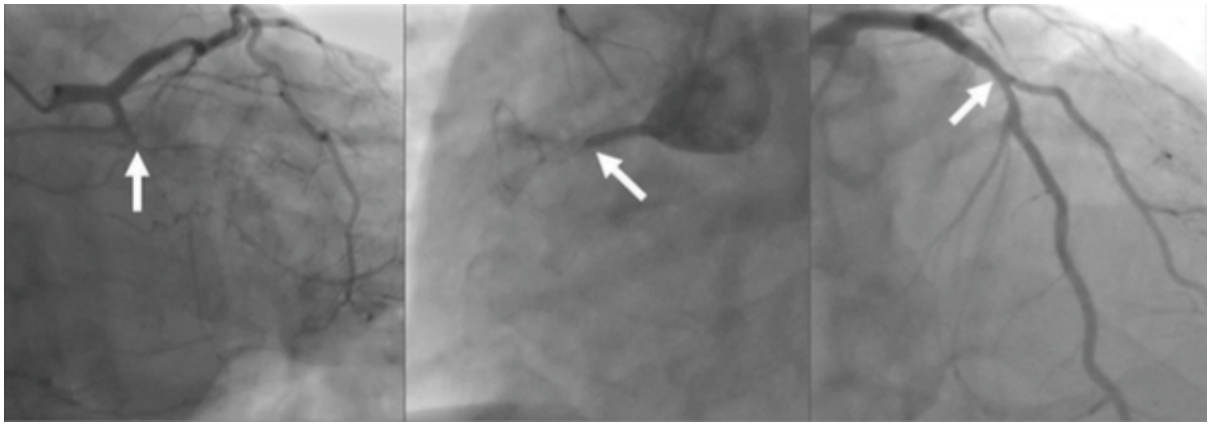
disability; significant memory, perceptual, or behavioural disorder or inability to provide informed consent.

The following demographic and clinical information were collected from participants prospectively: gender, age, height, weight, body mass index, waist circumference clinical presentation (such as ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, unstable angina, or stable angina), cardiovascular risk factors (smoking, diabetes mellitus, hypertension, hyperlipidaemia, or family history of premature coronary artery disease), previous myocardial infarction, previous stroke.

Coronary angiography was performed via the percutaneous radial or femoral approach using standard angiographic techniques. A maximum stenosis percentage was captured in patients with or without significant lesions, defined as greater than or equal to 50% stenosis in major epicardial vessels or their branches. Global CAD severity was expressed based on the Gensini score (265) (see Table 3.1 and Figure 3.1), a standardised and well validated measure of global obstructive burden, that has been demonstrated to associate with adverse cardiovascular outcomes (266, 267). CAD severity was classified as mild (less than 10), moderate (10-50), or severe (greater than 50). During the clinically indicated catheterisation procedure, 20 mls of blood were collected for measurement of serum lipids, standard biochemistry. The OSA50 (264) (see Figure 3.2) was administered as clinical OSA prediction questionnaire of low risk (0-5) and high risk (6-10). The ESS (268) (see Figure 3.3) was administered to detect daytime sleepiness with scores 11-24 classified as sleepier, and 0-10 less sleepy.

Table 3.1: Gensini Score calculation step by step algorithm adapted from Rampidis, G.P., et al (2019); *Atherosclerosis.* (269)

STEP 1 Calculation of the severity for each lesion ≥25% and adjustment for total occlusions or 99% obstructive lesions receiving collaterals			
Degree of stenosis	**Receiving collaterals**	**Adjustment for collaterals**	**Severity Score**
1%-25%	-	0	1
26%-50%	-	0	2
51%-75%	-	0	4
76%-90%	-	0	8
91%-99%	no	0	16
99%	yes	-8	8
100%	no	0	32
100%	yes, and normal source vessel	-16	32-16=16
100%	yes, and 25% stenosis source vessel	-12	32-12=20
100%	yes, and 50% stenosis source vessel	-8	32-8=24
100%	yes, and 75% stenosis source vessel	-4	32-4=28
100%	yes, and 90% stenosis source vessel	-2	32-2=30
100%	yes, and 99% stenosis source vessel	-1	32-1=31
STEP 2 A multiplying factor is applied to each lesion score based upon its location in the coronary tree			
Segment	**Right Dominance**	**Left Dominance**	
RCA proximal	1	1	
RCA mid	1	1	
RCA distal	1	1	
PDA	1	1	
PLB	0.5	0.5	
Left Main	5	5	
LAD proximal	2.5	2.5	
LAD mid	1.5	1.5	
LAD apical	1	1	
1st Diagonal	1	1	
2nd Diagonal	0.5	0.5	
LCx proximal	2.5	3.5	
LCx mid	1	2	
LCx distal	1	2	
Obtuse Marginal	1	1	
STEP 3 Sum of all of the lesion severity scores			



Total occlusion LCx proximal (receiving collaterals from LAD)	**Total occlusion RCA proximal (receiving collaterals from LAD)**	**LAD proximal = 50% stenosis**
STEP 1 Calculation of the severity for each lesion ≥25% and adjustment for total occlusions or 99% obstructive lesions receiving collaterals		
Severity score – Collateral factor = 32-8 **24**	Severity score – Collateral factor = 32-8 **24**	Severity score= (50% stenosis) **2**
STEP 2 A multiplying factor is applied to each lesion score based upon its location in the coronary tree		
Lesion score x Segment weighting factor = 24 x 2.5 **60**	Lesion score x Segment weighting factor = 24 x 1 **24**	Lesion score x Segment weighting factor = 2 x 2.5 **5**
STEP 3 Sum of all of the lesion severity scores		
Score (LCx proximal) + Score (RCA proximal) + Score (LAD middle) = 60 + 24 + 5 **89**		

Figure 3.1: Gensini Score calculation example adapted from Rampidis, G.P., et al (2019); ***Atherosclerosis.*** (269)

			If yes, SCORE
Obesity:	Waist circumference* or Females >88cm	Males >102cm	3
Snoring:	Has your snoring ever bothered other people?		3
Apneas:	Has anyone noticed that you stop breathing during sleep?		2
50:	Are you aged 50 years or over?		2
		Total Score:	/ 10 points

* Waist circumference to be measured at the level of the umbilicus

Figure 3.2: OSA50 screening questionnaire adapted from Chai-Coetzer, C.L., et al (2011); *Thorax.* (264)

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

0 = Would never dose
1 = Slight chance of dozing
2 = Moderate chance of dosing
3 = High chance of dozing

Situation	Chance of Dozing
Sitting and reading	_____
Watching TV	_____
Sitting, inactive in a public place (e.g. a theater or a meeting)	_____
As a passenger in a car for an hour without a break	_____
Lying down to rest in the afternoon when circumstances permit	_____
Sitting and talking to someone	_____
Sitting quietly after a lunch without alcohol	_____
In a car, while stopped for a few minutes in the traffic	_____

Figure 3.3: Epworth Sleepiness Scale adapted from Johns, MW (1991); Sleep. (268)

3.2.2 Statistical and data analysis

3.2.2.1 Sample size

This study is an observational review of the prevalence of OSA symptoms in a population of individuals going to the cath lab for a clinically indicated coronary angiogram. A sample size of 100 patients, 50 with low risk OSA and 50 with high risk OSA, provided 85% power at a 2-sided α of 0.05 to detect a difference of 15 in Genisini score between those with low risk OSA and with high risk OSA (266, 267).

3.2.2.2 Statistical methods

Participant data were de-identified, and statistical analysis was performed using Stata, version 14.2 (StataCorp). The D'Agostino-Pearson normality test was performed to determine whether continuous data were normally-distributed. Normally distributed data were analysed using the One-way Analysis of Variance (ANOVA). Results were expressed as mean ± standard error of the mean (SEM). If continuous data were not normally-distributed, analysis was performed using the Kruskal-Wallis test. Results were expressed as median + interquartile range (IQR). Statistical correlations were analysed using a linear regression model. Statistical significance was set at the 0.05 level.

3.2.3 Ethical and site approval

Ethics approval was obtained from the Royal Adelaide Human Research Ethics Committee, and site specific authorisation was obtained from the Central Adelaide Local Health Network.

3.3 Results

3.3.1 Participant characteristics

The demographics are summarised in Table 3.2. The mean age of participants was 68.1 years, and 23% of participants were female. Participants in the OSA low risk group were older than the OSA high risk group (70.14 vs 65.22 years; p=0.02). BMI (32.46 vs 28.20; p <0.001) and waist circumference (113.76 vs 98.2; p <0.001) were both higher in the OSA high risk group. Fifty-seven percent of participants had a history of hypertension, 28% were diabetic, and almost half of the participants were previous smokers. There were significantly more previous MIs (41% (17) vs 22% (13); p=0.05) in the OSA high risk group. There were no other significant differences in participant medical history between the groups. Forty-three percent of participants were receiving high-intensity therapy, and 66% of participants were receiving antiplatelet therapy. There were no significant differences in participant concomitant medications between the groups.

Table 3.2: Summary of participant characteristics by OSA risk

	Overall	OSA Low Risk	OSA High Risk	p value
Number of Participants	99	58	41	
Age, Mean (SD)	68.10 (10.27)	70.14 (10.87)	65.22 (8.69)	0.02
Female, n (%)	23 (23)	15 (26)	8 (20)	0.63
BMI (kg/m^2), Median (IQR)	30.41 (26.35, 32.81)	28.20 (24.74, 30.84)	32.46 (30.56, 34.72)	<0.001
Waist Circumference (cm), Mean (SD)	104.65 (18.28)	98.21 (17.98)	113.76 (14.61)	<0.001
Smoking, n (%)				
Never	31 (31)	21 (36)	10 (24)	0.46
Previous	47 (47)	26 (45)	21 (51)	
Current	21 (21)	11 (19)	10 (24)	
Hypertension, n (%)	56 (57)	30 (52)	26 (63)	0.31
Hyperlipidaemia, n (%)	31 (31)	19 (33)	12 (29)	0.83
Diabetes, n (%)	28 (28)	13 (22)	15 (37)	0.17
Atrial Fibrillation, n (%)	15 (15)	12 (21)	3 (7)	0.09
Previous MI, n (%)	30 (30)	13 (22)	17 (41)	0.05
CVD Family History, n (%)	10 (10)	4 (7)	6 (15)	0.31
Statin Intensity				
High, n (%)	43 (43)	27 (47)	16 (39)	0.54
Moderate, n (%)	20 (20)	11 (19)	9 (22)	0.80
Low, n (%)	2 (2)	1 (2)	1 (2)	1.00
No Statin Therapy, n (%)	34 (34)	19 (33)	15 (37)	0.83
Antiplatelet therapy, n (%)	65 (66)	37 (64)	28 (68)	0.67
ß-Blockers, n (%)	42 (42)	26 (45)	16 (39)	0.68
ACE Inhibitor, n (%)	30 (30)	21 (36)	9 (22)	0.18
ARB, n (%)	25 (25)	14 (24)	11 (27)	0.82

NB: Medication use was collected prior to catheterisation procedure. Antiplatelet use and other medications are likely to have been revised post procedure.

The biochemical measures of the participants are summarised in Table 3.3. There were no significant differences in participant lipid parameters, hs-CRP, and glucose levels between the two groups. HDL-C levels were at the lower end of normal, and hs-CRP levels were slighly elevated in the OSA low risk group.

Table 3.3: Summary of biochemical measures by OSA risk

	Overall	OSA Low Risk	OSA High Risk	p value
Number of Participants	99	58	41	
Total Cholesterol, median (IQR), mmol/L	3.63 (3.02, 4.95)	3.83 (3.14, 4.78)	3.45 (2.94, 5.00)	0.28
LDL-C, median (IQR), mmol/L	1.80 (1.50, 2.70)	1.90 (1.60, 2.80)	1.80 (1.40, 2.50)	0.33
HDL-C, median (IQR), mmol/L	1.04 (0.90, 1.24)	1.08 (0.90, 1.27)	0.97 (0.92, 1.10)	0.10
Triglycerides, median (IQR), mmol/L	1.34 (0.94, 1.84)	1.27 (0.94, 1.68)	1.40 (1.01, 1.89)	0.46
hs-CRP, median (IQR), mg/L	2.61 (1.61, 9.06)	3.28 (1.72, 8.69)	2.17 (1.38, 9.06)	0.49
Glucose, median (IQR), mmol/L	5.92 (4.94, 7.23)	5.61 (4.87, 6.42)	6.27 (5.27, 7.57)	0.08

The sleep parameters of the participants are presented in Table 3.4. Sixteen percent of participants were previously diagnosed with sleep apnoea. There were significantly more participants previously diagnosed with sleep apnoea and higher mean OSA50 scores in the OSA high risk group. Overall the participants scored low on the ESS.

Table 3.4: Summary of sleep parameters by OSA risk

	Overall	**OSA Low Risk**	**OSA High Risk**	**p value**
Number of Participants	99	58	41	
Sleep Apnoea, n (%)	16 (16)	3 (5)	13 (32)	<0.001
OSA50, Mean (SD)	5.85 (2.70)	3.90 (1.44)	8.61 (1.24)	<0.001
Epworth Sleepiness Scale, Median (IQR)	4 (2, 7)	3.5 (2, 7)	4 (2, 8)	0.46
Epworth Sleepiness Scale Low, n (%)	88 (89)	54 (93)	34 (83)	0.19
Epworth Sleepiness Scale High, n (%)	11 (11)	4 (7)	7 (17)	

The measures of CAD severity of participants are presented in Table 3.5. Global and focal measures of CAD severity were calculated as Gensini score and maximum stenosis, respectively. Gensini score interobserver variability was performed on 10 studies by two experienced angiogram readers. Bland-Altman analysis showed a mean difference of 2.3 (95% limit of agreement -12.2, 16.9) and an intra-class correlation coefficient of 0.981 (95% CI 0.974 - 0.990), indicating good reproducibility and reliability (266). Gensini score and maximum stenosis were not significantly different between the two groups. On average, Gensini score corresponded to overall moderate CAD, and the maximum stenosis corresponded to a significant single stenotic lesion.

Table 3.5: Summary of coronary artery disease severity by OSA risk

	Overall	**OSA Low Risk**	**OSA High Risk**	**p value**
Number of Participants	99	58	41	
Gensini Score				
Median (IQR)	35.0 (12.0, 65.5)	31.25 (6.50, 64.75)	41.00 (21.00, 66.50)	0.21
Mild, n (%)	22 (22)	16 (28)	6 (15)	0.31
Moderate, n (%)	40 (40)	22 (38)	18 (44)	
Severe, n (%)	37 (37)	20 (34)	17 (41)	
Maximum Stenosis				
Median (IQR)	70 (10, 90)	60 (10, 95)	70 (40, 90)	0.69
Non-significant Stenosis, n (%)	39 (39)	24 (41)	15 (37)	0.68
Significant Stenosis, n (%)	60 (61)	34 (59)	26 (63)	

3.3.2 Correlations between patient characteristics, lipids, CAD severity and symptoms suggestive of sleep apnoea

Symptoms suggestive of sleep apnoea as measured by the OSA50 questionnaire were correlated with the following parameters to determine whether any significant associations exist: patient characteristics, medical history, lipids, sleep parameters, and CAD severity measures.

Patient characteristics

Positive, significant associations were present between OSA50 score and both waist circumference and BMI. An inverse, significant association was present between OSA50 score and age (see Figure 3.4).

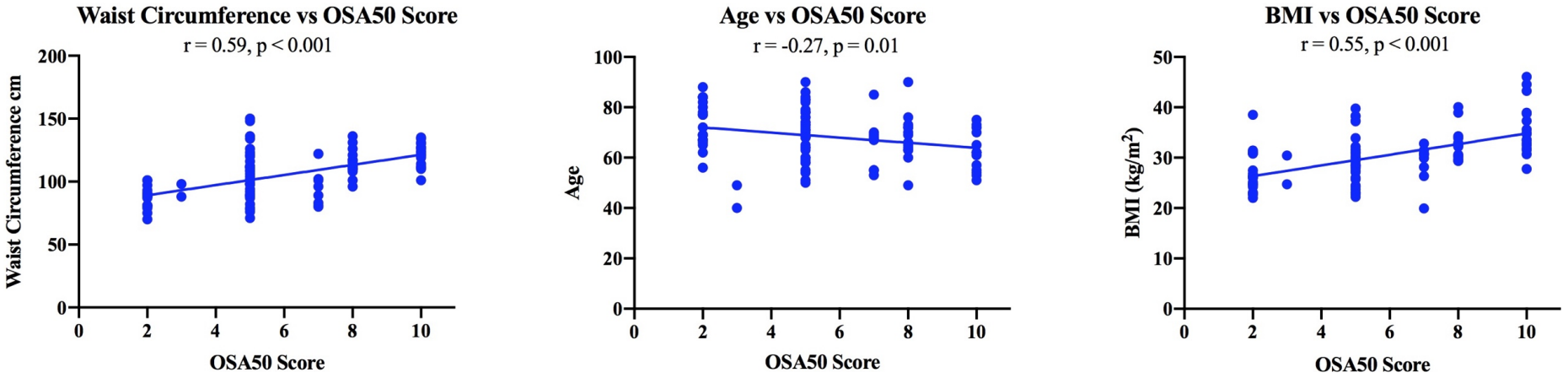


Figure 3.4: Correlations of patient characteristics of waist circumference, age, BMI and OSA50 score. n = 99 for each graph.

Medical history

A positive, significant association was present between OSA50 score and smoking, but not diabetes or hypertension. An inverse, significant correlation was present between OSA50 score and atrial fibrillation, and a non-significant positive correlation was present between OSA50 score and family history of CVD (see Table 3.6).

Table 3.6: Correlations of medical history of atrial fibrillation, family history of CVD, smoking, diabetes, hypertension and OSA50 score.

	r	p value
Atrial Fibrillation	-0.21	0.04
Family History of CVD	0.19	0.06
Smoking	0.24	0.02
Diabetes	0.14	0.16
Hypertension	0.16	0.11

Lipids

A non-significant inverse correlation was present between HDL-C levels and OSA50 score. No significant correlations were present between OSA50 score and either total cholesterol, LDL-C levels, and triglycerides (see Figure 3.5).

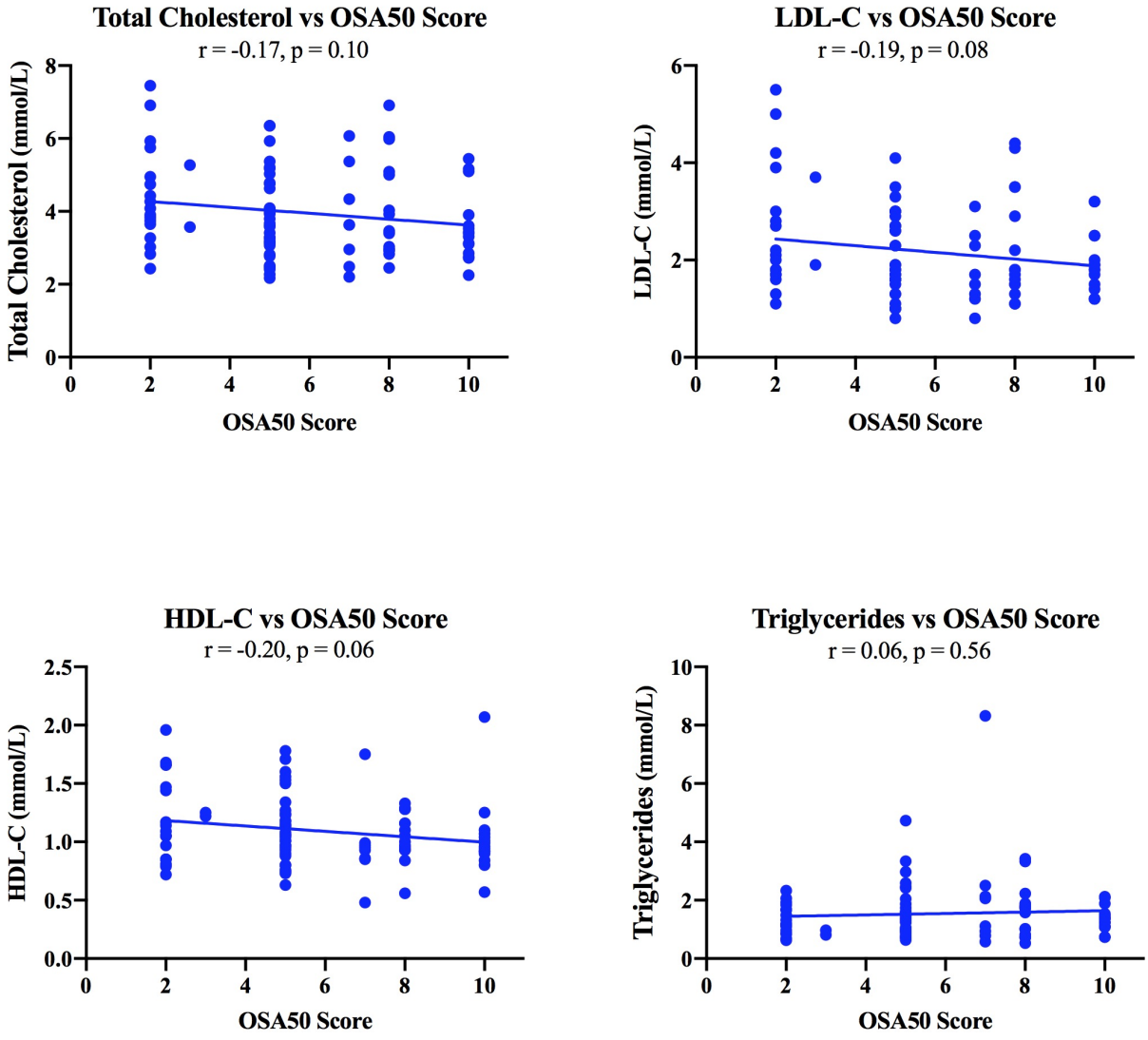


Figure 3.5: Correlations of total cholesterol, LDL-C, HDL-C, triglycerides and OSA50 score. n = 99 for each graph.

Sleep parameters

A positive, significant association was present between OSA50 score and both previous OSA diagnosis and CPAP treatment, but not ESS (see Table 3.7).

Table 3.7: Correlations of sleep parameters of Epworth sleepiness scale, previous OSA diagnosis, CPAP treatment and OSA50 score.

	r	P value
Epworth Sleepiness Scale	0.12	0.22
Previous OSA Diagnosis	0.38	< 0.001
CPAP Treatment	0.32	0.001

CAD severity

No significant correlations were present between OSA50 score and Gensini score or maximum stenosis (see Figure 3.6).

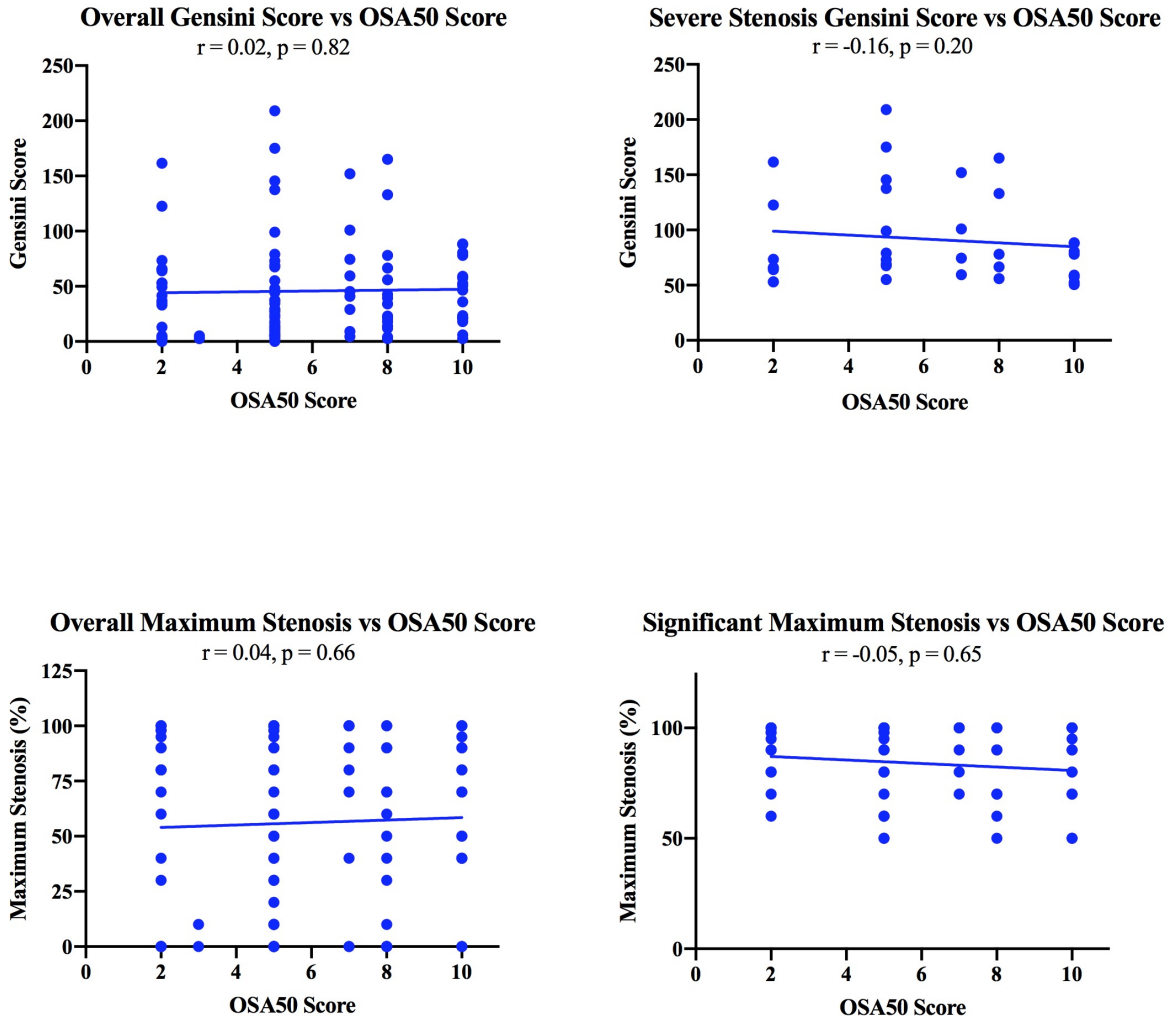


Figure 3.6: Correlations of CAD severity of overall Gensini score, severe stenosis Gensini score, overall maximum stenosis, significant maximum stenosis and OSA50 score. n = 99 for overall Gensini score and overall maximum stenosis graphs, n = 35 for severe stenosis Gensini score graph, and n = 60 significant maximum stenosis graphs.

3.4 Discussion

This study investigated the relationship between symptoms suggestive of sleep apnoea, and severity of coronary artery stenosis in a cohort of individuals 40 years and older undergoing a clinically indicated catheterisation at a single centre. Overall, this cohort included participants on average with moderately severe CAD, as measured by Gensini score (265), and a significantly stenotic lesion. Forty percent of participants had a prevalence of high risk for OSA, as measured by the OSA50 questionnaire (264). Risk of OSA did not associate with severity of angiographic measures of global or focal obstructive disease.

The OSA diagnosis gold standard continues to be PSG, yet it is expensive and access is limited. Therefore, questionnaires have been developed to screen for OSA, and have been utilised as screening tools to identify those at risk for OSA in cohorts with CVD (261, 270, 271). CAD was associated with OSA, as identified by the Berlin Questionnaire (BQ), in 44% of patients in an atrial fibrillation (AF) cohort and the recurrence rate of AF was higher in those with OSA compared to those without OSA after catheter arrhythmia ablation (270). Hypertensive populations have also been investigated, and CAD was associated with BQ identified OSA in 80% of resistant hypertension patients and 44% with controlled hypertension (271). The OSA50 questionnaire, used in the current study, is based on four predictors of OSA, waist circumference, snoring, witnessed apnoeas and age (Figure 3.1). In a primary care setting, the OSA50 questionnaire has been shown to be significantly predictive of moderate to severe OSA with an area under the curve of 0.84 (264). The current cohort includes a high prevalence of risk of OSA, however the relationship between CAD severity and OSA risk was not significant. The ESS (268), also administered to participants, assesses daytime sleepiness by ranking likelihood of dozing throughout the day in eight daytime scenarios. Scores greater than 10 out of 24 are considered to associate with OSA (271, 272). The overall daytime sleepiness

of this cohort was low, and there were no significant differences in scores between the low and high risk of OSA groups.

The relationship between risk of OSA and obesity was significant, with a significantly greater BMI and waist circumference in the OSA high risk group, and increased BMI and waist circumference significantly correlating with increase in OSA50 score. Obesity is the strongest risk factor for developing OSA. Risk of collapse and obstruction of the upper airway is increased as increased body fat contributes to the reduction of upper airway lumen size (273). A longitudinal study of more than 600 participants reported 10% weight gain was associated with 32% AHI increase and 10% weight loss associated with 26% AHI reduction after 4 years of follow-up (274). Conflicting results of the investigations of the relationship between OSA and BMI have been reported (274, 275), suggesting regional fat distribution instead associates with OSA. Increased BMI is a prevalent risk factor for developing CAD (276), and increased waist circumference, an indicator for total body fat, associates with CAD (277). Obesity and OSA share common cardiometabolic risks (99, 278, 279). However, in this study, there were no differences in BMI ($p=0.70$) or waist circumference ($p=0.19$) between severe and non-severe CAD, and there was no correlation between increased BMI ($r=0.12$; $p=0.24$) or waist circumference ($r=-0.05$; $p=0.64$) and severity of CAD. The lack of difference in these two measures of obesity may have contributed to the lack of relationship in CAD severity and risk of OSA.

A significant inverse correlation between risk of OSA and age was observed. Previous reports suggest that individuals with OSA under 50 years of age have more severe cardiovascular consequences (280), and cardiovascular risk associated with OSA decreases with age (29). Those at high risk of OSA were significantly younger than those at low risk of OSA, however this cohort consisted of participants 40 years and older with an average age of 68 years. Incidence of hypertension (102), AF (281), and a higher risk all cause-mortality (282)

have also been found to be more common in younger individuals with OSA. This cohort included several CAD comorbidities, with hypertension found in more than half of participants, almost one third had hyperlipidaemia, and 10% reported a family history of CVD. However, there were no significant differences in CAD comorbidities between low and high risk of OSA other than previous MI. Over time, recurrent IH may have cardioprotective effects. Ischaemic preconditioning may occur in the myocardium as a result of prolonged periods of time below the threshold required to cause significant injury, and adaptive pathways develop leading to myocardial survival during prolonged acute tissue hypoxia (193). Thus, the age of the cohort may have influenced the lack of relationship between CAD severity and risk of OSA observed. A younger cohort target should be considered for future study design.

Oxidative stress, present in OSA setting, generates dysfunctional oxidised lipids and reduces the capacity HDL-C to prevent LDL-C oxidation (36, 127, 128). IH also increases lipid delivery from the adipose tissue to the liver through an up-regulation of the sterol regulatory element-binding protein-1 and stearoyl-CoA desaturase-1, during the fasting state, increasing the synthesis of cholesterol esters and triglycerides (125, 126). However, results of investigations of the effects of OSA on lipid metabolism are conflicting, as lipid profile is not the primary point of investigation (129). The overall lipid profiles in this cohort were within normal ranges, and two thirds were treated with a statin, which may have some bearing on the lack of significant association between lipids and OSA risk. There were no significant differences in any lipids, glucose, or hs-CRP between OSA low risk and OSA high risk groups, and no significant correlations were present between OSA risk and total cholesterol, LDL-C levels, or triglycerides. A non-significant inverse correlation was present between HDL-C levels and OSA50 score.

There are limitations to this study that should be considered. This study enrolled participants referred for a clinically indicated catheterisation at a single centre, and selection

bias maybe present. A larger sample size may also be required to elucidate the association between OSA risk and CAD severity. The use of antiplatelets is low for the population under investigation. However, medication use was collected prior to catheterisation procedure. Antiplatelet use and other medications are likely to have been revised post procedure. Angiographic imaging produces a two-dimensional silhouette of the lumen that does not include the vessel wall. Therefore, quantification of atherosclerotic plaque volume was not possible in this analysis. However, the Gensini score, used in this study to measure CAD severity, is not only a standardised and well validated measure of global obstructive burden, but has also been demonstrated to associate with adverse cardiovascular outcomes (266, 267). This study was cross-sectional in nature and did not include outcome follow-up, a link between CAD parameters evaluated and OSA and its treatment effects could not be determined. The OSA50 questionnaire was developed as an OSA risk prediction tool, not a diagnosis of OSA. Participants did not go on to have overnight PSG for diagnosis of OSA as a part of this study. Therefore, prevalence nor severity of OSA could be compared to severity of CAD. The components of the OSA50 questionnaire of age, waist circumference, witnessed apnoeas, and snoring were validated as predictive of OSA with the intention of developing a simple questionnaire to be administered in the primary care setting. Other factors influencing the development of OSA not included in the questionnaire may have a stronger relationship in the setting of coronary atherosclerosis. Previous reports utilising the BQ resulted in associations with CAD (270) and hypertension (271). Despite the complex scoring system, the more detailed questions regarding snoring may have produced a correlation with atherosclerotic burden in this cohort, as the vascular consequences of inflammation and endothelial injury induced by the vibrations of snoring are transmitted through soft tissues surrounding the pharynx to the carotid artery wall (58) increasing the risk of developing CAD.

In conclusion, increased risk of OSA was not associated with CAD severity in patients presenting with symptomatic coronary artery disease. Symptoms suggestive of OSA correlate with cardiovascular events. Yet, the global or focal measures of obstructive disease as measured by angiography do not appear to be associated. Mechanisms other than obstructive disease maybe more likely to contribute to the relationship between OSA and CVD. In the future, large-scale serial studies are warranted that target the association between the risk of and diagnosis of OSA, and the effects of coronary artery disease treatment on OSA severity.

Chapter 4:

THE RELATIONSHIP BETWEEN INFLAMMATORY AND ANGIOGENIC FACTORS WITH SYMPTOMS SUGGESTIVE OF OBSTRUCTIVE SLEEP APNOEA AND SEVERITY OF CORONARY ARTERY STENOSIS

ABSTRACT

Background: Inflammatory and angiogenic factors play an important role in the pathogenesis of atherosclerosis. The pathogenesis of cardiovascular complications in OSA is not fully understood. This study aimed to assess whether symptoms suggestive of OSA are associated with coronary artery stenosis severity, angiogenic function, and vascular inflammation on a cellular level in an *in vitro* setting.

Methods: An OSA risk prediction questionnaire (OSA50) was administered to patients undergoing clinically indicated coronary angiography. Study participants (n=30) were divided into low, moderate, and high OSA risk. Coronary artery stenosis severity was determined by angiographic Gensini score, and the maximum stenosis was recorded. Serum collected at the time of the catheterisation was added to tumour necrosis factor-alpha-stimulated HUVECs in culture. Endothelial gene expression of markers of vascular inflammation (VCAM-1, ICAM-1), and angiogenesis (VEGFA, HIF-1α) were measured by RT-PCR (reverse transcription polymerase chain reaction). Angiogenesis capacity of treated HUVECs was assessed using the Matrigel tubulogenesis assay.

Results: The mean age was 65 years, the median BMI was 30.74 kg/m^2 (IQR (26.28, 33.91)), and 30% were female. The median LDL-C was 69.6 mg/dL (IQR 50.3, 104.4). There were no significant differences in lipid measures between the three groups of OSA risk. The median Gensini score was 22.75 (IQR 13, 53), and the mean maximum stenosis was 52.8% (SD 37.78). Tubule numbers were significantly lower in cells treated with serum from OSA high risk group (19.8±3.8) compared to low (56.6±4.6; p=0.0003) and moderate OSA risk (51.3±4.6; p=0.002). No significant differences were present between the gene expression of markers of inflammation and OSA risk.

Conclusion: Patients at high OSA risk demonstrated differences in angiogenic potential, but not in atherosclerotic disease burden or vascular inflammation.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

4.1 Introduction

The mechanisms of OSA associated with contributing to the development of atherosclerosis are complex as OSA is a heterogeneous disease characterised by multiple mechanisms and complications such as intermittent hypoxemia, hypercapnia, negative intrathoracic pressure increase, and arousal. IH caused by OSA is considered to contribute not only to the cascade of events leading to cardiovascular disease onset but also its progression. In the previous chapter we were unable to demonstrate a difference in measures of plaque burden between those deemed to be at lower and higher OSA risk. We were subsequently interested in investigating potential differences in mediators of CV risk beyond plaque burden.

Inflammation has been shown to play a central role in all phases of atherosclerosis (135, 136). Different types of inflammatory reactions are involved in the initiation and progress of atherosclerosis. Inflammatory cells, mainly monocytes, adhere to the endothelium and release inflammatory mediators, such as intracellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1). ICAM-1 and VCAM-1 have been considered as biomarkers for the detection of endothelial dysfunction in patients with CAD (283), and OSA has been associated with increase circulating levels of ICAM-1 and VCAM-1 (284).

Angiogenesis is the process by which new blood vessels are formed from pre-existing ones (285). It is an important physiological response to hypoxia following stimuli, such as increased oxygen demand in embryonic development or ischaemic conditions (286). Angiogenesis is driven by the main transcription factor, HIF-1α, in the setting of hypoxia (286). HIF-1α is able to be ubiquitinated and degraded in an oxygen-dependent manner when oxygen levels are normal. Under low oxygen conditions, HIF-1α is protected from degradation, allowing it to translocate into the cell nucleus, where it stimulates angiogenesis via the upregulation of several pro-angiogenic genes, including vascular endothelial growth factor A (VEGFA), a master regulator of angiogenesis (286). VEGFA augments angiogenesis by

binding and activating its receptor VEGFR2 to switch on downstream signalling pathways that drive endothelial cell proliferation, vascular permeability, migration, and tube formation (286). Angiogenesis may be impacted in patients with OSA as a result of repetitive night-time hypoxia.

4.1.1 Aims and rationale of study

The objective of this study was to investigate the relationship between angiogenic function and vascular inflammation on a cellular level in an *in vitro* setting by various degrees of OSA risk in patients undergoing clinically indicated angiography.

4.1.2 Hypothesis

The hypotheses of this study were that increases in endothelial cell makers of inflammation would associate with symptoms suggestive of OSA, and symptoms suggestive of OSA would result in reduced angiogenic function.

4.2 Methods

4.2.1 Study outline

This prospective cross-sectional study included 30 participants, and the participants were required to fulfil the eligibility criteria listed below.

Eligibility Criteria

- Males and females age ≥ 40 years of age
- A clinical indication for a coronary angiogram
- Patients with stable ischaemic heart disease (stable angina) or recent acute coronary syndrome (unstable angina, non-ST elevation myocardial infarction, ST-elevation myocardial infarction)
- No condition that in the opinion of the responsible physician or investigator renders the participant unsuitable for the study such as co-morbid disease with severe

disability; significant memory, perceptual, or behavioural disorder or inability to provide informed consent.

The following demographic and clinical information were collected from participants prospectively: gender, age, height, weight, body mass index, waist circumference clinical presentation (such as ST-elevation myocardial infarction, non-ST-elevation myocardial infarction, unstable angina, or stable angina), cardiovascular risk factors (smoking, diabetes mellitus, hypertension, hyperlipidaemia, or family history of premature coronary artery disease), previous myocardial infarction, previous stroke.

Coronary angiography was performed via the percutaneous radial or femoral approach using standard angiographic techniques. A maximum stenosis percentage was captured in patients with or without significant lesions, defined as greater than or equal to 50% stenosis in major epicardial vessels or their branches. Global CAD severity was expressed based on the Gensini score (265) and classified as mild [less than 10], moderate [10-50], or severe [greater than 50]. During the clinically indicated catheterisation procedure, 20 mls of blood were collected for measurement of serum lipids, standard biochemistry. The OSA50 (264) was administered as clinical OSA prediction questionnaire of low risk [2], moderate [5], and high [10] risk. The ESS (268) was administered to detect daytime sleepiness with scores 11-24 classified as sleepier, and 0-10 less sleepy.

4.2.2 Cell culture experiments

4.2.2.1 Endothelial cell markers

A cell culture model of endothelial cell markers was used to study the relationship between inflammatory and angiogenic factors with symptoms suggestive of OSA. The stored serum from each participant was added to cells in culture with the method described below.

HUVECs were obtained from fresh umbilical cords donated by the Women's and Children's Hospital, North Adelaide. They were plated in gelatin-coated flasks at a density of

10000 cells per cm^2. They were cultured using MesoEndo Cell Growth Medium (Cell Applications, San Diego, CA, USA) supplemented with an extra 5% of foetal bovine serum (FBS) to make a total of 10% FBS. Passage 3 HUVECs were plated onto 6-well plates until they reached 75% confluence, with 2 ml of media used per well. They were then washed twice with warm sterile PBS and then cultured for 24 hours in EBM-2 basal media plus SingleQuot kit supplements and growth factors without the serum aliquot (Lonza, Basel, Switzerland). The serum was taken from participants was added for 24 hours at a concentration of 10%. Control conditions used 10% foetal bovine serum. All wells were then washed twice with warm sterile PBS, and then fresh EBM-2 basal media plus SingleQuot kit supplements and growth factors except for serum was added again. For each condition, there was either TNF or no TNF added for 4 hours in serum-free media. For TNF conditions, human TNF-α (Sigma-Aldrich, St. Louis, MO, USA) was added at a concentration of 10 ng/ml. This dose and duration have been demonstrated to significantly increase cell adhesion molecule expression (287, 288).

The cell culture media was then aspirated from each well, immediately placed on dry ice and stored at -80°C. The cells were washed with PBS at 4°C, and the Tri-reagent method was then used to extract RNA (ribonucleic acid) from the cells. The media from cultured cells was aspirated, and then the cells were washed with 1 ml of cold (4°C) PBS. After the PBS was aspirated, 500 µl of TRI Reagent® (Sigma-Aldrich, St. Louis, MO, USA) was added to each well of a 6-well plate and immediately frozen at -80°C. After thawing at room temperature, cells were scraped off of 6-well plates using a cell scraper, and the cell/TRI reagent solution was transferred to sterile 1.5 ml microcentrifuge tubes. One tenth of the TRI reagent volume (50 µl) of 1-Bromo-3-chloropropane (Sigma-Aldrich, St. Louis, MO, USA) was added, and the mixture was vortexed for 15 seconds, ensuring complete mixing of both phases. The solution was centrifuged for 15 mins at 19000 x *g* at 4°C. The aqueous phase was transferred to another sterile 1.5 ml microcentrifuge tube, and 250 µl of isopropanol was added. This solution was

transferred to a -20°C freezer for RNA precipitation. At least 24 hours later, the solution was vortexed, and centrifuged at 19000 x *g* at 4°C for 15 mins. The supernatant was removed using a pipette, and the RNA pellet was then washed by adding 250 µl of ice-cold 75% ethanol. The solution was vortexed and centrifuged at 19000 x *g* for 10 mins at 4°C. The ethanol was removed, and the RNA pellet was air-dried for 10 mins. Pre-warmed (60°C) nuclease-free water (20 µl) was added to the RNA pellet, followed by vortexing and brief centrifugation. The RNA solution was kept on ice until RNA quantification was performed, using the NanoDrop 8000 spectrophotometer. RNA was stored at -80°C until use.

The RNA was quantified using a NanoDropTM 8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of RNA was normalised between all samples and then RNA was reverse transcribed to cDNA. Prior to conversion to cDNA, all RNA samples to be used for the same experiment were normalised to the same concentration. RNA was converted to cDNA using iScriptTM Reverse Transcription Supermix for RT-PCR (Bio-Rad, Hercules, CA, USA). A desired quantity of RNA for the cDNA synthesis reaction was determined. RNA was thawed on ice, and the required volume was added to iScript RT Supermix, which comprised 20% of the total reaction mix (i.e. 4 µl iScript for a 20 µl cDNA reaction). The remaining volume was nuclease-free water. The mix was vortexed and centrifuged, and then incubated in a thermal cycler (T100TM Thermal Cycler, Bio-Rad) using the following protocol: priming – 5 mins at 25°C, reverse transcription – 30 mins at 42°C, and reverse transcription inactivation – 5 mins at 85°C. cDNA was stored at 4°C until further use.

Reverse transcription PCR was performed to measure the relative expression of the following human genes: VCAM- 1, ICAM-1, VEGFA and HIF-1α, with GAPDH being used as a reference gene (see Table 4.1 for primers). RT-PCR was performed using a Bio-Rad CFX ConnectTM Real-Time PCR Detection System. Reactions were 20 µl in volume and were performed in 96-well plates. Reaction mixes consisted of 10 µl of Bio-Rad SsoAdvancedTM

Universal SYBR® Green Supermix, 6 µl of nuclease-free water, 1 µl of forward primer, 1 µl of reverse primer, and 2 µl of cDNA. The reaction protocol was set at: 50°C for 2 mins, 95°C for 15 mins, then 40 cycles of: 94°C for 15 seconds, 60-64°C (primer-specific based on optimisation) for 30 seconds, 72°C for 30 seconds, then 65°C to 95°C at 0.5°C increments for 5 seconds each. Primer stocks were diluted to a concentration of 100 µM, and the working solution was 10 µM. All RT-PCR reactions were performed with reference genes (B2M for human, and 36B4 for mouse). All PCR reactions used 100ng of cDNA. Due to the large number of 96-well PCR plates used for this experiment, control conditions were used on each PCR plate. These conditions were the culture conditions of (1) HUVECs cultured in 10% FBS, and (2) HUVECs cultured in 10% FBS plus TNF-α. Inter-PCR-plate calibration was performed using Bio-Rad CFX Manager™ software version 3.0.1224.1015 (Hercules, CA, USA). Data analysis was performed using GraphPad Prism 7 (La Jolla, CA, USA).

Table 4.1: Human Primers for qRT-PCR

	Forward	**Reverse**
GAPDH	5'- GAAGGCTGGGGCTCATTT-3'	5'-CAGGAGGCATTGCTGATGAT-3'
VEGFA	5'- TGTGAATGCAGACCAAAGAAAGA3'	5'-TGCTTTCTCCGCTCTGAGC-3'
HIF1-α	5'-AACGTCGAAAAGAAAAGTCTCG-3'	5'-CCTTATCAAGATGCGAACTCACA-3'
VCAM	5'-AAGGCAGGCTGTAAAAGAATTGC-3'	5'-AGGTCATGGTCACAGAGCCACC-3'
ICAM	CAGAGTTGCAACCTCAGCCT	GGACACAGA TGTCTGGGCA TT

4.2.2.2 Matrigel tubulogenesis assay

Cells were treated with human serum as previously described. A matrigel assay was conducted to observe angiogenesis *in vitro*. Matrigel Basement Membrane (Corning) was thawed out at 4°C overnight. A total volume of 40 µL/well of matrigel was added to a 96-well flat bottom plate, and was allowed to set for 1 hour at 37°C. Cells were cultured in 75cm^2 flask until 80% confluency, trypsinised and counted using a haemocytometer before seeding at a density of 1x10^5 cells/mL onto 40 µL of Matrigel. Cells were incubated at 37°C, and observed

at hourly time points after treatment using Axio Zeiss microscope at 40X magnification (see Figure 4.1). At 4 h tubules were observed and used as a final time point for analysis. Images were analysed using NIH Image J software (Bethesda), whereby number of tubules were recorded and measured.

4.2.3 Statistical and data analysis

4.2.3.1 Sample size calculation

The sample size of 10 participants per group was based on a previous study (289). The difference in TNF-α-induced VCAM gene expression in the active treatment group was 45% and was statistically significant. The power calculation for 3 groups with a significance level of 0.05, with 80% power, for a difference between groups of 0.45, requires a sample size of 10 subjects per group.

4.2.3.2 Statistical methods

Participant data were de-identified. Statistical analysis of patient characteristics, biochemical measures, sleep parameters, coronary artery disease severity was performed using Stata, version 14.2 (StataCorp). The D'Agostino-Pearson normality test was performed to determine whether continuous data were normally-distributed. Normally distributed data were analysed using the One-way ANOVA. Results were expressed as mean ± SEM. If continuous data were not normally-distributed, analysis was performed using the Kruskal-Wallis test. Results were expressed as median + IQR. Statistical correlations were analysed using a linear regression model. Statistical analysis of cell culture experiments was performed using GraphPad Prism 7 software. Data are expressed as mean ± SEM for all parameters. A two-way ANOVA (Tukey's *post hoc* comparison test) was used for tubulogenesis and gene expression analysis. Statistical significance was set at the 0.05 level.

4.2.4 Ethical and site approval

Ethics approval was obtained from the Royal Adelaide Human Research Ethics

Committee, and site specific authorisation was obtained from the Central Adelaide Local Health Network.

4.3 Results

4.3.1 Participant characteristics

The demographics of participants are summarised in Table 4.2. Overall, the mean age was 65 years, the median BMI was 30.74 kg/m^2 (IQR (26.28, 33.91)), and 30% were female. Participants in the OSA high risk group were significantly younger (p=0.01), and had a significantly higher BMI (p<0.001) than those in the low and moderate OSA risk groups. There were significantly more participants with diabetes (p=0.02) in the OSA high risk group compared to the low and moderate OSA risk groups. There were no other significant differences in medical history nor any concomitant medication use between the three groups.

Table 4.2: Summary of participant characteristics by OSA risk

	Overall	OSA Low Risk	OSA Moderate Risk	OSA High Risk	p value
Number of Participants	30	10	10	10	
Age, Mean (SD)	65.0 (4.66)	67.8 (3.88)	65.5 (4.17)	61.7 (4.06)	0.01
Female, n (%)	9 (30%)	3 (30%)	3 (30)	3 (30)	1.00
BMI (kg/m^2), Median (IQR)	30.74 (26.28, 33.91)	26.18 (25.16, 26.83)	29.99 (29.38, 31.49)	36.74 (33.46, 43.29)	<0.001
Waist Circumference (cm), Mean (SD)	105.57 (19.21)	90 (5.16)	103.6 (20.70)	123.1 (10.89)	<0.001
Smoking, n (%)					
Never	9 (30)	5 (50)	4 (40)	0 (0)	0.06
Previous	13 (43)	3 (30)	5 (50)	5 (50)	
Current	8 (27)	2 (20)	1 (10)	5 (50)	
Hypertension, n (%)	20 (67)	4 (40)	7 (70)	9 (90)	0.08
Hyperlipidaemia, n (%)	8 (27)	2 (20)	3 (30)	3 (30)	1.00
Diabetes, n (%)	9 (30)	3 (30)	0 (0)	6 (60)	0.02
Atrial Fibrillation, n (%)	5 (17)	3 (30)	1 (10)	1 (10)	0.57
Previous MI, n (%)	8 (27)	1 (10)	2 (20)	5 (50)	0.19
CVD Family History, n (%)	4 (13)	0 (0)	2 (20)	2 (20)	0.51
Statin Intensity					
High, n (%)	13 (43)	3 (30)	5 (50)	5 (50)	0.72
Moderate, n (%)	5 (17)	2 (20)	2 (20)	1 (10)	1.00
No Statin Therapy, n (%)	12 (40)	5 (50)	3 (30)	4 (40)	0.89
Antiplatelet therapy, n (%)	18 (60)	5 (50)	6 (60)	7 (70)	0.89
ß-Blockers, n (%)	9 (30)	3 (30)	3 (30)	3 (30)	1.00
ACE Inhibitor, n (%)	11 (37)	4 (40)	4 (40)	3 (30)	1.00
ARB, n (%)	8 (27)	2 (20)	2 (20)	4 (40)	0.67

The biochemical measures of the participants are summarised in Table 4.3. There were no significant differences in participant lipid parameters, hs-CRP, or glucose levels between the three groups.

Table 4.3: Summary of biochemical measures by OSA risk

	Overall	OSA Low Risk	OSA Moderate Risk	OSA High Risk	p value
Number of Participants	30	10	10	10	
Total Cholesterol, median (IQR), mmol/L	3.90 (2.83, 4.74)	4.25 (3.37, 4.85)	3.58 (2.49, 4.09)	3.28 (2.80, 4.53)	0.65
LDL-C, median (IQR), mmol/L	1.80 (1.30, 2.70)	2.40 (1.55, 2.90)	1.80 (1.10, 2.60)	1.65 (1.30, 2.50)	0.59
HDL-C, median (IQR), mmol/L	1.06 (0.95, 1.44)	1.27 (0.82, 1.56)	1.06 (0.97, 1.18)	1.00 (0.95, 1.16)	0.93
Triglycerides, median (IQR), mmol/L	1.27 (0.97, 1.49)	1.25 (0.91, 1.89)	1.27 (0.97, 1.49)	1.30 (1.18, 1.43)	0.88
hs-CRP, median (IQR), mg/L	3.28 (1.37, 4.40)	2.46 (0.82, 4.27)	3.79 (1.61, 4.21)	2.88 (1.65, 7.69)	0.75
Glucose, median (IQR), mmol/L	6.01 (5.31, 6.46)	6.38 (5.99, 6.85)	5.75 (5.21, 5.96)	6.22 (5.63, 7.50)	0.14

The sleep parameters of the participants are presented in Table 4.4. Twenty-three percent of participants were previously diagnosed with sleep apnoea. There were significantly more participants previously diagnosed with sleep apnoea the OSA high risk group. Overall the participants scored low on the Epworth Sleepiness Scale.

Table 4.4: Summary of sleep parameters by OSA risk

	Overall	**OSA Low Risk**	**OSA Moderate Risk**	**OSA High Risk**	**p value**
Number of Participants	30	10	10	10	
Sleep Apnoea, n (%)	7 (23)	0 (0)	1 (10)	6 (60)	0.006
Epworth Sleepiness Scale, Median (IQR)	3.5 (1, 8)	2.5 (0, 5)	4.5 (2, 7)	6 (1, 10)	0.49
Epworth Sleepiness Scale Low, n (%)	25 (83)	9 (90)	8 (80)	8 (80)	1.00
Epworth Sleepiness Scale High, n (%)	5 (17)	1 (10)	2 (20)	2 (20)	

The measures of CAD severity of participants are presented in Table 4.5. Global and focal measures of CAD severity were calculated as Gensini score and maximum stenosis, respectively. These parameters were not significantly different between the three groups. On average, Gensini score corresponded to overall moderate CAD, and the maximum stenosis corresponded to a significant single stenotic lesion.

Table 4.5: Summary of coronary artery disease severity by OSA risk

	Overall	OSA Low Risk	OSA Moderate Risk	OSA High Risk	p value
Number of Participants	30	10	10	10	
Gensini Score					
Median (IQR)	22.75 (13.00, 53.00)	24.00 (4.50, 53.00)	29.25 (16.50, 67.50)	21.50 (18.00, 52.50)	0.66
Mild, n (%)	7 (23)	4 (40)	1 (10)	2 (20)	0.66
Moderate, n (%)	14 (47)	3 (30)	6 (60)	5 (50)	
Severe, n (%)	9 (30)	3 (30)	3 (30)	3 (30)	
Maximum Stenosis					
Mean (SD)	52.8 (37.78)	45.0 (42.75)	63.0 (38.02)	50.5 (33.70)	0.57
Non-significant Stenosis, n (%)	13 (43)	5 (50)	3 (30)	5 (50)	0.72
Significant Stenosis, n (%)	17 (57)	5 (50)	7 (70)	5 (50)	

4.3.2 In vitro studies

4.3.2.1 Angiogenic capacity of HUVECs

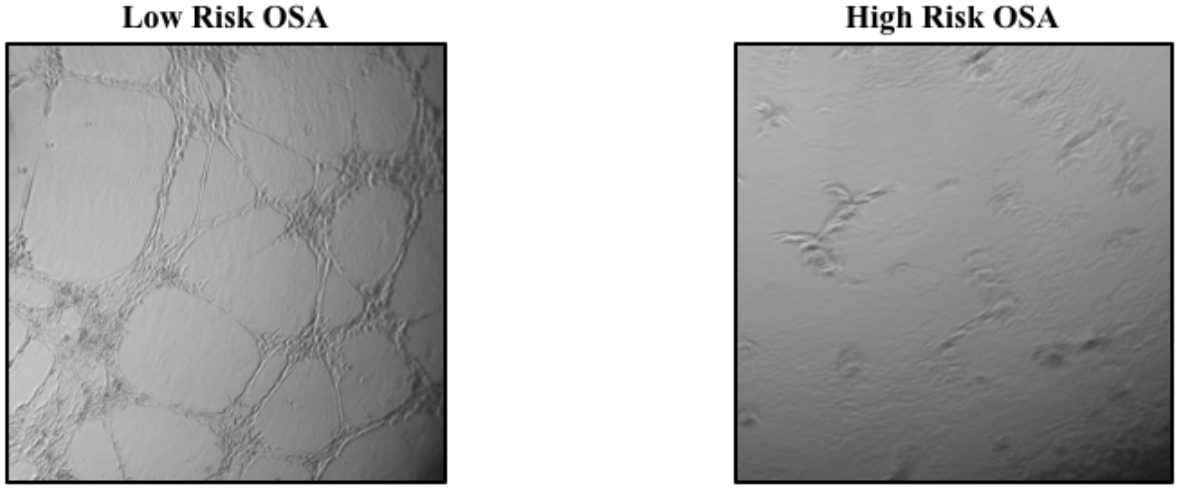


Figure 4.1: Matrigel tubulogenesis representative images. Example of low risk OSA (left), example of high risk OSA (right).

The number of tubules was significantly lower in the OSA high risk group compared to the low risk group (p= 0.0003) and the moderate risk group (p= 0.002, see Figure 4.2).

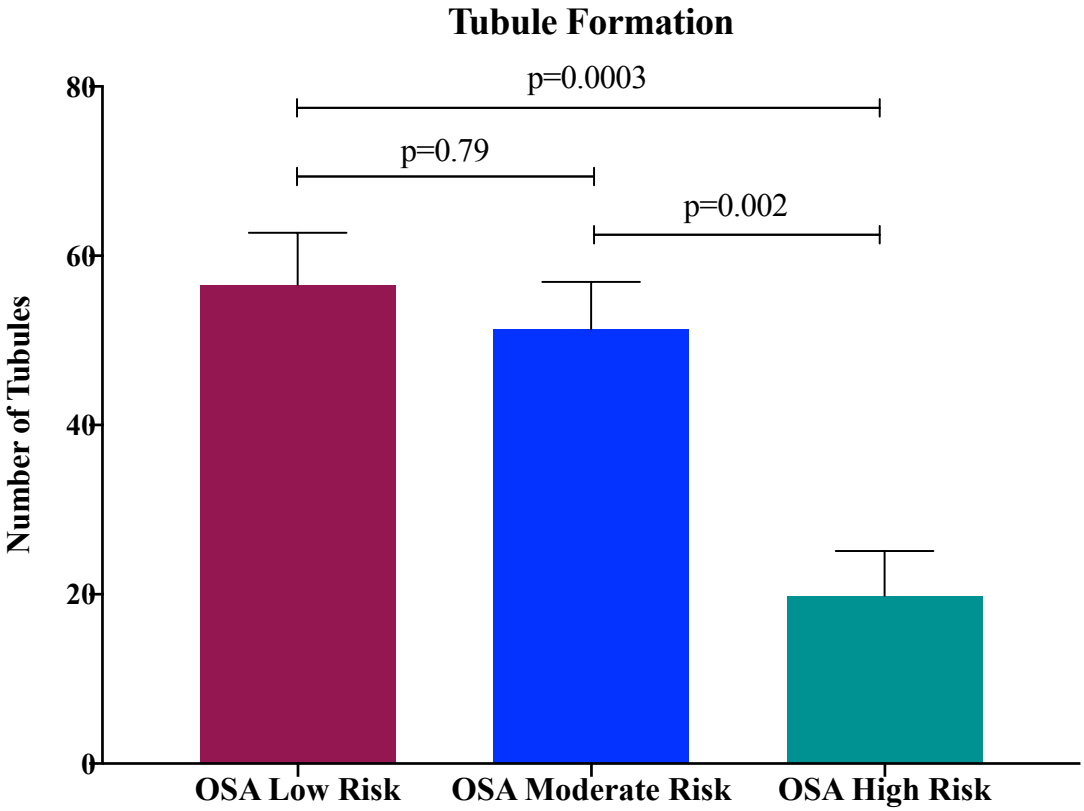


Figure 4.2: Tubule formation in HUVECs by OSA risk. n=10 per group. Results expressed as mean ± SEM.

4.3.2.2 Gene expression

Gene expression of VCAM-1, ICAM-1, VEGFA and HIF-1α was measured by RT-PCR in HUVECs co-incubated with serum from participants, and with or without TNF stimulation.

The VCAM-1 expression of TNF-stimulated HUVECs co-incubated with serum obtained from study participants was not significantly different between OSA risk groups (p=0.18; see Figure 4.3).

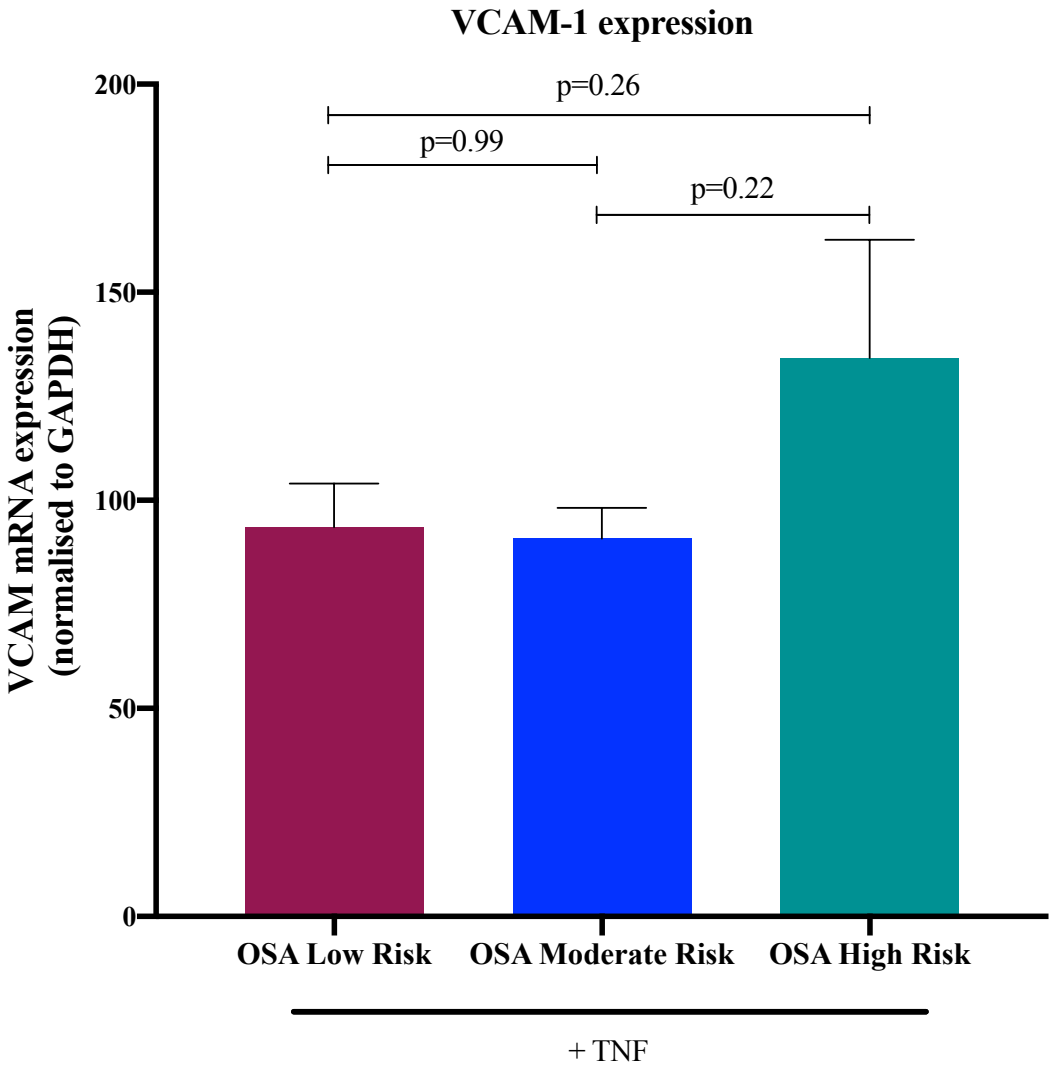


Figure 4.3: Gene expression of VCAM-1 by TNF-stimulated HUVECs co- incubated with serum, normalised to GAPDH. n=10 per group. Results expressed as mean ± SEM.

ICAM-1 expression was also not significantly different between OSA risk groups (p=0.36, see Figure 4.4).

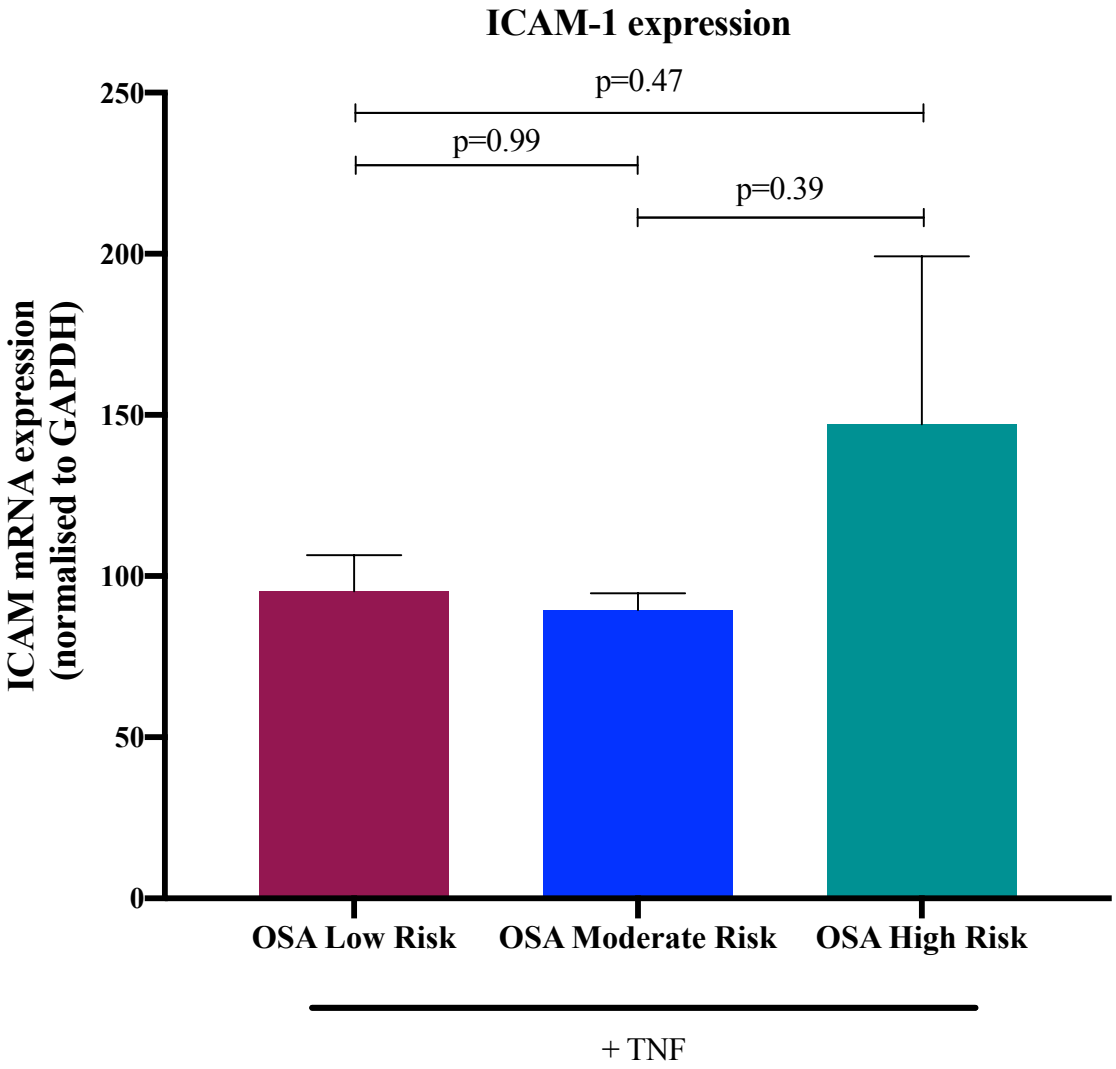


Figure 4.4: Gene expression of ICAM-1 by TNF-stimulated HUVECs co- incubated with serum, normalised to GAPDH. n=10 per group. Results expressed as mean ± SEM.

VEGFA expression was also not significantly different between OSA risk groups (p=0.79, see Figure 4.5).

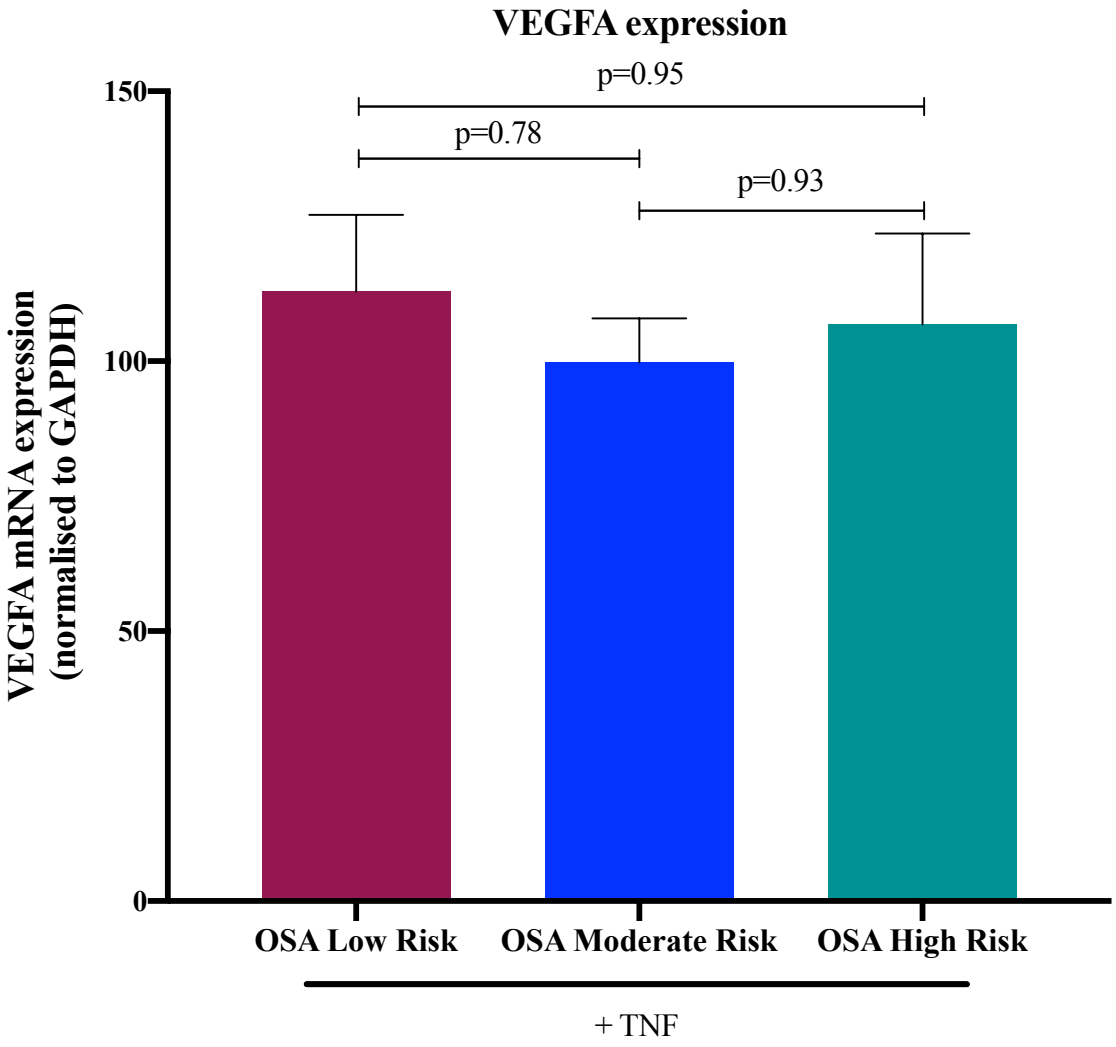


Figure 4.5: Gene expression of VEGFA by TNF-stimulated HUVECs co- incubated with serum, normalised to GAPDH. n=10 per group. Results expressed as mean ± SEM.

HIF-1α expression was also not significantly different between OSA risk groups (p=0.54, see Figure 4.6).

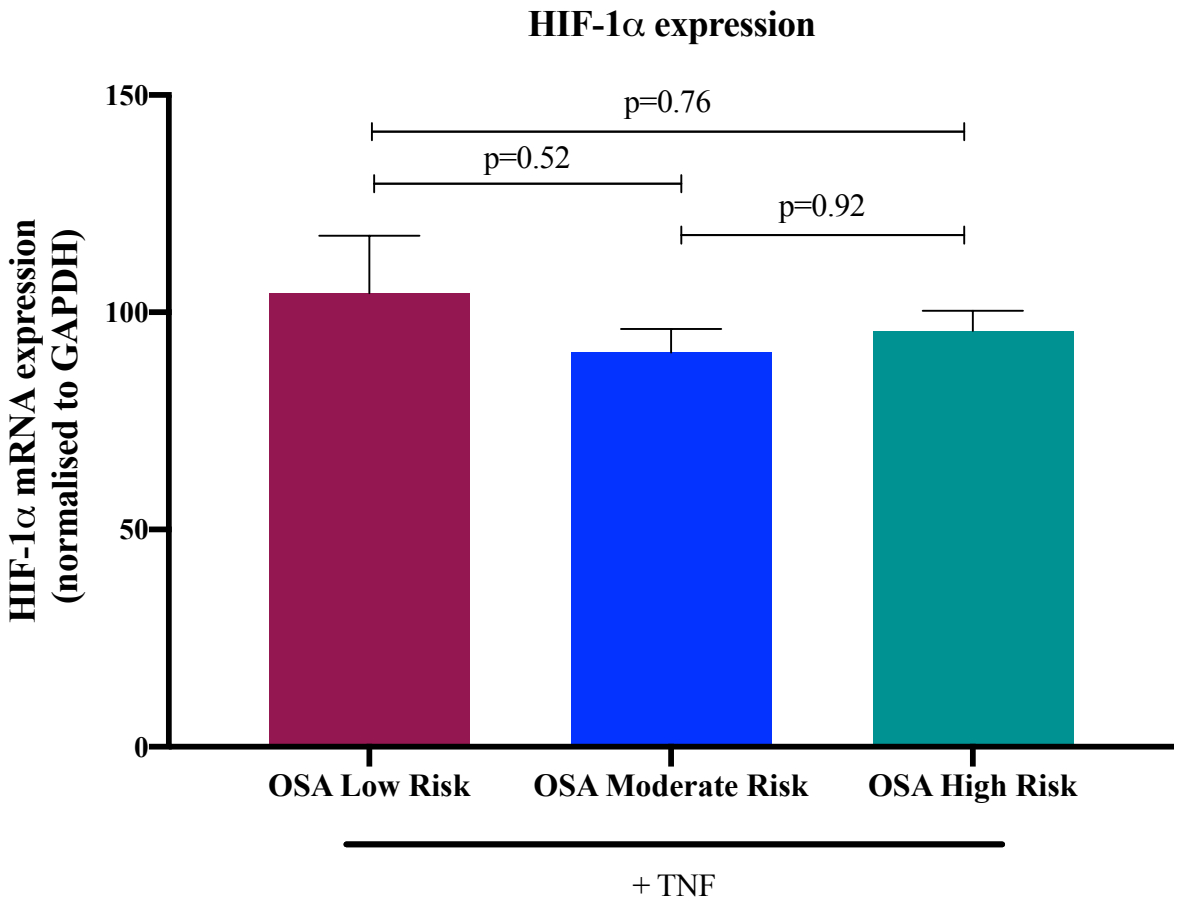


Figure 4.6: Gene expression of HIF-1α by TNF-stimulated HUVECs co- incubated with serum, normalised to GAPDH. n=10 per group. Results expressed as mean ± SEM.

4.3.3 Correlations between inflammatory markers and angiogenic factors, symptoms suggestive of sleep apnoea and CAD severity

The expression of VCAM-1, ICAM-1, VGFA and HIF-1α by stimulated HUVECs, and angiogenesis capacity of treated HUVECs were correlated with CAD severity as measured by Gensini score to determine whether any significant associations exist.

Gene expression

A significant inverse correlation was present between VEGFA expression and Gensini score. A non-significant inverse correlation was present between HIF-1α and Gensini score. No significant correlations were present between VCAM-1 or ICAM-1 and Gensini score (see Figure 4.7).

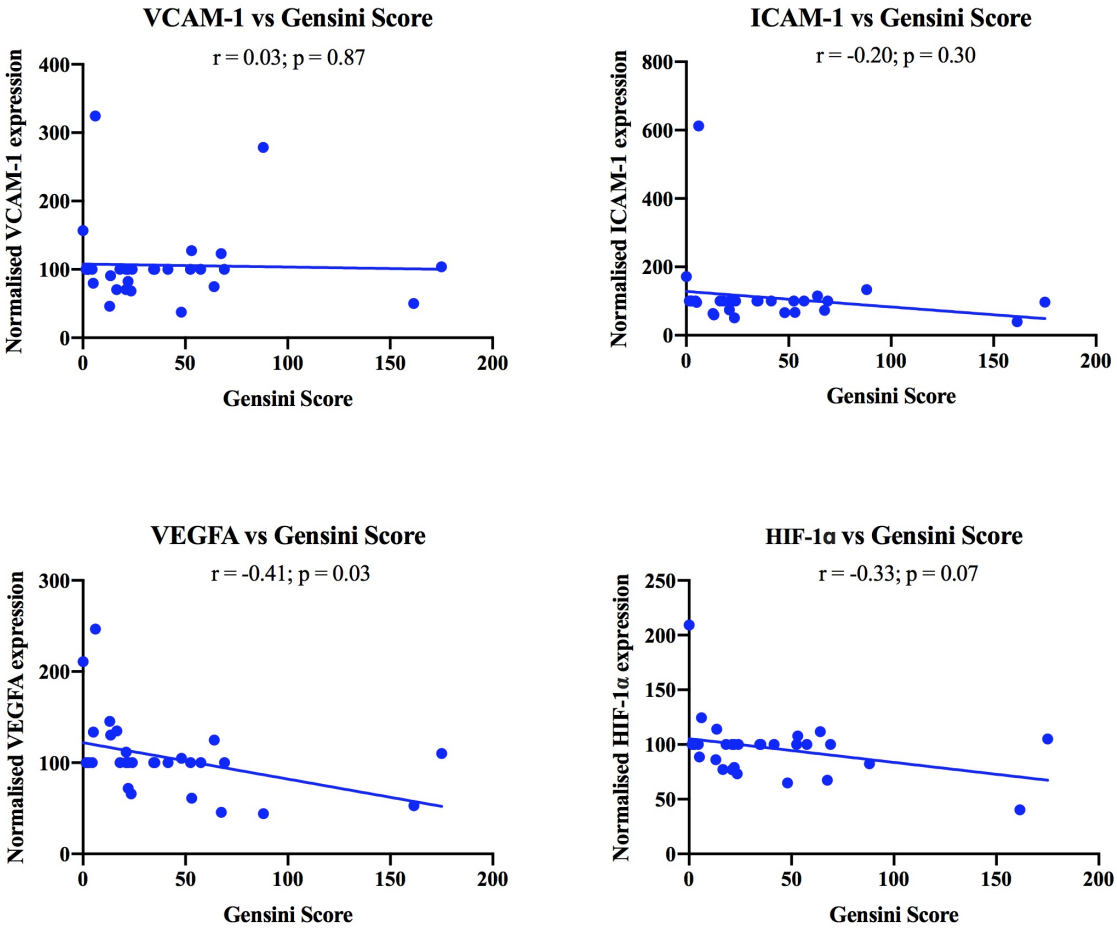


Figure 4.7: Correlations of gene expression of VCAM-1, ICAM-1, VEGFA, HIF-1α and Gensini score. n = 30 for each graph.

Tubule formation

There was no association between number of tubules and Gensini score (see Figure 4.8).

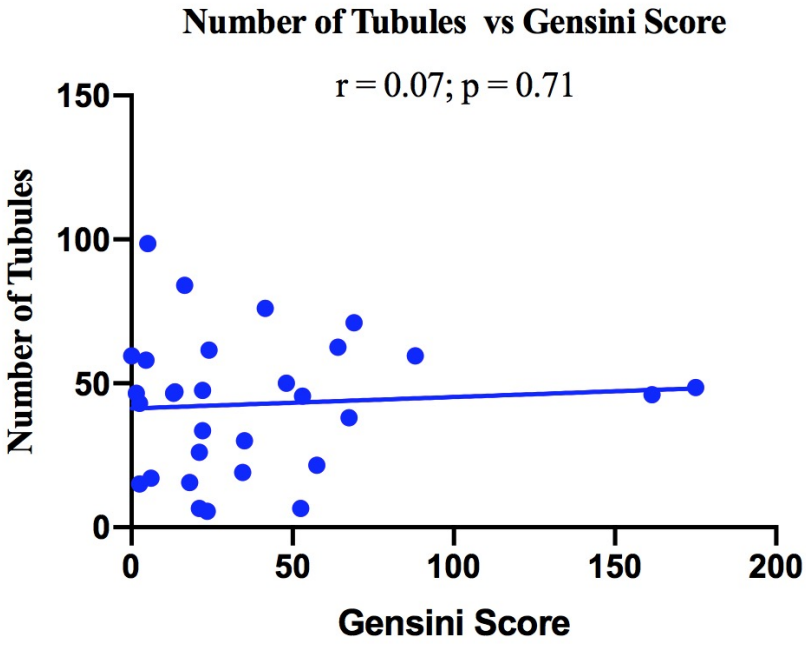


Figure 4.8: Correlations of tubule formation in HUVECs and Gensini score. n = 30.

4.4 Discussion

This study investigated the relationship between angiogenic function and vascular inflammation on a cellular level in an *in vitro* setting and OSA risk in patients undergoing clinically indicated angiography. The patients at a high risk of OSA demonstrated significantly less angiogenic potential than those at moderate and low risk of OSA with the reduced number of tubules formed. However, there were no differences in gene expression of markers of angiogenic function (VEGFA, HIF-1α) or vascular inflammation (ICAM-1, VCAM-1) or atherosclerotic disease burden as measured by Gensini score or the OSA risk groups.

Oxidative stress has been shown to be one of the main causes of endothelial impairment in patients with OSA, and hypoxia, a consequence of OSA, causes damage to the endothelium (290). The progression of endothelial damage mediated by inflammation is the widely accepted pathophysiology present in the setting of OSA (78, 291). The chronic IH of OSA causes anoxia and reoxygenation in which contributes to the production of oxygen radicals and elicits local and systemic inflammation. Increased ICAM-1 and VCAM-1, inflammatory mediators, have been implicated in endothelial dysfunction (283). However, there were no differences in terms of OSA risk on gene expression of ICAM-1 and VCAM-1 measured in TNF-stimulated HUVECs in culture. This study investigated risk of OSA, not the effects of OSA on inflammatory gene expression. Previously, serum levels of ICAM-1, VCAM-1 were significantly increased in individuals with OSA compared to the healthy control group, and significantly decreased after three months of treatment with CPAP (292). Upregulation of NF-κB expression, has also been previously observed in OSA patients. This upregulation increased the expression of downstream inflammatory mediators and cytokines, such as TNF-α and IL-6, and hs-CRP. In this study, there was also no difference in hs-CRP according to OSA risk. Results are conflicting in the association between OSA and elevated levels of the systemic inflammatory marker, CRP. Studies investigating patients with sleep disordered breathing have

reported higher levels of CRP in patients with OSA compared with age- and BMI-matched controls (143-147). Yet, other studies have not observed this relationship, finding obesity rather than OSA to be the key predictor of elevated CRP among OSA patients (150, 151).

The development of atherosclerosis is complex and involves various mechanisms such as inflammation and endothelial dysfunction (293), critical steps in the initiation in the pathogenesis of atherosclerosis (294, 295). There were no associations of gene expression with increased CAD severity found in this study. While the upregulation of the expression of ICAM-1 and VCAM-1 have been implicated in the initiation of endothelial injury (296) leading to atherosclerosis, a positive association of ICAM-1 and VCAM-1 gene expression and CAD severity may not be present in the established disease found in this cohort.

Blood-derived endothelial progenitor cells (EPCs) play a crucial role in maintaining vascular homeostasis by replacing dysfunctional endothelium and enhancing tissue repair after an ischemic vascular insult through an endogenous repair mechanism (297, 298). EPCs are mobilised by hypoxia through HIF-1α- and VEGF-dependent pathways (299), and they promote coronary collateral formation while improving endothelial functions by integrating into new capillaries or the injured vessels. In the setting of acute MI, EPCs go to the site of the ischemic myocardium and participate in vascular and cardiac repair (297, 300). Endothelial dysfunction, atherosclerosis, and poor cardiovascular outcomes have been associated with low EPC numbers. Growing EPCs *in vitro* can induce proliferation and differentiation, and colonies secret angiogenic growth factors such as VEGF inducing endothelial tube formation. Previously, endothelial tube formation was significantly higher in acute MI patients with OSA compared to acute MI patients without OSA [27]. This finding suggests that IH may have a role in promoting protective functions of EPCs in the setting of acute MI. The results of the present analysis show a significant reduction in tubule formation in the OSA high risk group, suggesting endothelial dysfunction.

Despite the reduction in tubule formation in the OSA high risk group, there were no significant differences in the gene expression of VEGFA or HIF-1α between the OSA risk groups. Previously, a significant increase in HIF-1α expression was found in patients with severe OSA, yet there were no significant changes were found in mild and moderate OSA (301). This result suggests that the longer periods of anoxia and shorter periods of reoxygenation, found in severe OSA, results in more HIF-1α produced and accumulated, while the necessary balance in mild and moderate OSA could be achieved through the downregulation of HIF-1α degradation. Hypoxia becomes more critical in severe cases of OSA. However, there were associations with VEGFA and HIF-1α and increased severity of CAD, suggesting endothelial dysfunction.

Hypoxia, a major consequence of OSA, is now recognised as a key driving force for angiogenesis by its induction of the HIF-1α/VEGFA angiogenic signalling pathway. However, angiogenesis is a dynamic and complex process that have yet to be explored in the setting of OSA. Various other signalling pathways beyond the classical pathway are implicated in new vessel formation. These include notch/delta, ephrin/Eph receptor, roundabout/slit, and netrin/UNC (uncoordinated) receptor families as well as intracellular proteins such as hedgehog and sprouty (302). Hypoxia in the setting of OSA may disrupt one of these pathways inhibiting tubule formation.

There are limitations to this study that warrant consideration. This was a small study, the numerical differences seen in gene expression may have been greater in the setting of a larger cohort, such as the differences in expression of VCAM-1 and ICAM-1 between the OSA high risk and lower risk groups, in particular. This study was an exploratory analysis, and the samples were from the study in the prior chapter. We sought to investigate the relationship between angiogenic function and vascular inflammation on a cellular level by various degrees of OSA risk. While this study had statistical power, this analysis should lead to larger studies

in the future. This study was also enrolled at a single centre that referred participants for a clinically indicated catheterisation, therefore a selection bias maybe present. The OSA50 questionnaire was developed as an OSA risk prediction tool, not a diagnosis of OSA. Participants did not go on to have overnight PSG for diagnosis of OSA as a part of this study. Therefore, possible prevalence and severity of OSA were not determined. The components of the OSA50 questionnaire were validated as predictive of OSA with the intention of developing a simple questionnaire to be administered in the primary care setting. Other factors influencing the development of OSA not included in the questionnaire may have a stronger relationship with gene expression of markers of angiogenic potential and vascular inflammation in the setting of coronary atherosclerosis.

In summary, patients at high OSA risk demonstrated differences in angiogenic potential, but not in atherosclerotic disease burden or vascular inflammation. Angiogenic potential is impaired in patients at high risk of OSA based on the low number of tubules formed in the functional assay. However, there were no differences in gene expression of angiogenic markers. The clinical implications of these findings require further investigation, as some evidence suggests protective effects of IH patterns with upregulation of HIF-1α and VEGFA leading to increased tubule formation, while others indicate a dysfunctional endothelium with low numbers of tubule formation.

Chapter 5:

THE RELATIONSHIP BETWEEN EPICARDIAL FAT VOLUME AND DENSITY WITH OBSTRUCTIVE SLEEP APNOEA AND CORONARY PLAQUE BURDEN

ABSTRACT

Background: OSA is frequently associated with obesity. However, BMI is not a good measure of body adiposity. EAT is a metabolically active fat depot, and its thickness has been shown to correlate with OSA. CT permits quantification of EAT volume and density. Lower epicardial fat density associates with the presence of inflammatory white EAT. No studies have evaluated the association between EAT parameters with coronary plaque burden in patients with OSA. The aim of this study was to compare the association between OSA severity with EAT volume, EAT density and BMI.

Methods: Participants who were referred for the investigation of possible OSA and scheduled for a clinically indicated cardiac CT underwent clinically indicated overnight PSG. The degree of OSA was determined by AHI, and severe OSA was defined as AHI greater than 30. Participants underwent clinically indicated CTCA. EAT volume and Leaman scores were quantified on CTCA, and significant coronary plaque burden was defined as Leaman score greater than 8.3.

Results: Participants (n=71, age 59.7 years, BMI 32.6 kg/m^2, 24% female, 31% severe OSA) had a median EAT volume 98 mL and Leaman score 6.57. EAT volume correlated with AHI (r=0.29; p=0.01), BMI (r=0.23, p=0.05) and Leaman score (r=0.29; p=0.01). EAT mean density correlated with Leaman score (r=-0.36; p=0.002) but not AHI (r=0.06; p=0.62) or BMI (r=0.01; p=0.95). AHI correlated with EAT volume (r=0.29; p=0.01) and BMI (r=0.28, p=0.02) but not mean EAT density (r=0.06; p=0.62), age (r = 0.12; p=0.96) or gender (r=0.10; p=0.43). On multivariate linear regression, EAT volume continued to independently associate with AHI (p=0.002). Patients with severe OSA had higher EAT volume (116 mL vs. 86 mL, p=0.048) compared to those without severe OSA. On multivariate analysis, EAT volume independently associated with the presence of severe OSA (p=0.01).

Conclusion EAT volume associated with OSA severity and may be a potential mediator of cardiovascular risk in these patients.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

5.1 Introduction

OSA is characterised by repetitive episodes of upper airway obstruction during sleep as a result of repetitive partial or complete collapse of the upper airway. The features accompanying OSA, sleep fragmentation, intermittent hypoxia (IH), daytime somnolence, are associated with increased CVD risk (303). However, the associations between cardiovascular and metabolic disorders along with obesity in OSA patients are complex (42). Studies investigating the relationship between BMI and the severity of OSA have reported conflicting results (304-307), indicating that perhaps the regional distribution of fat, as opposed to BMI may associate with OSA severity.

Patients with OSA not only have a high prevalence of central obesity, but also have increased visceral fat (308). Visceral adipose tissue associates with CV risk factors (309) and systemic markers of inflammation (310). EAT is the layer of visceral fat deposited around the heart between the myocardium and visceral pericardium. Eat surrounds the coronary arteries, and is considered metabolically active tissue as well as a local source of pro-inflammatory signalling by cytokines (311) linked to CVD development. Increased thickness of EAT has also been shown to associate with metabolic syndrome, (312) and to be an independent risk factor for CVD (313). Various methods have been used to determine EAT thickness (314, 315) and volume (316).

5.1.1 Aims and rationale of study

This study sought to investigate the relationship of OSA severity with EAT volume, EAT density and BMI.

5.1.2 Hypothesis

The hypothesis of this study was EAT volume would be greater in those with severe OSA compared to those without severe OSA in patients referred for the investigation of

possible OSA and scheduled for a clinically indicated cardiac CT, and increasing EAT volumes would associate with AHI increase.

5.2 Methods

5.2.1 Study design

Participants in this study included 71 patients referred for the investigation of possible OSA and scheduled for a clinically indicated cardiac CT, including coronary computed tomographic angiogram (CTA). This timeframe was the period over which both CCTA and PSG data were available on the medical record.

5.2.2 Overnight sleep studies

Patients who had a diagnostic PSG with at least 2 hours of recorded sleep or a split PSG with 2 hours of sleep in the diagnostic portion were included. A standard clinical recording montage was employed. This montage included: electroencephalogram (EEG); bilateral electrooculogram (EOG); mentalis/submentalis and anterior tibialis electromyogram (EMG); electrocardiogram (ECG); nasal pressure cannula; oronasal thermistor; thoracic and abdominal respiratory effort bands; and fingertip oximetry. Sleep studies were staged and scored according to standard criteria, using the American Academy of Sleep Medicine (AASM) 2007 Alternate criteria up until 31 December 2014 (47 sleep studies), and using the AASM 2012 Recommended criteria from 1 January 2015 (72 sleep studies) (317, 318). The degree of OSA was determined by AHI, the number of apnoea or hypopnoea events per hour of recording. Severe OSA was defined as AHI greater than 30. An ODI was calculated as the number of times per hour during the oximetry recording that the blood oxygen saturation level drops by greater than or equal to 4 percentage points from baseline. The daytime sleepiness questionnaire, ESS, was also administered.

5.2.3 CT imaging protocol

CT acquisition details have been previously described (319). In brief, patients underwent cardiac CT using a 320-row detector CT scanner (Aquilion ONE, Toshiba Medical Systems, Tochigi, Japan). The CT protocol consisted of rest CTA followed by CTP. Scan parameters for rest CTA were as follows: detector collimation, 320 x 0.5 mm; tube current, 300-500 mA (depending on BMI); tube voltage, 120 kV; gantry rotation time, 350 ms; and temporal resolution, 175 ms. Prospective electrocardiographic gating was used covering phases 70%-80% of the R-R interval.

5.2.4 CCTA-adapted Leaman score

The CT-LeSc methodology has been described previously (320). Briefly, the score is determined by 3 sets of weighting factors: (1) localisation of the coronary plaques, accounting for dominance, (2) type of plaque, with a multiplication factor of 1 for calcified plaques and of 1.5 for noncalcified and mixed plaques, and (3) degree of stenosis, with a multiplication factor of 0.615 for nonobstructive (less than 50% stenosis) and a multiplication factor of 1 for obstructive (greater than or equal to 50% stenosis) lesions. The CT-LeSc on a patient level was calculated as the sum of the partial CT-LeSc of all evaluable coronary segments. Significant coronary plaque burden was defined as Leaman score greater than 8.3.

5.2.5 Epicardial adipose tissue volume and density measurements

Measurement of EAT was performed according to previously described methods (321) using the QFAT software (Cedar Sinai, USA). The EAT was defined as tissue located between the pericardium and the myocardium from the bifurcation of the pulmonary arteries to the cardiac apex within the Hounsfield unit (HU) range of -200 to -50. The border of the pericardium was manually delineated in the axial plane and consequently corrected in the sagittal and coronal planes. EAT volume and density was then automatically quantified by the software (see Figure 5.1).

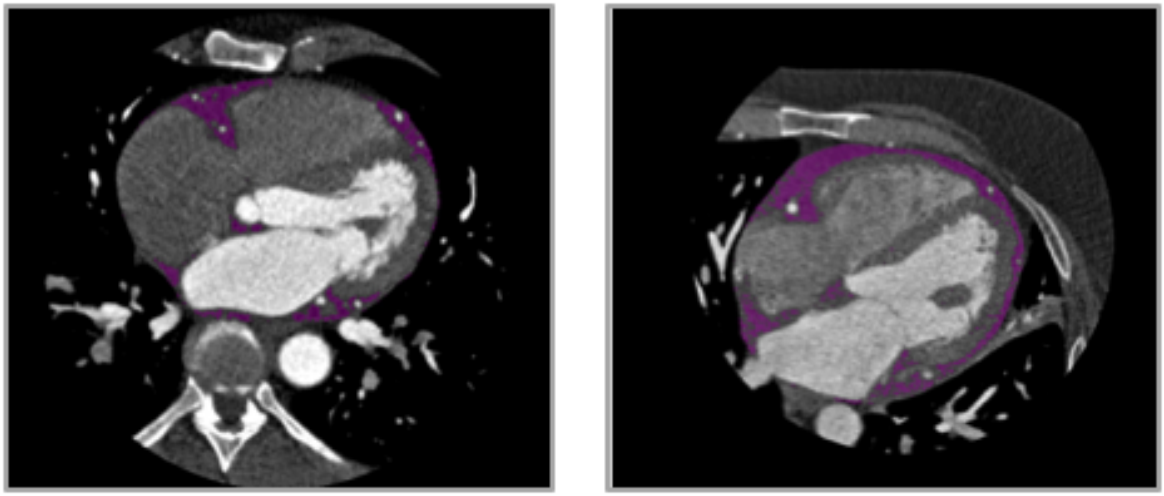


Figure 5.1: EAT volume representative images acquired from CTCA. Example of low EAT volume (left), high EAT volume (right); delineation of the EAT in purple surrounding the heart.

5.2.6 Statistical and data analysis

5.2.6.1 Sample size calculation

The sample used in this analysis consists of all available data from this cohort of participants, and done so with the understanding that variability and standard deviation would need to be taken into account. A sample size of 66 patients, 22 with severe OSA and 44 with non-severe OSA, provided 85% power at a 2-sided α of 0.05 to detect a difference of 30 ml in EAT volume between those with severe OSA and with non-severe OSA (322, 323).

5.2.6.2 Statistical methods

All statistical analyses were performed using Stata, version 14.2 (StataCorp). Continuous variables are presented as mean ± standard deviation if normally distributed or median with interquartile range if non-parametric. Categorical variables are displayed as frequencies (percentage). Normality was assessed visually using a histogram and using the Shapiro-Wilk test. The t-test or Wilcoxon Rank Sum test was used to compare continuous and

categorical variables and ANOVA, or Kruskal-Wallis test was used to compare means/medians across more than 2 groups as appropriate. Pearson's correlation coefficient or the Spearman's rank correlation were used to compare continuous variables. Data was analysed on a per-patient basis. A two-sided p-value of 0.05 was adopted to demonstrate statistical significance.

5.3 Results

5.3.1 Clinical characteristics of the study population

The demographics and clinical characteristics of the participants are summarised in Table 5.1. Overall, the mean age was 59.7 years, the median BMI was 32.6 kg/m^2, 24% were female, and 31% had severe OSA. There were no significant differences in the demographics of age (57.6 years vs. 60.6 years; p = 0.30), gender (14 % female vs. 29 % female; p = 0.23), BMI (33.79 kg/m^2 vs. 32.04 kg/m^2; p = 0.17) history of diabetes (18% vs. 24%; p = 0.76) or hypertension (45% vs. 63%; p = 0.20), in those with and without severe OSA. Those with severe OSA had significantly higher AHI (58.79 vs. 13.23; p <0.001), ODI 43.92 vs 4.40; p <0.001), and EES scores (9.5 vs. 7; p = 0.01) compared to those without severe OSA (see Table 5.1).

Table 5.1: Demographics, Clinical Characteristics by OSA severity

	Overall	Non-severe OSA	Severe OSA	p value
Number of participants	71	49	22	
Age, Mean (SD)	59.7 (11.0)	60.6 (10.3)	57.6 (12.4)	0.30
Female, n (%)	17 (24)	14 (29)	3 (14)	0.23
BMI (kg/m^2), Median (IQR)	32.58 (29.03, 37.39)	32.04 (28.89, 35.71)	33.79 (30.48, 40.59)	0.17
Smoking, n (%)	10 (14)	5 (10)	5 (23)	0.27
Hypertension, n (%)	41 (58)	31 (63)	10 (45)	0.20
Hyperlipidaemia, n (%)	35 (49)	26 (53)	9 (41)	0.44
Diabetes, n (%)	16 (23)	12 (24)	4 (18)	0.76
CVD Family History, n (%)	29 (41)	20 (41)	9 (41)	1.00
Obstructive Sleep Apnoea Characteristics				
Severe OSA, n (%)	22 (31)	0 (0)	22 (100)	
Oxygen desaturation index, Mean (SD)	11.1 (3.1, 24.0)	4.4 (2.0, 13.2)	43.9 (21.3, 64.5)	< 0.001
Apnoea–hypopnea index, Median (IQR)	21.0 (11.7, 44.7)	13.2 (10.0, 21.8)	58.8 (44.7, 64.2)	< 0.001
Epworth Sleepiness Scale score, Median (IQR)	8.0 (6.0, 11.0)	7.0 (4.0, 10.0)	9.5 (7.0, 15.0)	0.01

5.3.2 Coronary plaque burden and epicardial fat measures

Coronary plaque burden and epicardial fat volume and mean density were measured in all participants. Overall, the median Leaman score was 6.57, and 39% of the cohort had significant plaque burden. The overall median EAT volume was 98 ml, and the mean EAT mean density was -85.2 HU. There were no significant differences in Leaman scores (9.01 vs. 6.39; p = 0.21) or EAT mean density (-84.91 HU vs. -85.35 HU; p = 0.78) between those with and without severe OSA. EAT volume (116 ml vs. 86 ml; p = 0.048) was significantly higher in those with severe OSA compared to those without severe OSA (see Table 5.2).

Table 5.2: Coronary plaque burden and epicardial fat measures by OSA severity

	Overall	Non-severe OSA	Severe OSA	p value
Number of participants	71	49	22	
Leaman Score, Median (IQR)	6.57 (2.15, 10.43)	6.39 (0.92, 9.46)	9.01 (3.22, 10.58)	0.21
Leaman Score > 8.3, n (%)	28 (39)	16 (33)	12 (55)	0.12
Leaman Score > 5, n (%)	41 (58)	27 (55)	14 (64)	0.61
Epicardial Fat Volume (ml), Median (IQR)	98 (58, 129)	86 (58, 119)	116 (65, 160)	0.048
Epicardial Fat Mean Density (HU), Mean (SD)	-85.2 (6.2)	-85.35 (6.47)	-84.91 (5.55)	0.78

5.3.3 Correlations of AHI, EAT volume, and EAT mean density

AHI, EAT volume, and EAT mean density each correlated with a number of parameters to determine whether any significant associations exist.

Apnoea–hypopnea index

Positive, significant associations were present between AHI and both BMI and Leaman score. There was no significant association between AHI and EAT density (see Figure 5.2).

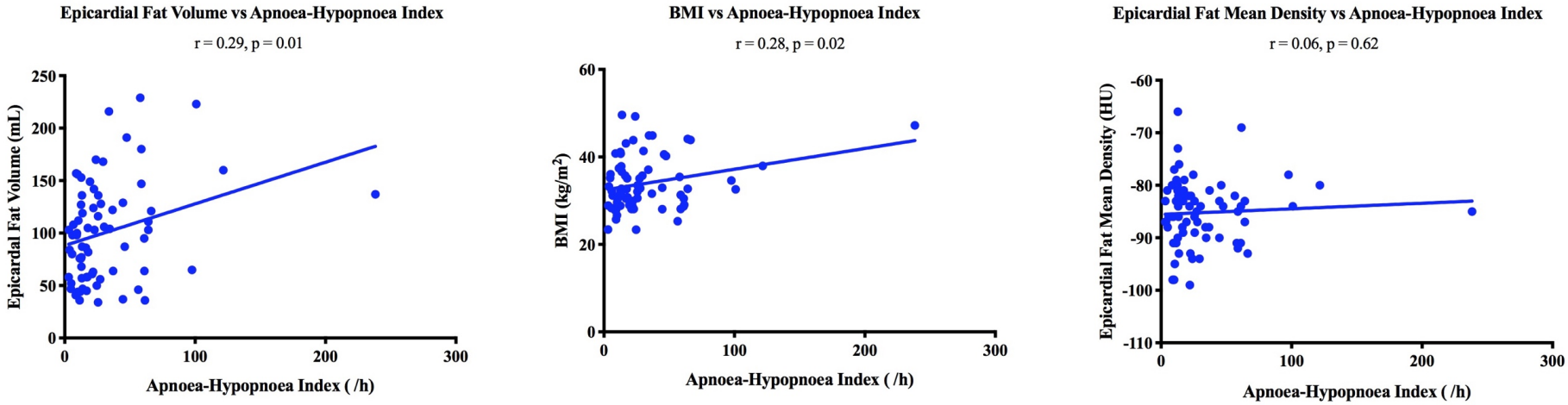


Figure 5.2: Correlations of apnoea–hypopnea index, BMI, Leaman score and epicardial fat volume. n = 71 for each graph.

Epicardial fat volume

Positive, significant associations were present between ODI, BMI, and Leaman scores and EAT volume (see figure 5.3).

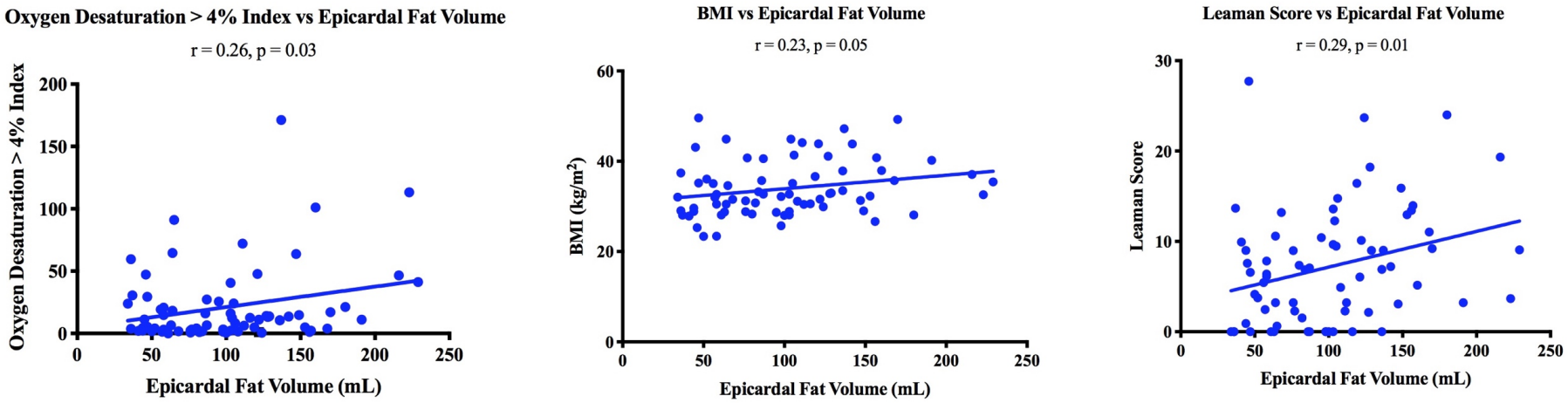


Figure 5.3: Correlations of oxygen desaturation >4% index, BMI, Leaman score and epicardial fat volume. n = 71 for each graph.

Epicardial fat mean density

An inverse, significant association was present between Leaman scores and EAT density. No significant correlations were present between ODI or BMI and EAT mean density (see Figure 5.4).

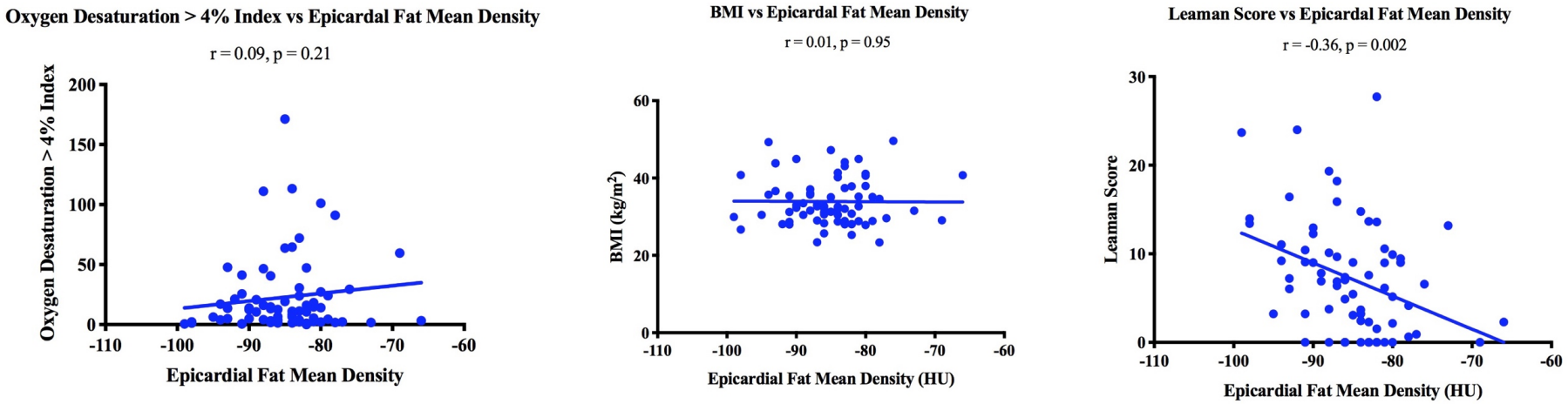


Figure 5.4: Correlations of oxygen desaturation >4% index, BMI, Leaman score and epicardial fat mean density. n = 71 for each graph.

5.3.4 Independent predictors of AHI and OSA severity

Independent predictors of AHI and OSA severity were tested to determine whether any significant associations exist.

Apnoea–hypopnea index

EAT volume (β = 0.008; p = 0.002) was independently associated with AHI. BMI (β = 0.24; p = 0.15) and EAT density (β = 0.037; p = 0.06) did not significantly associate with AHI (see Table 5.3).

Table 5.3: Multivariable linear regression model of determinants of apnoea–hypopnea index

	β	Standard Error	p-value
Epicardial Fat Volume	0.008	0.002	0.002
Epicardial Fat Mean Density	0.037	0.019	0.06
Body Mass Index	0.24	0.017	0.15

OSA severity

EAT volume (β = 0.01; p = 0.01) was independently associated with OSA severity. BMI (β = 0.03; p = 0.45) and EAT density (β = 0.08; p = 0.11) did not significantly associate with OSA severity (see Table 5.4).

Table 5.4: Multivariable binary regression model of determinants of OSA severity

	β	Standard Error	p-value
Epicardial Fat Volume	0.01	0.007	0.01
Epicardial Fat Mean Density	0.08	0.052	0.11
Body Mass Index	0.03	0.045	0.45

5.4 Discussion

This study used CTCA imaging to measure EAT volume and coronary plaque burden in patients with OSA. Patients with severe OSA had higher EAT volume compared to those without severe OSA. EAT volume demonstrated to be associated with AHI, BMI, and plaque burden. In addition, EAT volume associated with the presence of severe OSA, independent of BMI.

EAT is now understood to not only be storage depot for adipose tissue, but a metabolically active organ with unique characteristics compared to subcutaneous and other visceral fat subtypes. EAT, directly surrounding the coronary arteries, has been suggested to promote the development of arterial stiffness (324), calcification (325), and atherosclerosis (326). Epicardial fat thickness is reported to be an independent risk factor for major adverse cardiac events (313). However, investigations exploring the relationship between EAT and severity of coronary stenosis have reported mixed results. One study found EAT volume to be the strongest independent determinant of the presence of totally occluded arteries (327), and another study did not find significant associations between EAT volume and coronary calcium scores, presence of significant stenosis, or abnormal myocardial perfusion (328). Furthermore, an investigation reported a significant relationship between EAT volume and presence of coronary atherosclerosis, but there was no relationship between increased EAT volume and increasing severity of atherosclerosis (329). Yet, consistent with previous reports (330), EAT volume significantly correlated with plaque burden in the present study.

CT imaging attenuation has been shown to reflect metabolic activity, composition and lipid content of adipose tissue within the fat range [−250 to −50 Hounsfield units (HU)], and higher density reflects increased vascularity (331). Preclinical data has demonstrated significant increases in CT attenuation of non-cardiac adipose tissue in rodents upon metabolic activity as determined by histopathological analysis (332). While a population-based cross-

sectional study found associations, independent of BMI and fat mass, between abdominal fat density and cardiometabolic risk markers (333). While the current analysis found a significant relationship between epicardial fat mean density and EAT volume and plaque burden, there was no relationship in regard to severity of OSA. Epicardial fat density is linked to adipose lipid content and vascularity, and is associated with insulin resistance and diabetes (333). The cohort included in this analysis did not have any differences between those with and without severe OSA in those with hyperlipidaemia or diabetes.

The inflammatory process in OSA has been suggested to be initiated by intermittent hypoxia, a unique kind of hypoxia (334-336), characterised by desaturations related to respiratory events occurring during sleep and arousal, and reperfusion following them. Inflammatory markers, such as CRP, IL-6 and IL-8, as well as TNF-α have been found to be higher in patients with OSA (337). Endothelial dysfunction related to the inflammatory process present in OSA has also been implicated in the development of cardiovascular diseases (338, 339). A few imaging studies using coronary angiography and CT imaging have also shown a positive association between OSA and CAD (340). Intravascular plaque imaging studies have also found a correlation between sleep induced breathing abnormalities and coronary atherosclerotic plaque volume (72) as well as moderate to severe OSA has been independently associated with a larger TAV when compared to those with no to mild OSA (73). However, there were no differences in plaque burden as measured by CTCA between those with and without severe OSA in this study.

As a result of the close proximity to coronary vessels, epicardial fat acts as a paracrine gland that releases inflammatory markers, promoting the inflammatory process within atherosclerotic plaques (341). Inflammatory adipokines, such as TNF-α, IL-6 and 1b, and monocyte chemoattractant protein-1, are released from epicardial fat, resulting in the development of cardiovascular events (342). In this analysis, there was an association with

increased AHI and increased EAT volume, independent of BMI. The chronic IH present in OSA has also been shown to influence the function of adipocytes and appears to be a key factor in adipocyte dysfunction, proliferation and hypertrophy (343). As such, adiposity is stimulated increasing fat accumulation in the neck that contributes to upper airway narrowing, and thus to OSA severity. While there are common pathophysiological pathways shared by obesity and OSA, and many patients with OSA are overweight or obese, BMI has not been shown to be a good predictor of OSA severity (304). In accordance with previous reports, this analysis did not result in an association between OSA severity and BMI, yet did show a relationship between EAT volume and OSA severity.

Patients with cardiac adiposity are usually obese and more likely to have hypertension, diabetes mellitus, arrhythmias, and atherosclerosis (344). OSA has also been associated with CAD and hypertension (42). Previously, studies have investigated the relationship between OSA and epicardial fat. One investigation found a correlation between OSA and epicardial fat thickness in obese individuals with OSA. Additionally, epicardial fat was reported to be thicker in patients with OSA and metabolic syndrome compared to those with OSA alone (345). While another study reported that epicardial fat thickness increased as OSA severity increased in a cohort of obese individuals with OSA (346). Epicardial fat thickness has also been reported to be greater in those with OSA compared to controls in a cohort of non-obese individuals (347). When investigating differences in gender, significant differences in epicardial fat thickness between severe OSA patients and controls, and mild OSA patients and controls were found in females. There was no significant correlation between epicardial fat thickness and OSA severity found in males (348). Treatment with CPAP has also been shown to reduce EAT volume in patients with OSA (349).

This study has limitations that should be considered. The sample size is small, and there is not an equal distribution of participants between the severe OSA and non-severe OSA

groups, nor did we include a control group. The study was cross-sectional; thus, a link between EAT parameters evaluated and OSA and its treatment effects could not be determined. Medications were not reported in the analysis. Therefore, the influence of medications, such as statins, on the relationship between OSA severity and EAT volume cannot be taken into account. Biochemical measures were also not captured, and associations between lipid profiles, inflammatory markers, and EAT volume cannot be evaluated. Potential population sampling biases may exist as the study was conducted at a single centre.

In summary, EAT volume associated with OSA severity, independent of BMI. The local inflammatory effect of EAT volume on the coronary arteries may play an important role in the development of atherosclerosis, and should be considered as a possible mediator of cardiovascular risk in patients with OSA. Furthermore, serial investigations are warranted to elucidate the effect of treatment for OSA on changes in EAT volume and density.

Chapter 6:

THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA ON SHORT TERM CHANGES IN CORONARY ATHEROSCLEROTIC PLAQUE IN PATIENTS WITH ACUTE CORONARY SYNDROME

ABSTRACT

Background: OSA has been identified as a major CVD risk factor. Several factors link OSA and atherosclerosis, including metabolic abnormalities, and the activation of inflammatory and oxidative pathways. However, the relationship between OSA and the burden and progression of coronary atherosclerotic plaque has not been investigated.

Methods: Serial coronary IVUS was used to compare changes in TAV and PAV (percent atheroma volume) in male patients presenting with an ACS after 12 weeks of treatment. Participants included in a subgroup analysis of a randomised multi-centre study with (n = 16) and without (n = 32) OSA, after propensity score matching for age (median 59.3 yrs).

Results: Participants in the OSA group had a significantly greater BMI [median (IQR) 29 (29, 33) kg/m^2 vs. 27 (26, 30) kg/m^2; p=0.03)], significantly more prior PCI (38 vs. 13%; p=0.04), and significantly less were treated with a high intensity statin (31 vs. 78%; p=0.002), compared to the non-OSA group. LDL-C ([median (IQR) 2.17 (1.84, 3.15) mmol/L vs. 1.81 (1.45, 2.17) mmol/L; p=0.01) levels were significantly higher at baseline in the OSA group compared to the non-OSA group. There were no significant differences in changes in blood pressure, glucose, or lipid parameters between the two groups at follow-up. Significantly greater progression of TAV and PAV was observed in the OSA group (TAV [median (IQR)] +1.0 mm^3 (-9.1, 5.8) vs. -11.0 mm^3 (-15.5, -4.6) (p=0.007)); (PAV [median (IQR)] +0.4% (-1.9, 1.2) vs. -1.3% (-2.8, -0.3) (p=0.03)) compared to the non-OSA group. However, the between group differences in the changes in each of the measures of plaque burden were no longer significant (p=0.14) in a multivariate model after adjustment for cardiovascular risk factors and statin use.

Conclusion: OSA was associated with progression of atheroma volume in the short term in patients after an acute coronary syndrome.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

6.1 Introduction

OSA, characterised by repeated partial or complete collapse of the upper airway during sleep, leads to IH and frequent arousals (350). These breathing disturbances may result in increased sympathetic activation, blood pressure alterations, and vascular atherogenic changes (351-353). In the last decade, evidence has revealed OSA to be a risk factor contributing to adverse cardiovascular events with patients presenting with ACS (354).

Several mechanisms have been considered to cause ACS. Plaque rupture within an inflammatory setting and without, superficial plaque erosion, and microvascular spasms due to an imbalance of vasoconstrictor and vasodilator agents have all been implicated in the development of ACS. The properties that the coronary endothelium poses that play a role in the pathogenesis and course of ACS are anti-thrombotic, anti-proliferative and anti-inflammatory and most likely represent the linking element to OSA.

Studies performed on the carotid and peripheral arteries have shown an independent association between OSA and early markers of atherosclerosis (57, 63). A few imaging studies using coronary angiography and CT imaging have also shown a positive association between OSA and coronary artery disease (71, 340). However, these non-intravascular imaging modalities provide a limited measurement of the extent of coronary artery disease in overweight patients and preclude the accurate measurement of total atheroma volume.

Serial IVUS imaging of the coronary arteries has contributed to the current understanding of the factors that influence atherosclerotic disease progression and regression (355), including its association with clinical events (356, 357). The technique of measuring atheroma volume with the high imaging resolution of coronary IVUS has been described and validated previously (358).

6.1.1 Aim and rationale of study

The primary objective of this analysis was to determine if there are differences in short-term changes in plaque burden after an ACS in patients with and without OSA.

6.1.2 Hypothesis

The hypothesis of this study was that greater short-term progression of atherosclerotic plaque would be observed in patients with OSA compared to patients without OSA after an ACS event.

6.2 Methods

6.2.1 Study Population

A subgroup analysis was conducted in patients with and without OSA. The design of the CARAT study has been described previously (359). In brief, ACS patients with angiographic evidence of CAD with a PAV of at least 30% in the proximal 10-mm of the target artery at baseline were randomised to the high-density lipoprotein mimetic, CER-001 (3 mg/kg) or placebo. Coronary IVUS imaging was obtained at both baseline and following 2 weeks preceding treatment with 10 weekly intravenous infusions of CER-001 (3 mg/kg) or placebo. The patients with OSA (n = 16) were those that reported OSA in their medical history during initial trial enrolment.

6.2.2 Acquisition and analysis of serial IVUS images

The acquisition and serial analysis of IVUS images has been previously described in detail (360-362). Briefly, target vessels for imaging were selected if they contained no luminal stenosis greater than 50% angiographic severity within a segment of at least 30 mm length. Imaging was performed within the same coronary artery at baseline and at study completion. Patients meeting pre-specified requirements for image quality were eligible for randomisation. An anatomically matched segment was defined at the 2 time points on the basis of proximal and distal side branches (fiduciary points). Cross-sectional images spaced precisely 0.5 mm apart were selected for measurement. Leading edges of the lumen and external elastic

membrane (EEM) were traced by manual planimetry. Plaque area was defined as the area occupied between these leading edges. The accuracy and reproducibility of this method have been reported previously (363). The PAV was determined by calculating the proportion of the entire vessel wall occupied by atherosclerotic plaque, throughout the segment of interest as follows:

$$\text{PAV} = \frac{\sum(EEM_{area} - Lumen_{area})}{\sum EEM_{area}} \text{X } 100$$

TAV was calculated using the equation below to determine the summation of plaque area calculated for each image and subsequently normalised to account for differences in segment length between subjects (356).

$$\text{TAV}_{\text{normalised}} = \frac{\sum(EEM_{area} - Lumen_{area})}{Number\ of\ images\ in\ pullback} \text{X } Median\ number\ of\ images\ in\ cohort$$

EEM and lumen volumes were calculated by summation of their respective areas in each measured image and subsequently normalised to account for differences in length of arterial segments between subjects.

The post hoc analyses pooled results from both treatment groups. Infusing CER-001 did not promote atherosclerotic plaque regression in the primary analysis (364), thus allowing for a pooled analysis of patients in the characterisation of the natural history of patients with contemporary therapy post ACS. I played a pivotal role in image analysis for several serial regression/progression studies. I conceived the research question for this chapter and performed the analysis. I have worked closely with Professor Stephen Nicholls, the Global PI for the CARAT Study and my primary PHD supervisor, on the CARAT Study which gave me the unique opportunity to include this analysis in my thesis and perform this work on a short-term follow up study.

6.2.3 Statistical and data analysis

6.2.3.1 Sample size calculation

The sample used in this analysis consists of all available data from this cohort of participants, and done so with the understanding that variability and standard deviation would need to be taken into account. A sample size of 48 patients, 16 with OSA and 32 without OSA provided 80% power at a 2-sided α of 0.05 to detect a nominal difference of 1.0% in change in PAV assuming a 1.15% SD (361, 362, 364-366).

6.2.3.2 Statistical methods

All statistical analyses were performed using Stata 14.2 (StataCorp, Texas, USA). Descriptive statistics are presented as mean and standard deviation for normally distributed continuous variables; as median and inter quartile ranges for non-normally distributed continuous variables; and as frequency and percentage for categorical variables. A patient with OSA was age-matched with two patients without OSA using the propensity score method. Propensity score for the OSA was created using logistic regression model with age as a predictor. All OSA patients were matched with 2 non-OSA patients using the nearest neighbour method with replacement. Patients demographics including medications, lipid parameters and baseline and follow-up IVUS parameters were compared using independent t-test for normally distributed continuous variables; Mann-Whitney test for non-normally distributed continuous variables; Chi-squared or Fishers' Exact test for categorical variables. Analysis of Covariance (ANCOVA) was used to compare the change in IVUS parameters adjusted for the baseline measurement. ANCOVA was conducted using the rank transformation of IVUS variables. A two-sided p-value of less than 0.05 was considered significant.

6.2.4 Ethical and site approval

Independent ethics boards at each of the centres participating in the study approved the protocol and patients provided written, informed consent. The trial was registered with ClinicalTrials.gov (Identifier: NCT02484378).

6.3 Results

6.3.1 Clinical characteristics of the study population

Baseline demographics, clinical characteristics, and concomitant medication use are described in Table 6.1. Previously diagnosed OSA reported in the medical history of this cohort were male participants. Significant trends for between-group differences were noted across various baseline variables. The BMI (29 kg/m^2 vs. 27 kg/m^2; p=0.03) of the OSA group was significantly greater compared to the non-OSA group. There were significantly less participants with hypertension (38% vs. 81%; p=0.002), and significantly more participants had undergone a previous PCI (38% vs. 13%; p=0.04) in the OSA group compared to the non-OSA group. There was a significantly higher number of participants in the OSA group treated with a moderate intensity statin (63% vs. 22%; p=0.006) compared to the non-OSA group (see Table 6.1). Prior statin use was defined as statin use on any occasion prior to index ACS event.

Table 6.1: - Demographics, baseline clinical characteristics and baseline and concomitant medications by OSA status

	Non-OSA	OSA	p value
Number of participants	32	16	
Age, Mean (SD), y	59.3 (7.7)	59.3 (7.8)	1
White, n (%)	31 (97)	13 (81)	0.07
BMI kg/m^2, Median (IQR)	27 (26, 30)	29 (29, 33)	0.03
Hypertension, n (%)	26 (81)	6 (38)	0.002
Previous PCI, n (%)	4 (13)	6 (38)	0.04
Previous MI, n (%)	1 (3)	3 (19)	0.07
Smoking, n (%)	7 (22)	4 (25)	0.81
Diabetes, n (%)	4 (13)	5 (31)	0.12
Baseline statin use, n (%)	32 (100)	15 (94)	0.15
High intensity statin, n (%)	25 (78)	5 (31)	0.002
Moderate intensity statin, n (%)	7 (22)	10 (63)	0.006
New to Statin, n (%)	24 (75)	8 (50)	0.08
Antiplatelet therapy, n (%)	32 (100)	16 (100)	-
Anti-hypertensive therapy, n (%)	21 (66)	7 (44)	0.15

6.3.2 Baseline and changes in biochemical measures and blood pressure

The baseline, follow-up, and changes in laboratory biochemical and blood pressure measures are summarised in Table 6.2. Significant trends for between-group differences were noted across a number of the baseline laboratory variables. The baseline total cholesterol (4.03 mmol/L vs. 3.49 mmol/L; p=0.01), and low-density lipoprotein cholesterol (LDL-C) (2.17 mmol/L vs. 1.81 mmol/L; p=0.01) levels were significantly higher in the OSA group compared to the non-OSA group. There were nonsignificant differences in free cholesterol ((OSA) 1.06 mmol/L vs. (non-OSA) 0.88 mmol/L; p=0.07) and ApoB (2.2 mmol/L vs. 1.74 mmol/L; p=0.06) levels at baseline. There were significantly lower levels of HDL-C (0.91 mmol/L vs. 1.24 mmol/L; p=0.006) and ApoA-I (3.33 mmol/L vs. 3.68 mmol/L; p=0.03), and significantly higher triglycerides (1.90 mmol/L vs. 1.20 mmol/L; p=0.05) in the OSA group compared to the non-OSA group at follow-up. The follow-up systolic (126 mm Hg vs. 146.5 mm Hg; p=0.03) blood pressure was significantly lower in the OSA group compared to the non-OSA group. There were no significant differences in changes from baseline in laboratory values between the two groups (see Table 6.2).

Table 6.2: - Laboratory findings baseline and changes in biochemical measures and blood pressure by OSA status

	Baseline			Follow-up			Change from Baseline		
	Non-OSA	**OSA**	**p value**	**Non-OSA**	**OSA**	**p value**	**Non-OSA**	**OSA**	**p value**
Number of participants	32	16		32	16		32	16	
Cholesterol									
Total Cholesterol, mmol/L	3.49 (3.18, 3.85)	4.03 (3.44, 5.33)	0.01	3.67 (3.27, 4.27)	4.31 (3.04, 4.72)	0.42	0.10 (-0.34, 0.41)	0 (-1.06, 0.47)	0.30
LDL-C, mmol/L	1.81 (1.45, 2.17)	2.17 (1.84, 3.15)	0.01	1.86 (1.47, 2.37)	1.93 (1.34, 2.90)	0.61	0.08 (-0.16, 0.23)	-0.08 (-0.91, 0.75)	0.91
HDL-C, mmol/L	1.03 (0.91, 1.16)	1.03 (0.93, 1.27)	0.86	1.24 (0,98, 1.37)	0.91 (0.75, 1.16)	0.006	0.10 (-0.05, 0,23)	-0.03 (-0.16, 0.05)	0.11
Triglycerides, mmol/L	1.44 (0.93, 1.67)	1.81 (1.03, 2.54)	0.14	1.20 (0.92, 1.82)	1.90 (1.15, 2.78)	0.05	0.10 (-0.43, 0.44)	0.34 (-0.14, 0.57)	0.10
Phospholipid, mmol/L	5.44 (5.02, 5.99)	3.99 (5.17, 7.10)	0.15	5.64 (5.27, 6.01)	6.50 (4.68,7.27)	0.31	-0.03 (-0.36, 0.06)	0.29 (-0.84, 0.91)	0.22
Free cholesterol, mg/dL	0.88 (0.80, 1.05)	1.06 (0.81, 1.36)	0.07	0.91 (0.83, 1.01)	1.11 (0.81, 1.46)	0.17	0.01 (-0.09, 0.12)	0.04 (-0.19, 0.23)	0.68
Apolipoprotein									
ApoB, mmol/L	1.74 (1.37, 2.05)	2.2 (1.61, 2.85)	0.06	1.83 (1.48, 2.18)	2.10 (1.55, 2.36)	0.29	0.03 (-0.18, 0.26)	0.03 (-0.49, 0.16)	0.57
ApoA-I, mmol/L	3.34 (2.95, 3.47)	3.39 (3.00, 3.55)	0.62	3.68 (3.26, 3.95)	3.33 (2.78, 3.55)	0.03	0.28 (-0.13, 0.65)	0 (-0.05, 0.31)	0.25
hsCRP, nmol/L	37.14 (13.33, 96.19)	39.05 (14.29, 77.14)	0.99	13.33 (6.67, 34.29)	23.81 (11.43,52.38)	0.10	-28.57 (-61.91, -4.76)	-12.38 (-31.43, -0.95)	0.004
Glucose, mmol/L	5.63 (5.24, 6.60)	5.91 (5.19, 6.72)	0.65	5.69 (5.28, 6.55)	6.05 (5.47, 7.77)	0.29	0 (-1.33, 4.72)	0.39 (-0.28, 1.36)	0.81
$HbA1_c$, %	5.7 (5.4, 5.8)	5.9 (5.7, 6.4)	0.11	5.6 (5.5-5.9)	5.8 (5.5, 6.4)	0.50	0.1 (-0.2, 0.1)	-0.1 (-0.2, 0.1)	0.48
Blood pressure									
Systolic, mm Hg	127.5 (121, 147)	128 (120.5, 140)	0.62	146.5 (117.5-150.5)	126 (111.5, 135)	0.03	14.5 (-4, 22)	-5 (-16.5, 3.5)	0.35
Diastolic, mm Hg	80.5 (76.5, 84)	76 (71, 83)	0.17	86.5 (74.5-93.5)	78 (69, 86.5)	0.09	9.5 (-5, 14)	1 (-7, 6.5)	0.58

Values are presented using median and interquartile range (IQR).

6.3.3 Baseline and changes in coronary atheroma volume

Baseline and follow-up IVUS measures in plaque burden are summarised in Table 6.3. Significantly greater baseline PAV (41% vs. 35.6%; p=0.05) was observed in the OSA group compared to the non-OSA group. There were no significant differences observed in baseline TAV (169.7 mm^3 vs. 188.2 mm^3; p=0.56) and TAV in the most diseased 10-mm segment (73.5 mm^3 vs. 84.4 mm^3; p=0.36) between the two groups (see Table 6.3). The changes in coronary atheroma burden are displayed in Figure 6.1. A nonsignificant increase in PAV (p=1.0 compared with baseline) of 0.4 % was observed in the OSA group, and a significant PAV (p=0.001 compared with baseline) decrease of 1.3 % was observed in the non-OSA group. There was a significant difference in the change in PAV (between groups difference p=0.03) between the two groups. A nonsignificant increase in TAV (p=0.80 compared with baseline) of 1 mm^3 was observed in the OSA group, and a significant TAV (p<0.001 compared with baseline) decrease of 11 mm^3 was observed in the non-OSA group. There was a significant difference in the change in TAV (between groups difference p=0.007) between the two groups (see Figure 6.1). There were significant decreases in TAV in the most diseased 10-mm segment in both groups ((OSA) 6.1 mm^3; p=0.04 vs (non-OSA) 5.6 mm^3; p<0.001, compared with baseline), and the change from baseline was not significant (p=0.58) between the two groups (see Table 6.3). The difference between groups in atheroma volume changes was no longer significant (p=0.14), in a multivariate model, after adjustment for cardiovascular risk factors and statin use.

Table 6.3: Ultrasound parameters by OSA status

	Non-OSA	OSA	p value
Number of participants	32	16	
Percent atheroma volume, %			
Baseline	41.0 (37.4, 46.4)	35.6 (31.9, 41.7)	0.05
Follow-up	38.1 (35.6, 46.1)	36.0 (32.2, 42.2)	0.22
Total atheroma volume, mm^3			
Baseline	169.7 (129.6, 232.4)	188.2 (162.2, 236.9)	0.56
Follow-up	165.7 (123.3, 225.2)	187.7 (154.0, 235.0)	0.31
Total atheroma volume most diseased 10-mm segment, mm^3			
Baseline	73.5 (57.8, 95.3)	84.4 (67.0, 103.2)	0.36
Follow-up	67 (53.5, 97.6)	78.9 (59.7, 101.1)	0.35
Change from Baseline	-5.6 (-8.6, -1.0)	-6.1 (-9.0, 3.3)	0.58
p value for change from baseline	< 0.001	0.04	

Values are presented using median and interquartile range (IQR)

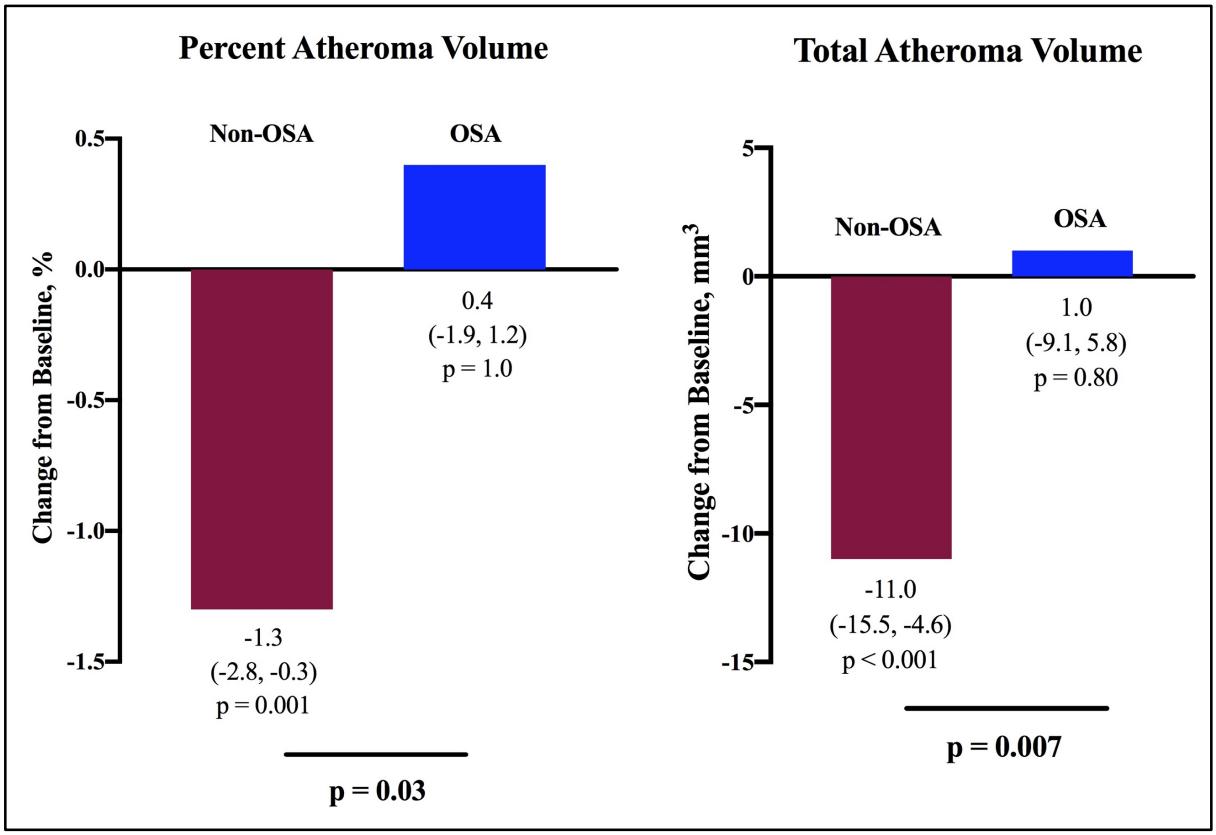


Figure 6.1: Changes in coronary atheroma volume by OSA status

Values are presented using median and interquartile range (IQR)

6.4 Discussion

The present analysis showed that atherosclerotic plaque burden did not regress in patients with OSA after short-term treatment for ACS compared with patients without OSA. There were no significant differences in the changes from baseline in any of the lipid parameters, glucose, or blood pressure. Yet, there was significantly less of a decrease in hs-CRP in patients with OSA.

Despite the use of established therapies adverse cardiovascular events continue to occur, highlighting the need to treat factors that are driving residual CAD risk. A body of evidence suggesting that sleep apnoea is risk factor for patients with CAD (354) has accumulated over the last decade. Cardiovascular impairment has now been linked in those experiencing IH as a result of repetitive episodes of apnoeas and hypopnoeas**,** which lead to sympathetic activation, increased oxidative stress, proinflammatory responses, platelet activation, and endothelial dysfunction (367). European guidelines on cardiovascular disease prevention in clinical practice now recommend screening for and treating OSA in patients with chronic coronary artery disease and hypertension (368). Yet, the influence of OSA in the setting of ACS is not as clear. On one hand, OSA has also been shown to be a comorbidity in patients with ACS with more severe adverse effects on the course of the disease (192). A study was done in a cohort of ACS patients that revealed those with OSA had a higher incidence of death, myocardial infarction, or revascularisation than those without OSA (369). However, another study reported that although sleep disordered breathing was highly prevalent in the setting of ACS, the investigators did not observe a difference in outcomes between those with and without OSA after a 6-month follow-up (370).

While there is considerable interest in the relationship between OSA and CAD, there have only been a few studies done using coronary imaging. A study of 19 stable coronary artery disease patients found that there was a significantly positive correlation between sleep induced

breathing abnormalities and coronary atherosclerotic plaque volume (72). A case-control study of 29 males with no known history of CAD referred to a sleep clinic, observed that coronary plaque volume was significantly greater in the high-AHI group compared to those with a low AHI as measured by CTCA (371). More recently, a study done comparing coronary plaque volume and characteristics in patients with angiographically proven CAD as measured by virtual histology intravascular ultrasound (VH-IVUS), reported that compared to patients with no to mild OSA, moderate to severe OSA was independently associated with a larger total atheroma volume. On the other hand, there were no significant differences in the prevalence of thin cap fibroatheroma, a sign of plaque vulnerability, between the patients with moderate to severe OSA and no to mild OSA (73). While these data support the relationship between OSA and the severity of CAD, none of the studies done previously were serial in design, and therefore did not investigate the effect of OSA on the changes in plaque volume over time.

Studies have shown that coronary plaques in ACS have vulnerable morphology that is prone to more regression in response to treatment than more stable plaques, and these changes are evident in a relatively short period of time (372). In response to treatment shortly after an ACS, the current analysis found that atherosclerotic plaque volume, as measured by IVUS, did not regress in those with sleep apnoea. This finding highlights the possible difference in the regressive mechanisms of plaques in patients with sleep apnoea compared to those without it. While there were no significant differences in other clinical parameters such as LDL-C, HDL-C, and triglycerides, concomitant intensive improvement of these parameters by several interventions including high intensity statins, sleep apnoea treatment, such as CPAP, and lifestyle changes are required to exert more favourable effects in terms of plaque regression.

There has been recent interest in the interaction of OSA and metabolic syndrome, as altered lipid metabolism in OSA may be the pathway by which cardiovascular risk is promoted. While conflicting results have been observed in studies evaluating the impact of OSA on lipid

profiles, the present study showed significantly greater total cholesterol and LDL-C levels just after an ACS event compared to those without OSA. At follow-up however, HDL-C and triglyceride levels significantly improved in those without OSA compared to those with OSA. These observations are consistent with studies previously done in OSA cohorts (373). While the follow-up period for this study was short, significant changes in blood lipid levels have been shown after one month of treatment with statins in patients with ACS (374).

Previous findings have highlighted the role the inflammatory cascade plays in the cardiovascular effects of OSA (375). The reduction of serum oxygen levels observed in OSA have shown an increase in inflammatory cell adhesion to the vascular endothelium, and at the same time promotes the activation of pro-inflammatory cytokines and other inflammation markers involved in atherosclerosis (375). Inflammatory mediators such as homocysteine, B-type natriuretic peptide, and CRP play a key role (375-377). Prior studies have shown OSA to contribute to increased levels of CRP independent of BMI (146, 147). On the other hand, other investigations have found the influence of obesity to play a role in the increase of CRP, not OSA (150, 151). While results of the present analysis may support an inflammatory connection between OSA and CAD, as levels of hs-CRP decreased significantly less in those with OSA than those without OSA at follow-up, BMI was greater in the OSA group at baseline. High levels of hs-CRP have a close relationship with cardiovascular disease mortality in patients with OSA. While hs-CRP lacks specificity, its increase is one of the most significant predictors of cardiovascular risk and prognosis (378).

The limitations of this study merit consideration. This study is a post hoc analysis of a randomised study. This analysis included males only, therefore results may not apply to females. The small sample size has also reduced the power to detect clinically relevant differences in the endpoints. This study focused on the differences in plaque burden between those with and without OSA, and did not include plaque characterisation. Observations of

differences in short-term changes in plaque composition after an ACS in patients with and without OSA was not possible, as the resolution of grayscale IVUS is limited to plaque burden quantification. Event rates were also not included in the analysis as the short follow-up period and small sample size did not allow for detecting differences in clinical outcomes between those with and without OSA. The diagnosis of OSA in this study was based on self-reported information, and severity, duration, and treatment were not recorded. In the future, a study of serial IVUS imaging conducted in ACS patients that also require treatment for OSA would facilitate the investigation of the relationship between the presence of OSA and plaque burden, as well as investigate the impact of CPAP therapy on plaque progression. In summary, OSA was found to be associated with a greater increase in atheroma volume compared to those without OSA after short-term treatment for an ACS event. This finding is consistent with prior cross-sectional human vascular imaging data. Additional larger studies with a longer follow-up period are required to further elucidate the effects that the cardiovascular consequences of OSA have on atherosclerotic plaque.

Chapter 7:

THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA ON CHANGES IN CORONARY PLAQUE VOLUME

ABSTRACT

Background: CAD and its long-term consequences continue to be important contributors to morbidity and mortality. Factors other than traditional comorbidities that might contribute to the development and progression of CAD continue to be evaluated. OSA is highly prevalent in patients with CAD. The aim of this investigation was to determine the association between OSA and coronary plaque volume in patients presenting with CAD.

Methods: Serial coronary IVUS was used to compare changes in TAV and PAV in patients presenting with CAD with (n = 42) and without (n = 84) OSA in a subgroup analysis of an 18-month, randomised, multi-centre study.

Results: BMI (34.5 kg/m^2 vs. 28.6 kg/m^2; p<0.001) was significantly higher in the OSA group compared to the non-OSA group. There were significantly more participants with diabetes (50% vs 14.3%; p<0.001) and treated with a high-intensity statin (71.4% vs. 53.6%; p=0.03), and significantly less participants that had a previous MI (19.0% vs 40.5%; p=0.02) in the OSA group compared to the non-OSA group. There were no significant differences at baseline, follow-up or changes from baseline in lipid levels between the two groups. Significantly higher glucose levels at baseline (5.99 mmol/L vs. 5.38 mmol/L; p=0.005) and follow-up (6.50 mmol/L vs. 5.56 mmol/L; p<0.001) were observed in the OSA group compared to the non-OSA group. HbA_{1c} levels were signifcantly higher in the OSA group compared to the non-OSA group at baseline (5.9 % vs. 5.7 %; p=0.03), and follow-up (6.1 % vs. 5.7 %; p=0.001). There were no significant differences in PAV or TAV between the two groups at baseline. The nominal change in PAV decreased 2.3% in the OSA group and decreased 1.1% in the non-OSA group (difference, −1.1% [95%CI, -2.18% to -0.04%]; p=0.04). TAV decreased -4.9 mm^3 in the OSA group, and decreased -2.9 mm^3 in the non-OSA group (difference, -2.0 mm^3 [95%CI, -7.8 to 3.7]; p=0.48). After multivariate analysis, OSA remained a significant (p=0.04) factor for PAV regression.

Conclusion: Greater regression of percent atheroma volume was observed in patients with OSA after treatment for CAD. The clinical implications of these finding require further investigation.

I, Jordan Andrews, conceived, designed, executed and analysed all of the work included in this chapter.

7.1 Introduction

IH and frequent arousals present in the setting of OSA as a result of upper airway obstruction may culminate in increased sympathetic activation, blood pressure alterations, and vascular atherogenic changes, the mechanisms linking OSA directly to atherosclerosis (60). Furthermore, studies now show that OSA may negatively affect cardiovascular outcomes, independent of comorbidities such as obesity and diabetes (379).

Previous studies have used various invasive (70, 72, 73) and non-invasive (66, 67) imaging techniques to investigate the relationship between OSA and CAD. These studies showed that CAD burden was greater in those with OSA compared to those without. However, these studies were cross-sectional in design, and did not consider the effects OSA has on atherosclerotic plaque changes over time. Serial studies that have utilised IVUS to measure coronary plaque volume have been integral to our understanding of the factors related to the contribution to the progression and regression (355) of coronary atherosclerosis along with their relationships with clinical events (356, 357). Coronary atheroma as measured by high resolution IVUS imaging has been validated previously, and it has been well described (358).

Proprotein convertase subtilisin kexin type 9 (PCSK9) limits removal of LDL particles from the circulation by reducing LDL receptor recycling to the hepatic surface (380, 381). PCSK9 monoclonal antibodies reduce LDL-C levels when administered alone or when combined with a statin (382, 383). Recently, PCSK9 inhibition reduced progression of atherosclerosis as measured by IVUS (362). The effects of OSA on atherosclerotic plaque progression have not been studied in the setting of such extensive lipid lowering therapies. In the previous chapter we reported a potential difference between patients with and without a reported history of OSA in terms of early changes in plaque burden following an ACS. We were subsequently interested in determining whether this relationship continues to be observed on longer follow up.

7.1.1 Aim and rationale of study

The present analysis aimed to investigate the influence of OSA on coronary plaque burden in patients with CAD, and compare changes in plaque volume after treatment for CAD between those with and without OSA.

7.1.2 Hypothesis

The hypothesis of this study was that OSA would negatively impact the changes in coronary plaque volume after treatment for CAD.

7.2 Methods

7.2.1 Study population

A subgroup analysis was conducted in patients with and without OSA. The Design of the GLAGOV study has been described previously (384). In brief, patients with a clinical indication for coronary angiography were eligible, provided that they demonstrated at least 1 epicardial coronary stenosis of 20% or greater. Patients were randomised to treatment with evolocumab (420 mg) or placebo, administered monthly via subcutaneous injection for 76 weeks. Coronary IVUS imaging was obtained at both baseline and at the end of the treatment period. The patients with OSA (n = 42) were selected as those that reported OSA in their medical history during initial trial enrolment.

7.2.2 Acquisition and analysis of serial ultrasound images

The acquisition and serial analysis of IVUS images has been previously described in detail (360, 361, 385-389). Briefly, target vessels for imaging were selected if they contained no luminal stenosis greater than 50% angiographic severity within a segment of at least 30 mm length. Imaging was performed within the same coronary artery at baseline and at study completion. Patients meeting pre-specified requirements for image quality were eligible for randomisation. An anatomically matched segment was defined at the 2-time points on the basis

of proximal and distal side branches (fiduciary points). Cross-sectional images spaced precisely 1.0 mm apart were selected for measurement. Leading edges of the lumen and external elastic membrane were traced by manual planimetry. Plaque area was defined as the area occupied between these leading edges. The accuracy and reproducibility of this method have been reported previously (363). PAV was determined by calculating the proportion of the entire vessel wall occupied by atherosclerotic plaque, throughout the segment of interest as follows:

$$\text{PAV} = \frac{\sum(EEM_{area} - Lumen_{area})}{\sum EEM_{area}} \text{X } 100$$

TAV was calculated using the equation below to determine the summation of plaque area calculated for each image and subsequently normalised to account for differences in segment length between subjects (356).

$$\text{TAV}_{\text{normalised}} = \frac{\sum(EEM_{area} - Lumen_{area})}{Number\ of\ images\ in\ pullback} \text{X } Median\ number\ of\ images\ in\ cohort$$

EEM and lumen volumes were calculated by summation of their respective areas in each measured image and subsequently normalised to account for differences in length of arterial segments between subjects. This post hoc analysis pooled results from both treatment groups. I played a pivotal role in image analysis for several serial regression/progression studies. I conceived the research question for this chapter and performed the analysis. I have worked closely with Professor Stephen Nicholls, the Global PI for the GLAGOV Study and my primary PHD supervisor, on the GLAGOV Study which gave me the unique opportunity to include this analysis in my thesis and perform this work on a long-term follow up study.

7.2.3 Statistical and data analysis

7.2.3.1 Sample size calculation

The sample used in this analysis consists of all available data from this cohort of participants, and done so with the understanding that variability and standard deviation would need to be taken into account. A sample size of 126 patients, 42 with OSA and 84 without OSA, provided 80% power at a 2-sided α of 0.05 to detect a nominal difference of 1.0% in change in PAV assuming a 1.88% SD (361, 362, 364-366).

7.2.3.2 Statistical methods

All statistical analyses were performed using SAS version 9.4 (SAS Inc). Descriptive statistics are presented as mean and standard deviation for normally distributed continuous variables; as median and inter quartile ranges for non-normally distributed continuous variables; and as frequency and percentage for categorical variables. A patient with OSA was age-matched with two patients without OSA using the propensity score method. Propensity score for the OSA was created using logistic regression model with age as a predictor. All OSA patients were matched with 2 non-OSA patients using the nearest neighbour method with replacement. Patients demographics including medications, lipid parameters and baseline and follow-up IVUS parameters were compared using independent t-test for normally distributed continuous variables; Mann-Whitney test for non-normally distributed continuous variables; Chi-squared or Fishers' Exact test for categorical variables. ANCOVA was used to compare the change in IVUS parameters adjusted for the baseline measurement. ANCOVA was conducted using the rank transformation of IVUS variables. A two-sided p-value of less than 0.05 was considered significant.

7.2.4 Ethical and site approval

Independent ethics boards at each of the centres participating in the study approved the protocol and patients provided written, informed consent. The trial was registered with ClinicalTrials.gov (Identifier: NCT01813422).

7.3 Results

7.3.1 Clinical characteristics of the study population

Baseline demographics, clinical characteristics, and concomitant medications are summarised in Table 7.1. Overall, the mean age was 59.8 years, and 14% of the cohort was female. Significant trends for between-group differences were noted across various baseline variables. BMI (34.5 kg/m^2 vs. 28.6 kg/m^2; $p<0.001$) was significantly higher in the OSA group compared to the non-OSA group, and there were significantly more participants with hypertension (90.5% vs 71.4%; p=0.02) and diabetes (50% vs 14.3%; $p<0.001$), and were treated with a high-intensity statin (71.4% vs. 53.6%; p=0.03) in the OSA group compared to the non-OSA group. There were significantly less participants that were current smokers (2.4% vs 25.0%; p=0.002), had previously had an MI (19.0% vs 40.5%; p=0.02), and treated with a beta blocker (66.7% vs 83.3%; p=0.03) in the OSA groups compared to the non-OSA group (see Table 7.1).

Table 7.1: Baseline demographics, clinical characteristics, and concomitant medications by OSA status

	Non-OSA	OSA	p value
Number of participants	84	42	
Age, mean (SD), y	59.8 (7.71)	59.8 (7.73)	0.99
Female, n (%)	12 (14)	6 (14)	1.0
White, n (%)	80 (95)	39 (93)	1.0
BMI kg/m^2, mean (SD)	28.6 (4.50)	34.5 (5.40)	<0.001
Hypertension, n (%)	60 (71.4)	38 (90.5)	0.02
Previous PCI, n (%)	38 (45.2)	16 (38.1)	0.45
Previous MI, n (%)	34 (40.5)	8 (19.0)	0.02
Current Smoker, n (%)	21 (25.0)	1 (2.4)	0.002
Diabetes, n (%)	12 (14.3)	21 (50.0)	<0.001
Baseline statin use, n (%)	32 (100)	15 (94)	0.15
Intensity			0.03
High intensity, n (%)	45 (53.6)	30 (71.4)	
Moderate intensity, n (%)	39 (46.4)	11 (26.2)	
No Statin Therapy, n (%)	0 (0.0)	1 (2.4)	
Antiplatelet therapy, n (%)	80 (95.2)	39 (92.9)	0.69
ß-Blockers, n (%)	70 (83.3)	28 (66.7)	0.03
ACE Inhibitor, n (%)	50 (59.5)	22 (52.4)	0.45
ARB, n (%)	10 (11.9)	11 (26.2)	0.04

7.3.2 Baseline and changes in biochemical measures and blood pressure

Baseline, follow-up, and changes in laboratory biochemical measures are summarised in Table 7.2. Significant trends for between-group differences were noted across the laboratory variables. Significantly higher glucose (5.99 mmol/L vs 5.38 mmol/L; p=0.005), and HbA_{1c} (5.9% vs 5.7%; p=0.03) levels were observed at baseline in the OSA group compared to the non-OSA group. At follow-up, hs-CRP (20.95 nmol/L vs 13.33 nmol/L; p=0.04), glucose (6.50 mmol/L vs 5.56 mmol/L; p<0.001), HbA_{1c} (6.1% vs 5.7%; p=0.001) levels were significantly higher in the OSA group compared to the non-OSA group. The single significant difference in change from baseline in biochemical measures between the two groups observed was $HbA1_c$ ((OSA) 0.11% vs (non-OSA) 0.02%; p=0.04). There were no statistical differences in systolic pressure between the two groups at either time point. However, a nonsignificant difference was observed in diastolic blood pressure ((OSA) 80.5 mm Hg vs (non-OSA) 77.5 mm Hg; p=0.07) at baseline, and a significant difference in change from baseline in in diastolic blood pressure ((OSA) -1.6 mm Hg vs (non-OSA) 2.4 mm Hg; p=0.002) was observed between the two groups (see Table 7.2).

Table 7.2: Baseline and changes in biochemical measures and blood pressure by OSA status

	Baseline			Follow-up			Change from Baseline		
	Non-OSA	**OSA**	**p value**	**Non-OSA**	**OSA**	**p value**	**Non-OSA**	**OSA**	**p value**
Number of participants	84	42		84	42		84	42	
Cholesterol									
Total Cholesterol, mmol/L	4.27 (3.52, 4.65)	4.09 (3.57, 4.78)	0.83	3.67 (2.46, 4.47)	3.35 (2.58, 4.24)	0.58	-0.52 (-1.51, 0.23)	-1.03 (-1.47, 0.11)	0.72
LDL-C, mmol/L	2.37 (1.89, 2.72)	2.17 (1.97, 2.69)	0.79	1.73 (0.72, 2.43)	1.51 (0.73, 2.42)	0.64	-0.70 (-1.47, 0.15)	-0.85 (-1.40, 0.00)	0.76
HDL-C, mmol/L	1.14 (0.96, 1.35)	1.06 (0.85, 1.19)	0.06	1.25 (1.04, 1.41)	1.14 (0.98, 1.29)	0.11	0.10 (-0.03, 017)	0.09 (-0.03, 0.21)	0.80
Triglycerides, mmol/L	1.35 (0.97, 1.90)	1.52 (1.14, 2.08)	0.10	1.37 (0.99, 1.90)	1.49 (1.08, 2.18)	0.30	0.04 (-0.21, 0.33)	0.02 (-0.46, 0.28)	0.75
non-HDL-C, mg/dL	3.05 (2.40, 3.47)	2.84 (2.53, 3.62)	0.72	2.41 (1.22, 3.23)	2.06 (1.20, 3.21)	0.85	-0.45 (-1.62, 0.19)	-1.16 (-1.59, 0.06)	0.75
TC:HDL-C	3.5 (3.0, 4.4)	3.8 (3.4, 4.5)	0.12	2.8 (2.0, 3.8)	2.7 (2.0, 4.3)	0.36	-0.7 (-1.7, 0.1)	-1.2 (-1.8, -0.1)	0.76
Apolipoprotein									
Apo-B, mmol/L	2.01 (1.76, 2.41)	2.15 (1.83,2.59)	0.30	1.66 (0.90, 2.24)	1.50 (0.85, 2.33)	0.99	-0.45 (-1.09, 0.06)	-0.72 (-1.13, -0.08)	0.55
Apo-A-I, mmol/L	3.55 (3.13, 4.01)	3.44 3.16,3.94)	0.45	3.88 (3.53, 4.12)	3.64 3.39, 3.94)	0.08	0.24 (-0.01, 0.55)	0.19 (-0.08, 0.42)	0.15
ApoB:A-I	0.61 (0.47, 0.72)	0.61 (0.53, 0.72)	0.18	0.42 (0.25, 0.59)	0.37 (0.25, 0.69)	0.47	-0.17 (-0.34, -0.01)	-0.27 (-0.33, -0.03)	0.89
hsCRP, nmol/L	13.33 (7.62, 27.62)	18.10 (11.43, 35.24)	0.07	13.33 (6.67,23.81)	20.95 (7.61, 52.38)	0.04	0.00 (-10.47, 4.76)	-0.95 (-7.62, 6.67)	0.62
Lp (a), µmol/L	0.32 (0.15, 1.85)	0.31 (0.18, 2.31)	0.52	0.24 (0.10, 1.56)	0.24 (0.13, 2.10)	0.51	-0.06 (-0.27, 0.0)	-0.05 (-0.22, -0.01)	0.96
Glucose, mmol/L	5.38 (5.00,5.83)	5.99 (5.27, 6.88)	0.005	5.56 (5.26, 6.02)	6.5 (5.61, 7.61)	<0.001	0.16 (-0.08, 0.51)	0.24 (-0.48, 0.78)	0.47
$HbA1_c$, %	5.7 (5.5, 5.9)	5.9 (5.5, 6.6)	0.03	5.7 (5.5, 6.0)	6.1 (5.6, 7.0)	0.001	0.02 (-0.10, 0.19)	0.11 (-0.03, 0.44)	0.04
Blood pressure									
Systolic, mm Hg	132.0 (120.0, 140.0)	129.5 (118.0, 142.0)	0.78	130.1 (123.8, 139.6)	129.4 (122.1, 137.4)	0.36	2.9 (-6.5, 11.2)	0.1 (-8.6, 5.9)	0.21
Diastolic, mm Hg	77.5 (70.5, 83.5)	80.5 (72.0, 86.0)	0.07	78.0 (74.0, 83.6)	76.9 (72.5, 80.9)	0.21	2.4 (-2.1, 8.0)	-1.6 (-6.9, 2.3)	0.002

Values are presented using median and interquartile range (IQR).

7.3.3 Baseline and changes in coronary atheroma volume.

Baseline and follow-up IVUS measures of plaque burden are summarised in Table 7.3. There were no significant between-group differences at baseline in PAV ((OSA) 37.1% [Median (IQR), (31.2, 43.3)] vs (Non-OSA) 36.4% [Median (IQR), (31.3, 43.8)]; p=0.75) or TAV ((OSA) 171.7 mm^3 [Median (IQR), (125.2, 237.9)] vs (Non-OSA) 177.0 mm^3 [Median (IQR), (127.7, 244.4)]; p=0.42). There were no significant between-group differences at follow-up in PAV ((OSA) 37.0% [Median (IQR), (29.4, 42.3)] vs (Non-OSA) 37.6% [Median (IQR), (31.1, 42.5)]; p=0.30) or TAV ((OSA) 171.7 mm^3 [Median (IQR), (120.9, 229.5)] vs (Non-OSA) 168.9 mm^3 [Median (IQR), (130.9, 237.7)]; p=0.46) (see Table 7.3). There were nonsignificant decreases in TAV from baseline observed in the OSA (-4.9 mm^3 [95% CI, -11.7 to 1.9]; p=0.16) and non-OSA (-2.9 mm^3 [95% CI, -8.7 to 2.9]; p=0.33) groups, and the between-group difference in the change in TAV (-2.0 mm^3 [95%CI, -7.8 to 3.7]; p=0.48) from baseline was also not significant. There were significant decreases in PAV from baseline observed in the OSA (2.3% [95% CI, -3.5 to -1.0]; p=0.0006) and non-OSA (-1.1 % [95% CI, -2.2, -0.1]; p=0.04) groups, and the between-group difference in the change in PAV (−1.1% [95%CI, -2.18% to -0.04%]; p=0.04) from baseline was also significant (see Figure 7.1). The difference in PAV reduction continued to remain significant (p=0.04) between the two groups in a multivariate model, despite adjustment for cardiovascular risk factors and statin use.

Table 7.3: Ultrasound parameters by OSA status

	Non-OSA	OSA	p value
Number of participants	84	42	
Baseline			
Percent Atheroma Volume; %, Median (IQR)	36.4 (31.3, 43.8)	37.1 (31.2, 43.3)	0.75
Total Atheroma Volume; (mm^3), Median (IQR)	177.0 (127.7, 244.4)	171.7 (125.2, 237.9)	0.42
Follow-up			
Percent Atheroma Volume; %, Median (IQR)	37.6 (31.1, 42.5)	37.0 (29.4, 42.3)	0.30
Total Atheroma Volume; (mm^3), Median (IQR)	168.9 (130.9, 237.7)	171.7 (120.9, 229.5)	0.46

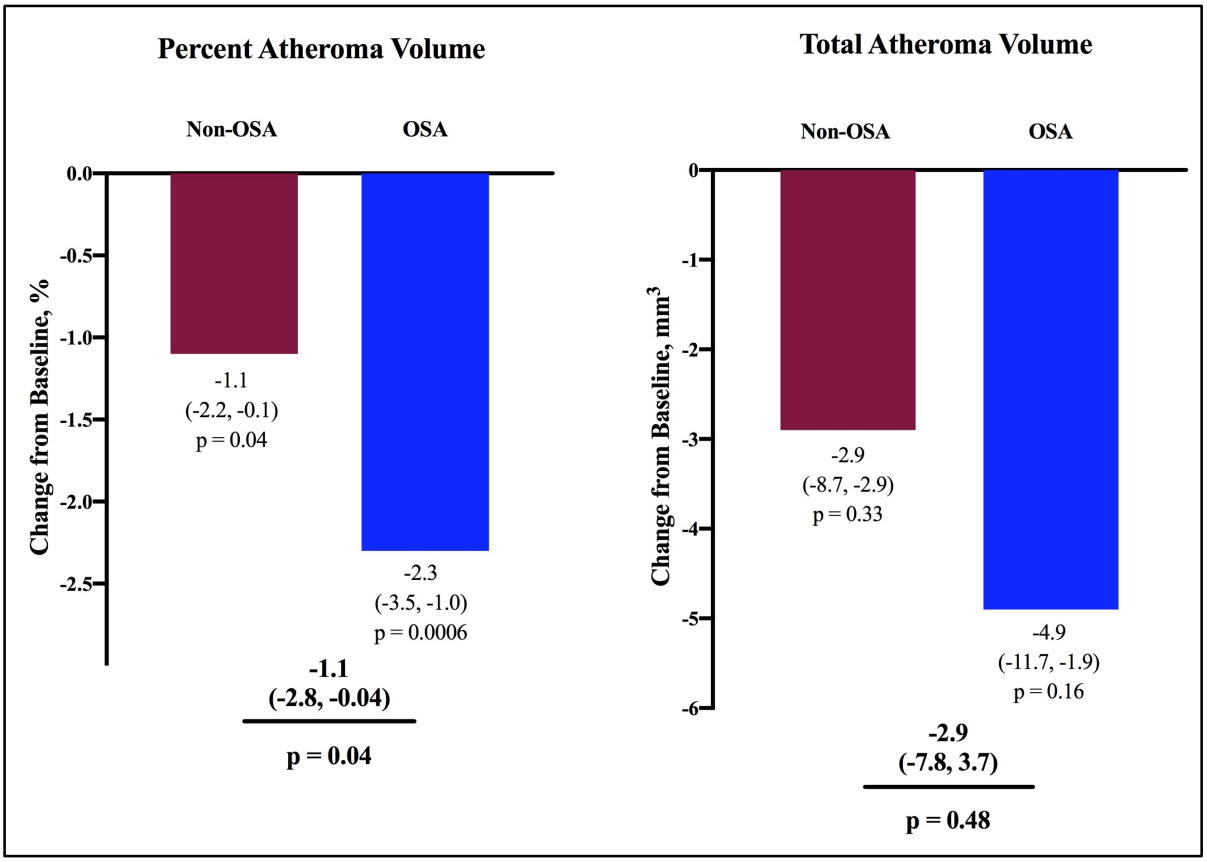


Figure 7.1 Changes in coronary atheroma volume by OSA status

Values are presented using median and interquartile range (IQR)

7.4 Discussion

The present analysis demonstrated significantly greater PAV regression in patients with OSA after treatment for CAD compared to patients without OSA. There were no significant differences in the changes from baseline in any of the lipid parameters. Glucose, $HbA1_c$, and diastolic blood pressure were all significantly higher at baseline in those with OSA compared to those without OSA. Glucose and $HbA1_c$ levels remained significantly higher in those with OSA at follow-up, while the difference in change from baseline in $HbA1_c$ and diastolic blood pressure was also significantly higher in those with OSA compared to those without OSA.

CAD continues to be a leading cause of mortality and morbidity worldwide. Despite the use of established therapies, adverse cardiovascular events still occur, highlighting the need to target additional risk factors. Several lines of evidence spanning across *in vitro*, animal, clinical, and epidemiological studies now indicate that OSA may have direct adverse effects on cardiovascular outcomes (379). A few imaging studies have investigated the relationship between CAD and OSA (70, 72, 73). The mean TAV as measured by IVUS was found to be larger in subjects with OSA than those without in a cohort of 19 individuals with stable CAD (72). While another investigation of 93 CAD patients resulted in a significantly greater TAV in those with moderate to severe OSA compared to those with no to mild OSA. However, there were no significant differences in the prevalence of thin cap fibroatheroma in the culprit lesions between those with moderate to severe OSA and no to mild OSA (73). In contrast to these results, there were no differences in plaque burden between the two groups in the current analysis. The current population has less severe disease, as the TAV observed in the previous studies in those with OSA was also much greater than in the current population studied. While the studies previously done examined the effects of OSA on coronary atheroma, serial changes in the development of the atherosclerotic process were not investigated as in the current analysis.

Dyslipidaemia has been shown to be prevalent among patients with OSA. Hyperlipidaemia has been shown to be caused by IH in murine models as well as the upregulation of genes of lipid biosynthesis in the liver (390, 391). While intermittent hypoxemia has been shown to play a major role in the pathogenesis of the dysregulation of lipoprotein metabolism, the precise mechanism by which OSA induces dyslipidaemia is not well understood. On one hand, the dysregulation of lipoprotein metabolism associated with OSA has been found to be independent of adiposity (131) and to be partially reversible with CPAP therapy, even without body weight changes (203, 392). However, the majority of studies investigating the relationship between OSA and dyslipidaemia were not specifically designed to evaluate lipid profile and have been influenced by potential confounders (393, 394) such as obesity (395). Guidelines have recommended lowering LDL-C to less than 1.8 mmol/L in patients with CAD (396, 397), particularly those at greater cardiovascular risk (368). In the present analysis, nearly three quarters of those with OSA were treated with high-intensity statins and 60% were also treated with PCSK9 inhibitor, resulting in median LDL-C levels less than 1.8 mmol/L at follow-up, suggesting that lowering LDL-C to less than 1.8 mmol/L in patients with OSA significantly modulates the progressive nature of coronary atherosclerosis.

IH and sleep fragmentation are thought to be the features in the causal pathway leading to metabolic dysfunction. The prevalence of T2D in individuals with OSA has been estimated to be between 15% and 30% (110). Previous studies have also shown independent associations between the severity of OSA and insulin resistance in individuals without T2D (202, 398). These findings are supported by the current cohort of individuals with OSA, as there were significantly more individuals with diabetes in the group with OSA, and therefore significant differences in glucose and $HbA1_c$ levels. CAD patients with concomitant comorbidities represent a high-risk population, and investigations had not been able to show regression of coronary atheroma (399). However, recently an analysis of individuals with diabetes treated

with high-intensity statins for 24 months reported regression of coronary atherosclerosis (400). The current population studied reported not only significant PAV regression, but significantly more regression than those without OSA after 24 months of treatment for CAD. More than 70% of those with OSA were treated with high-intensity statin. This finding provides further evidence that aggressive long-term treatment of CAD patients with concomitant comorbidities is required to positively impact disease progression.

Inflammation has been speculated to play a role in the development of atherosclerosis in patients with OSA. This premise is based on the notion of the pro-inflammatory state of obesity, and the physiological derangements during sleep, such as IH, in subjects with OSA further aggravate inflammation. One study found serum levels of CRP and CIMT to be significantly higher in patients with OSA syndrome than the obese control subjects (57). However, conflicting results have been reported regarding the relationship between inflammation and OSA, such as the association of CRP and OSA, whereas the strength of the relationship may be influenced by obesity rather than OSA alone (153, 401). While there were differences in hs-CRP in this analysis, BMI was also significantly greater in those with OSA, limiting our ability to draw reliable conclusions.

The bidirectional relationship between hypertension and OSA has revealed that patients with hypertension appear to be more likely to suffer OSA, and patients with OSA present with a high prevalence of hypertension (278, 402, 403). Pathophysiological features support this relationship between OSA and hypertension, as mechanisms based on sympathetic and reninangiotensin-aldosterone activation, as well as oxidative stress and endothelial dysfunction, implicate OSA as an independent cause of hypertension (404). While acute increases in blood pressure may cause an inhibition of the upper airway muscles. This phenomenon, together with volume overload and its displacement to the upper body during sleep can lead to pharyngeal oedema, and may explain the link between hypertension and OSA

(92, 93). Sympathetic hyperactivity also increases the risk of thrombotic events through platelet activation and also contributes to hypertension (352, 402), which may in turn contribute to the development of coronary atherosclerosis. The results of the current analysis support these findings as 90% of those with OSA have a history of hypertension.

The cohort included in this analysis exhibit multiple risk factors that in previous studies have correlated to significant atherogenic disease progression, as well as the results of the short-term analysis included in this thesis. However, those with OSA exhibited significantly more of a reduction in atherosclerotic plaque burden than those without OSA in this study. There are a few of factors that may have contributed to the different outcomes. The previous study was a short-term analysis performed in post ACS patients, this study is a long-term investigation conducted the setting of stable CAD. The biology may be fundamentally different. Additionally, background drug use is different and more dynamic in the ACS patients. The findings in this study provide further insight into the dynamic nature of atherosclerotic burden in patients thought to harbor the most vulnerable form of CAD with inflammatory infiltrate and lipid-laden plaque. Statins possess not only potent LDL-C–lowering properties but also significant antioxidant and anti-inflammatory effects (405). Such properties may have rendered patients with OSA more susceptible to the antiatherosclerotic effects of potent statins when compared with patients without OSA, who may harbor greater degrees of less modifiable fibrocalcific plaque. The extended duration of the use of statins in this study could have magnified both the delipidating and the pleiotropic actions of statins on plaque as opposed to the short-term use in the previous study.

The limitations of this analysis should be considered. The small sample size has reduced the power to detect outcomes and clinically relevant differences in the endpoints. This study focused on the differences in plaque burden between those with and without OSA in a cohort with established CAD; therefore, these results should not be applied across all individuals with

OSA. The resolution of the imaging modality of grayscale IVUS utilised in this study allows for plaque burden quantification, but not plaque characterisation. Observations of the effects of OSA and changes after treatment for CAD on plaque composition were not possible. The severity, duration, and treatment of OSA is unknown in this cohort, as diagnosis of OSA was based on self-reported information at the start of the study. In the future, a study of serial IVUS imaging conducted in patients with established CAD that also require treatment for OSA would facilitate the investigation of the relationship between the presence of OSA and plaque burden, as well as investigate the impact of CPAP therapy on plaque progression. This study is a post hoc analysis of a randomised study. Therefore, differences such as treatment, diabetes, BMI, and statin dose and duration of treatment were not controlled for in the initial study design. Despite this, there was still a statistically significant difference in the change in coronary atheroma volume after adjustment for cardiovascular risk factors and statin use in a multivariate model.

In summary, patients with OSA had a greater decrease in atheroma volume compared to those without OSA after treatment for CAD. This finding sheds more light on the importance of aggressively treating CAD patients, especial those with concomitant comorbidities such as OSA that may be more susceptible to the antiatherosclerotic effects of treatment. Additional, larger studies are required to expand our knowledge on the extent to which the cardiovascular consequences of OSA affect atherosclerotic plaque progression.

Chapter 8:

DISCUSSION

8.1 Rationale for body of work

CVD continues to be the primary cause of death worldwide, despite the advances in atherosclerosis prevention and treatment. Several factors underscore this observation. The global rise in obesity has a deleterious impact on metabolic and vascular mediators implicated in atherosclerosis. The use of conventional CVD therapies continues to be suboptimal, and CV events occur even when therapies are used as directed. Therapy targets are based on pathways associated with atherosclerosis, yet other factors may also play important mechanistic roles in plaque formation, progression and rupture. There is a need to more clearly define the factors driving disease progression and understand how targeting these factors reduces CVD risk to develop more effective, individualised approaches to risk prediction, disease prevention and intervention.

There is a growing body of evidence that exists suggesting that OSA is associated with an increased burden of atherosclerosis in patients that present with symptomatic CAD, found in sleep laboratory cohorts, and community based study populations. The frequent nocturnal apnoeic episodes experienced by OSA patients over time cause repetitive periods of hypoxaemia, sleep deprivation, intrathoracic pressure changes and sympathetic activation. The vibrations of chronic snoring transmitted through the soft tissues surrounding the pharynx to the carotid artery wall induce vascular inflammation and endothelial injury. These stressors have the potential to lead to the development hypertension, arrhythmias, stroke and atherosclerosis. However, no studies have systematically investigated the clinical and mechanistic links between OSA and the development of atherosclerosis.

Imaging advances enable characterisation of factors implicated in the pathogenesis of atherosclerosis by investigating the functional and anatomical changes within the vascular tree. Clinical research programs have included imaging to investigate the impact of disease states on the vasculature. Vascular imaging has been used to study OSA and its association with the

abnormalities across the atherosclerotic disease spectrum. The majority of studies that have utilised PWV, a validated assessment of arterial stiffness, have reported an independent relationship between PWV and OSA severity. Studies have also demonstrated an inverse relationship between FMD, a validated measure of endothelium-dependent vascular relaxation representing a physiologic change of the artery wall preceding plaque formation, and OSA severity. Despite lack of direct evidence that thickening is a precursor of plaque formation, CIMT has been shown to be a CVD risk biomarker, and most CIMT studies have demonstrated a direct relationship with OSA severity. There is a direct association with the burden of calcium found in the coronary arteries and CVD risk factors and CV event rates. Non-contrast enhanced CT coronary imaging enables quantification of calcium burden, and CT calcium scoring is often integrated into risk prediction algorithms, as a result of the ability to reclassify risk in individuals, otherwise determined to be of intermediate risk. Studies using calcium scoring have reported direct associations with OSA severity. The experience of arterial imaging in OSA patients supports a direct relationship between OSA severity and vascular disease, however a systematic investigation has been not performed to directly characterise atherosclerotic plaque across vascular territories.

8.2 Findings of individual studies

8.2.1 The impact of CPAP on measures of subclinical atherosclerosis in patients with obstructive sleep apnoea - a systematic review

This systematic review, which included high quality evidence from clinical trials of the effects of CPAP therapy of markers of subclinical atherosclerosis indexed in the Cochrane Library, PubMed, and Embase with an NHMRC Evidence Level of I, II, or III demonstrated that treatment with CPAP in patients with OSA favourably effects the progression of subclinical atherosclerosis as assessed by CIMT, FMD, and PWV. The library searches yielded 125 results, of which 32 studies eligible for inclusion in the final analysis. These comprised of

4 randomised controlled clinical trials, 3 randomised sham-controlled clinical trials, 1 randomised crossover clinical trial, and 24 prospective observational studies. Each imaging modality utilised was assessed individually, and the overall effect of CPAP treatment was evaluated qualitatively due to the heterogeneity of study types included. Significant improvements in the development of subclinical atherosclerosis as a result of treatment with CPAP therapy were noted for each imaging modality evaluated, CIMT, FMD, and PWV.

The examination of the literature in this review highlights that current evidence does not fully answer the question of whether or not treatment with CPAP therapy can yield greater benefit in halting the the development of subclinical atherosclerosis. There was significant heterogeneity among the studies included in regards to sample size, OSA severity, the amount of compliance and duration of treatment with CPAP therapy. Studies included in this systematic review also included populations with cardiometabolic comorbidities that may have influenced the study outcomes. The examination of cardiovascular endpoints is still required to better understand the implications of the effects of OSA and the development of CVD to develop effective preventive strategies that may alter the natural course of cardiovascular disease.

8.2.2 The relationship between symptoms suggestive of obstructive sleep apnoea and severity of coronary artery stenosis

This prospective cross-sectional study recruited 99 participants that were at least 40 years of age and referred for a clinically indicated coronary catheterisation. Risk of OSA was measured after the administration of the OSA prediction questionnaire, OSA50, global angiographic stenosis was measured by Gensini score, biochemical values were measured in each participant. Participants were divided into two groups, high and low risk of OSA. Participants in the OSA low risk group were older than the OSA high risk group. BMI and waist circumference were both higher, and classified as obese in the OSA high risk group.

There were no significant differences in participant lipid parameters, high-sensitivity CRP, and glucose levels between the high and low risk of OSA groups. HDL-C levels were at the lower end of normal, and hsCRP levels were slighly elevated in the OSA low risk group.

There were significantly more participants previously diagnosed with sleep apnoea in the OSA high risk group. Overall, Gensini score corresponded to moderate CAD, and the individual maximum stenosis corresponded to a significant single stenotic lesion. Neither global nor focal measures of CAD severity as measured by Gensini score and maximum stenosis value were significantly different between the OSA high and low risk groups.

Positive, significant correlations were present between OSA50 score and both waist circumference and BMI. An inverse, significant association was present between OSA50 score and age. A positive, significant association was present between OSA50 score and smoking, and an inverse, significant correlation was present between OSA50 score and atrial fibrillation. A non-significant inverse correlation was present between HDL-C levels and OSA50 score. No significant correlations were present between OSA50 score and Gensini score or maximum stenosis.

Despite the evidence that OSA significantly impacts patients with CAD, there was no association between severity of CAD and increased risk of OSA. Mechanisms apart from plaque burden are more likely to underscore this relationship in an older cohort referred for a clinically indicated coronary catheterisation.

8.2.3 The relationship between inflammatory and angiogenic factors with symptoms suggestive of obstructive sleep apnoea and severity of coronary artery stenosis

This study consisted of 30 participants referred for a clinically indicated coronary angiogram. Coronary artery stenosis severity was determined by angiographic Gensini score, and the maximum stenosis was recorded. An OSA risk prediction questionnaire (OSA50) was

administered, and participants were divided into low, moderate, and high OSA risk. Serum collected at the time of the catheterisation was added to TNFα stimulated HUVECs in culture. Endothelial gene expression of markers of vascular inflammation (VCAM-1, ICAM-1), and angiogenesis (VEGFA, HIF-1α) were measured by RT-PCR. Angiogenesis capacity of treated HUVECs was assessed using the Matrigel tubulogenesis assay.

Participants in the OSA high risk group were younger, had a higher BMI, and more diabetics than those in the low and moderate OSA risk groups. There were no other significant differences in medical history nor any concomitant medication use between the three groups. There were no significant differences in participant lipid parameters, high-sensitivity CRP, or glucose levels between the three groups. There were significantly more participants previously diagnosed with sleep apnoea the OSA high risk group. Overall the participants scored low on the EES. Global and focal measures of CAD severity were calculated as Gensini score and maximum stenosis, respectively. On average, Gensini score corresponded to overall moderate CAD, and the maximum stenosis corresponded to a significant single stenotic lesion. In accordance with the study in the previous chapter, there were no differences in global or focal CAD severity in relation to OSA risk.

The number of tubules was significantly lower in the OSA high risk group compared to the low risk group and the moderate risk group. The VCAM-1, ICAM-1, VEGFA, and HIF-1α expression of TNF-stimulated HUVECs co-incubated with serum obtained from study participants was not significantly different between the three groups of OSA risk. There were no associations between OSA50 score and VCAM-1, ICAM-1, VGFA or HIF-1α gene expression. A significant inverse correlation was present between VEGFA expression and Gensini score, and A significant inverse correlation was present between number of tubules and OSA50 score.

Patients at high OSA risk demonstrated differences in angiogenic potential, but not in atherosclerotic disease burden or vascular inflammation. Previous evidence suggests protective effects of IH patterns with upregulation of HIF-1α and VEGFA leading to increased tubule formation, while others indicate a dysfunctional endothelium with low numbers of tubule formation.

8.2.4 The relationship between epicardial fat volume and density with obstructive sleep apnoea and coronary plaque burden

This study included 71 participants that were scheduled for a clinically indicated cardiac CT, and underwent clinically indicated overnight PSG for the investigation of possible OSA. The degree of OSA was determined by AHI, and severe OSA was defined as AHI greater than 30. Participants underwent clinically indicated CTCA, and EAT volume and density and Leaman scores, a global measure of coronary plaque burden, were quantified on CTCA. Significant coronary plaque burden was defined as Leaman score greater than 8.3.

There were no significant differences in demographics of age, gender, BMI, and medical history in those with and without severe OSA. Those with severe OSA had significantly higher measures of the sleep parameters of AHI, ODI, and EES scores compared to those without severe OSA. There were no significant differences in Leaman scores or EAT mean density between those with and without severe OSA. Those with severe OSA had significantly higher EAT volume compared to those without severe OSA.

Positive, significant associations were present between apnoea–hypopnea index and both BMI and Leaman score. Positive, significant associations were present between ODI, BMI, and Leaman scores and EAT volume. An inverse, significant association was present between Leaman scores and EAT density. EAT volume was an independent predictor of AHI and OSA severity. BMI did not significantly associate as a predictor of AHI or OSA severity.

Increased volume of EAT, now understood to be a metabolically active organ, significantly correlated with plaque burden in this study and previously reported results (330). The local inflammatory effect of EAT volume on the coronary arteries may play an important role in the development of atherosclerosis, and warrants consideration as a possible mediator of cardiovascular risk in OSA patients. Serial investigations are needed to elucidate the effect of treatment for OSA on changes in EAT volume and density.

8.2.5 The impact of obstructive sleep apnoea on short term changes in coronary atherosclerotic plaque in patients with acute coronary syndrome

To investigate if differences exist in short-term change in plaque burden in patients with and without OSA following treatment for ACS, serial coronary IVUS was used to compare changes in TAV and PAV in male patients presenting with an ACS after 12 weeks of treatment. This subgroup analysis of a randomised multi-center study included 16 participants with and 32 participants without OSA.

Participants in the OSA group had a greater BMI, more prior PCI, and less were treated with a high intensity statin compared to the non-OSA group. LDL-C levels were higher at baseline in the OSA group compared to the non-OSA group. There were no differences in changes in blood pressure, glucose, or lipid parameters between the two groups at follow-up. Significantly greater progression of TAV and PAV was observed in the OSA group compared to the non-OSA group. However, the between group differences in the changes in each of the measures of plaque burden were no longer significant in a multivariate model after adjustment for cardiovascular risk factors and statin use.

OSA was found to be associated with a greater increase in atheroma volume compared to those without OSA after short-term treatment for an ACS event, and this finding is consistent with prior cross-sectional human vascular imaging data.

8.2.6 The impact of obstructive sleep apnoea on changes in coronary plaque volume

To investigate if differences exist in long-term change in plaque burden in patients with and without OSA following 18 months of CAD treatment, serial coronary IVUS was used to compare changes in TAV and PAV in patients referred for a clinically indicated coronary angiogram. This subgroup analysis of a randomised multi-center study included 42 participants with and 84 participants without OSA.

BMI was higher in the OSA group compared to the non-OSA group. There were more participants with diabetes and treated with a high-intensity statin, and less participants that had a previous MI in the OSA group compared to the non-OSA group. There were no differences at baseline, follow-up or changes from baseline in lipid levels between the two groups. Higher glucose levels at baseline and follow-up were observed in the OSA group compared to the non-OSA group. HbA_{1c} levels were higher in the OSA group compared to the non-OSA group at baseline, and follow-up.

There were no significant differences in PAV or TAV between the two groups at baseline. The nominal change in PAV decrease was greater in the OSA group compared to the non-OSA group. The TAV decrease was not significantly different between the two groups. After multivariate analysis, OSA remained a significant factor for PAV regression.

Patients with OSA had a greater decrease in atheroma volume compared to those without OSA after treatment for CAD. However, previous studies have correlated to significant atherogenic disease progression, such as the results of the short-term analysis included in this thesis. The current finding sheds more light on the importance of aggressively treating CAD patients for a longer period of time, especial in those with concomitant comorbidities such as OSA that may be more susceptible to the antiatherosclerotic effects of treatment.

8.3 Overarching conclusions

The vasculature of patients with OSA is affected by the chronic exposure to frequent nocturnal apnoeic episodes. OSA associated with a greater increase in atheroma volume compared to those without OSA after short-term treatment for an ACS event, while patients with OSA had a greater decrease in atheroma volume compared to those without OSA after long-term optimal treatment for CAD. The longer follow-up period and the more aggressive treatment for CAD in the second study resulted in a greater decrease in atheroma volume compared to the first in the short-term treatment for ACS. EAT volume, a metabolically active fat depot that has been linked to CV risk factors and systemic markers of inflammation, associated with OSA severity, independent of BMI. Despite improvement in markers of subclinical atherosclerosis established in the systematic review, large randomised clinical trials have not resulted in a benefit of treatment with CPAP therapy on CV event rates (208-210).

Symptoms suggestive of OSA correlate with CV events. However, increased risk of OSA did not associate with either global or focal measures of increased severity of CAD. Though a significant inverse correlation between risk of OSA and age was observed, and patients with OSA under 50 years of age have more severe CV consequences. A younger cohort and a more detailed questionnaire addressing symptoms of OSA such as snoring, may have produced a correlation with atherosclerotic burden in a cohort of patients undergoing a clinically indicated catheterisation. Increased risk of OSA also did not associate with gene expression of inflammatory and angiogenic factors. Yet, those at a high risk of OSA have decreased function of angiogenic potential. However, a larger sample size may result in a significant difference in the markers of inflammation as OSA risk increases.

8.4 Clinical implications of research findings

Incidence of OSA is common in the clinical setting of STEMI, and OSA negatively impacts coronary plaque burden, microvascular obstruction, and recovery of left ventricular

function after PCI (182). OSA has also been reported to be a predictor of restenosis and target vessel revascularisation (189, 262). OSA is still largely underdiagnosed and undertreated. Untreated OSA is a known risk factor contributing to CVD, and leading to MI (9, 23, 28, 263). Untreated severe OSA significantly increases the risk of fatal and non-fatal CV events compared with healthy participants (28). Therefore, effective OSA screening tools are required in the setting of CAD. In this body of work, increased risk of OSA was not associated with CAD severity in patients presenting with symptomatic CAD. The components of the OSA50 questionnaire used in the study were validated as predictive of OSA with the intention of developing a simple questionnaire to be administered in the primary care setting. Other factors influencing the development of OSA not included in the questionnaire may have a stronger relationship in the setting of coronary atherosclerosis. Despite the complex scoring system, the more detailed questions found in the BQ regarding the OSA symptom of snoring may have produced a correlation with atherosclerotic burden in this cohort, as the vascular consequences of inflammation and endothelial injury induced by the vibrations of snoring are transmitted through soft tissues surrounding the pharynx to the carotid artery wall (58) increasing the risk of developing CAD.

A significant inverse correlation between risk of OSA and age was observed. Previous reports suggest that individuals with OSA under 50 years of age have more severe cardiovascular consequences (280), and cardiovascular risk associated with OSA decreases with age (29). Those at high risk of OSA were significantly younger than those at low risk of OSA, however this cohort consisted of participants with an average age of 68 years. The age of the cohort may have influenced the lack of relationship between CAD severity and risk of OSA observed. Therefore, a younger cohort target, and a more CAD focused questionnaire should be considered for future study design.

The mechanisms of OSA associated with contributing to the development of atherosclerosis are complex as OSA is a heterogeneous disease characterised by multiple mechanisms and complications such as intermittent hypoxemia, hypercapnia, negative intrathoracic pressure increase, and arousal. IH caused by OSA is considered to contribute the cascade of events leading to CVD onset its progression. Inflammation has been shown to play a central role in all phases of atherosclerosis (135, 136), and OSA has been associated with increase circulating levels of markers of inflammation (284). Angiogenesis, the process by which new blood vessels are formed from pre-existing ones (285) is an important physiological response to hypoxia following stimuli (286). Angiogenesis may be impacted in patients with OSA as a result of repetitive night-time hypoxia. In this body of work, patients at high OSA risk demonstrated differences in angiogenic potential, but not in atherosclerotic disease burden or vascular inflammation. Angiogenic potential is impaired in patients at high risk of OSA based on the low number of tubules formed in the functional assay. However, there were no differences in gene expression of angiogenic markers. The clinical implications of these findings require further investigation, as some evidence suggests protective effects of IH patterns with upregulation of HIF-1α and VEGFA leading to increased tubule formation, while others indicate a dysfunctional endothelium with low numbers of tubule formation.

PWV, a marker of aterial stiffness has also been shown to be elelvated in those with OSA (38). Blood pressure has been shown to be one of the determinants of arterial stiffness (258, 259), and blood pressure has been improved in those treated with CPAP (260). Vascular inflammation and endothelial injury are possibly induced by the vibrations of snoring, a symptom of OSA, transmitted through the soft tissues surrounding the pharynx to the carotid artery wall (58). FMD, the validated measure of endothelium-dependent vascular relaxation, has been impaired in minimally symptomatic patients with OSA (48), and has been shown to be inversely related to AHI (38). The studies included in the systematic review investigated the

effects of CPAP therapy on the development of subclinical CVD, and improvements in the early stages of atherosclerosis were observed. However, a reduction CV events after treatment with CPAP compared to usual care in a population with developed CVD require much more aggressive treatment, such as better adherence and longer follow-up period. Patients with CVD with concomitant comorbidities represent a high-risk population, and treatment benefit in the form of coronary atheroma regression had not been reported (399). However, patients with diabetes treated with high-intensity statins for 24 months reported regression of coronary atherosclerosis (400). The long-term serial IVUS study included in this body of work not only showed significant PAV regression in patients with OSA, but significantly more regression than those without OSA after 24 months of treatment for CAD. More than 70% of those with OSA were treated with high-intensity statin, providing further evidence that aggressive long-term treatment of CAD patients with concomitant comorbidities is required to positively impact disease progression.

Patients with OSA have a heterogeneous disease etiology with variable combinations of abnormalities in airway anatomy, neuromuscular responsiveness, respiratory chemosensitivity, and loop gain (15). Each of these components require different single or combinations of therapeutic interventions. Treatment strategies must also consider the co-morbidities of patients with heart disease, and integrate behavioural, pharmacological and device-based treatments to individually optimise treatment.

8.5 Suggestions for future research

The serial IVUS studies included in this body of work showed differences in changes in plaque burden between those with and without OSA after short-term and long-term treatment. However, treatment for OSA was not incorporated into the study design. The systematic review included in this body of work investigated the effects of CPAP therapy on the development of subclinical CVD, and improvements were observed in each of the measures

of subclinical CVD after a relatively short period of time. Yet, large studies investigating the impact of CPAP therapy on cardiovascular events failed to show reductions in CV event rates in those treated with CPAP compared to usual care (208-210).

Advances in arterial wall imaging enable visualisation of the full plaque burden, providing a unique opportunity to determine the clinical and pharmacological factors influencing the natural history of disease progression. This approach has led to observations that plaque burden and progression on serial imaging directly associate with adverse CV outcomes (356). A non-invasive imaging approach would allow a broader focus beyond patients undergoing angiography, to asymptomatic individuals and other CAD patients.

Increased interest has focused on CT coronary imaging. With administration of intravenous contrast and resolution advances, CT coronary angiography permits measurement of lumen stenosis, plaque burden and composition providing incremental prognostic information (406). Radioisotopes permit direct imaging of molecular elements implicated in the transition of plaque from the stable, clinically quiescent state to vulnerability and rupture. As a result, there has been interest in the use of molecular plaque imaging in research and clinical settings. ^{18}F-sodium fluoride plaque imaging has been validated as a tool to visualise vulnerability within the coronary arteries (407). ^{18}F-sodium fluoride detects early calcification associating with activation of inflammatory pathways within plaque (408).

Serial imaging studies conducted in newly diagnosed OSA patients across a range of OSA severity and treatment requirements with established CAD would facilitate the investigation of the relationship between the presence and severity of OSA and plaque burden, composition, and functionality, investigate the impact of the degree of OSA therapy with plaque progression, and define the factors associated with disease progression in OSA patients.

Despite the body of evidence that exists for OSA as a risk factor for CAD, significant knowledge gaps exist that are open for continued investigation. The findings of this body of

work provide insights into the effects of OSA across vascular territories and may inform the design of future clinical studies.

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