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Arterial Stiffness and Endothelial Function in Obstructive Sleep Apnoea: The Effect of Continuous Positive Airway Pressure (CPAP) Therapy

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Submitted for the degree of Doctor of Medicine

University of Edinburgh

2015

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Abstract

Arterial Stiffness and Endothelial Function in Obstructive Sleep Apnoea: The Effect of Continuous Positive Airway Pressure (CPAP) Therapy

Introduction: Obstructive sleep apnoea (OSA) is common and is caused by repetitive obstruction of the upper airway during sleep. OSA is associated with increased cardiovascular morbidity and mortality and is an independent risk factor for hypertension. The immediate physiological effects of OSA include intermittent hypoxia, repeated arousal from sleep and intra-thoracic pressure swings. The resulting activation of the sympathetic nervous system, systemic inflammation and oxidative stress may result in increased arterial stiffness and endothelial dysfunction, potentially explaining any causal link between OSA and cardiovascular disease (CVD). Continuous positive airway pressure (CPAP) therapy improves excessive daytime sleepiness (EDS) and in non-randomised studies, reduces cardiovascular mortality. Prior to starting this study, there was a limited amount of evidence suggesting that CPAP therapy improved arterial stiffness and endothelial function, but the effects in subjects without pre-existing CVD were unclear.

Aims: i) to determine whether CPAP therapy has an effect upon measures of arterial stiffness and endothelial function in patients with OSA, in the absence of known CVD. ii) To compare arterial stiffness and endothelial function in a subset of patients with OSAHS (defined as OSA and EDS), with a group of well-matched control subjects.

Methods: Fifty three patients with OSA, defined as an apnoea/hypopnoea index of ≥15, and without known CVD, entered a double-blind placebo-controlled crossover trial of 12 weeks CPAP therapy, of whom forty three completed the study protocol. Sham CPAP was used in the placebo arm of the study and vascular assessments were made at baseline and after each arm of the study. Arterial stiffness was determined by measuring aortic distensibility using cardiovascular magnetic resonance imaging and by measuring the augmentation index (AIx) and aortic pulse wave velocity (PWV) by applanation tonometry. Endothelial function was assessed non-invasively by measuring vascular reactivity after administration of salbutamol and glyceryl trinitrate. In a subset of twenty patients with OSAHS, arterial stiffness and endothelial function at baseline were compared to readings obtained from healthy control subjects, matched on a one-to-one basis for age, sex and BMI.

Results: Patients with OSAHS (n=20) had increased arterial stiffness [AIx 19.3(10.9) vs. 12.6(10.2) %; p=0.017] and impaired endothelial function, measured as the change in AIx following salbutamol [-4.3(3.2) vs. -8.0(4.9) %; p=0.02] compared to controls. Twelve weeks of CPAP therapy had no significant effect upon any measure of arterial stiffness or endothelial function in patients with OSA (n=43). A trend towards a reduction in AIx following CPAP therapy was seen, but this was non-significant. There was a reduction in systolic blood pressure following CPAP therapy [126(12) vs. 129(14) mmHg]. Sub group analysis showed CPAP to have no

effect on arterial stiffness or endothelial function in patients with EDS or in patients using CPAP for ≥4 hours per night.

Conclusions: This study demonstrates that even in the absence of known CVD, patients with OSAHS have evidence of increased arterial stiffness and impaired endothelial function. However, in patients with OSA, free from CVD, CPAP therapy did not lead to an improvement in any measure of arterial stiffness or endothelial function after 12 weeks.

Lay Summary

Background

Obstructive sleep apnoea (OSA) is a common condition in which the upper airway intermittently becomes obstructed during sleep. This leads to disturbed sleep, snoring, breathing pauses and can cause patients with OSA to be excessively sleepy during the daytime. Studies have shown that OSA is associated with high blood pressure and cardiovascular disease. The breathing pauses seen in OSA can lead to a reduction in blood oxygen levels, frequent awakenings from sleep and changes in pressure within the chest. These immediate consequences of OSA in turn are thought to lead to other changes within the body which may result in stiffening of the blood vessels (arterial stiffening) and an impairment of the way that blood vessels work, called endothelial dysfunction. Arterial stiffening and endothelial dysfunction may explain why OSA is associated with cardiovascular disease.

Continuous positive airway pressure (CPAP) therapy is the treatment for OSA and this involves the delivery of a high pressure of air via a tight fitting face mask. This keeps the upper airway open during sleep. This treatment is highly effective in treating the symptoms of OSA, namely snoring, breathing pauses and excessive daytime sleepiness. Some studies have also suggested that CPAP therapy reduces the risk of cardiovascular disease in patients with OSA, but this is less certain and more high quality evidence is needed. Before this study started there were some small studies showing that CPAP therapy improved arterial stiffness and endothelial dysfunction. Many patients in these previous studies however already had evidence of cardiovascular disease.

Aims

The aim of this study was to look at whether CPAP therapy could improve arterial stiffness and endothelial dysfunction in patients who had OSA but who did not have a history of cardiovascular disease. In addition, arterial stiffness and endothelial function were examined in 20 patients with the most symptomatic OSA (i.e. patients with OSA who also had evidence of excessive daytime sleepiness) and compared to a control group of well-matched volunteers who did not have OSA.

Methods

Measurements of arterial stiffness and endothelial function were taken from 53 patients with OSA at the start of the study. Patients were then randomly allocated to receive either standard CPAP therapy or a placebo CPAP in which the pressure of air applied through the mask was lower than would be needed to hold the upper airway open. After 12 weeks the measurements were repeated and patients switched over to receive the other form of CPAP for a further 12 weeks, after which the measurements were again repeated. Neither the patients, nor the researcher performing the measurements knew the order in which the patients received each treatment. Measurements were also performed once in a group of 20 healthy volunteers without OSA who served as the control group.

Results

Patients with OSA and excessive daytime sleepiness had increased arterial stiffness and impaired endothelial function compared to the 20 volunteers without OSA. Despite this, 12 weeks of CPAP therapy did not lead to an improvement in either arterial stiffness or endothelial function in the 43 patients with OSA who completed the study. In keeping with previous studies, a small reduction in blood pressure following CPAP therapy was seen.

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Publications:

Jones A, Vennelle M, Connell M, McKillop G, Newby DE, Douglas NJ, Riha RL. Arterial stiffness and endothelial function in obstructive sleep apnoea/hypopnoea syndrome. Sleep Med 2013;14(5):428-432

Jones A, Vennelle M, Connell M, McKillop G, Newby DE, Douglas NJ, Riha RL. The effect of continuous positive airway pressure therapy on arterial stiffness and endothelial function in obstructive sleep apnea: a randomized controlled trial in patients without cardiovascular disease. Sleep Med 2013;14(12):1260-1265

Presentations:

Jones A, Vennelle M, Connell M, McKillop G, Newby DE, Douglas NJ, Riha RL Arterial stiffness in the obstructive sleep apnoea/hypopnoea syndrome. 12/2008. Oral presentation. British Thoracic Society Winter Meeting, London.

Jones A, Vennelle M, Connell M, McKillop G, Newby DE, Douglas NJ, Riha RL Arterial stiffness in the obstructive sleep apnoea/hypopnoea syndrome. 05/2009. Poster discussion. American Thoracic Society Meeting, San Diego.

Acknowledgements

I am very grateful to the many people who have provided me with assistance whilst I undertook the work presented in this thesis.

Firstly I would like to thank my supervisor, Dr Renata Riha for her advice, encouragement and support during my research and in the writing of this thesis.

I would also like to thank Professor Neil Douglas, Professor David Newby and Dr Graham McKillop who always had time to offer advice and assistance.

I am grateful to Marjorie Vennelle for her work in the randomisation of patients, setting up of the CPAP and sham units and all the other help she provided me with during the study. Thanks also go to Martin Connell for his MRI expertise and aortic distensibility measurements.

I would also like to thank all the staff working in the Department of Sleep Medicine at the Royal Infirmary of Edinburgh, who helped me on numerous occasions and were kind enough to answer all my questions.

I am indebted to the research nurses in the Clinical Research Facility at the Royal Infirmary of Edinburgh and particularly Finny Paterson for accommodating me and for their patience in teaching me the technique of applanation tonometry. I would also like to thank the radiographers who work in MRI scanning and Lynne Thomson in particular for her help in arranging the MRI scans.

I am grateful to Kelly McGorm at the Scottish Primary Care Research Network and Inchpark Medical Practice, Edinburgh for their help in recruiting control subjects for the study.

I would also like to thank Dr Tom Mackay for all his help and support and Dr Jakki Faccenda for her encouragement during the writing of this thesis.

The funding for this study came from the British Heart Foundation and I am very grateful to them for this. I would also like to extend my thanks to all the participants in the study who gave up their time freely.

Last but not least I would like to thank my family, which has grown a little since I started this research. I am grateful to my mum and dad for their support over the years. To Tim, a massive thank you- this would not have been possible without you. Finally to Eve and Harry, thank you for being you while I wrote this.

Abbreviations

AASM, American Academy of Sleep Medicine

ABPM, ambulatory blood pressure

AHI, apnoea hypopnoea index

AIx, augmentation index

AJ, Anne Jones

A_{max}, maximum aortic area

A_{min}, minimum aortic area

ANOVA, analysis of variance

AoD, aortic distensibility

AP, augmentation pressure

APPLEs, Apnea Positive Pressure Long-term Efficacy study

BP, blood pressure

BMI, body mass index

CI, confidence interval

CPAP, continuous positive airway pressure

CRP, C-reactive protein

CSA, central sleep apnoea

CVA, cerebrovascular accident

CCF, congestive cardiac failure

CVD, cardiovascular disease

DM, diabetes mellitus

DVLA, Driver and Vehicle Licensing Agency

ECG, electrocardiogram

EDS, excessive daytime somnolence

EEG, electroencephalography

EMG, electromyography

EMP, endothelial microparticle

eNOS, endothelial nitric oxide synthetase

EOG, electrooculography

EPC, endothelial progenitor cell

ESS, Epworth Sleepiness Score

FMD, flow mediated dilatation

GMP, guanosine monophosphate

GTN, glyceryl trinitrate

HbA1c, glycated haemoglobin

HDL, high density lipoprotein

HIF-1, hypoxia inducible factor-1

ICAM, intercellular adhesion molecule-1

ICC, intraclass correlation coefficient

IH, intermittent hypoxia

IHD, ischaemic heart disease

Il-6, interleukin-6

IL-8, interleukin-8

IQR, interquartile range

LVEF, left ventricular ejection fraction

MAP, mean arterial pressure

MC, Martin Connell

MOSAIC, Multicentre Obstructive Sleep Apnoea Interventional Cardiovascular study

MRI, magnetic resonance imaging

MSLT, multiple sleep latency test

MV, Marjorie Vennelle

MWT, maintenance of wakefulness test

NFκB, nuclear factor κB

NICE, National Institute for Health and Care Excellence

NO, nitric oxide

ODI, oxygen desaturation index

OSA, obstructive sleep apnoea

OSAHS, obstructive sleep apnoea/hypopnoea syndrome

PP, pulse pressure

PSG, polysomnography

PWA, pulse wave analysis

PWV, aortic pulse wave velocity (sometimes referred to as carotid-femoral pulse wave velocity)

RCT, randomised controlled trial

RDI, respiratory disturbance index

REM, rapid eye movement

ROS, reactive oxygen species

SAQLI, sleep apnoea quality of life index

SIGN, Scottish Intercollegiate Guideline Network

SMC, Scottish Medicines Consortium

SNA, sympathetic nerve activity

SPCRN, Scottish Primary Care Research Network

TE, echo time

TIA, transient ischaemic attack

TNFα, tumour necrosis factor α

TR, repetition time

UPPP, uvulopalatopharyngoplasty

WMA, World Medical Association

Chapter 1: General Introduction

1.1 Introduction

Obstructive sleep apnoea (OSA) is caused by repetitive partial or complete obstruction of the upper airway during sleep. When it leads to excessive daytime sleepiness (EDS) then it is termed obstructive sleep apnoea/hypopnoea syndrome (OSAHS). OSAHS is common, affecting 2-4% of the middle aged population (Young 1993), with at least 20% of the population having frequent apnoeas and hypopnoeas in the absence of EDS (Young 2002a). Given the association with obesity in more than 40% of cases (Young 2005), then the prevalence of OSA is likely to increase.

The repetitive airway obstruction seen in OSA leads to sleep fragmentation and subsequently may cause EDS, the consequences of which are well documented and include cognitive dysfunction (Engleman 2004) along with a significant increase in road traffic accidents (George 2004). However, it is becoming increasingly apparent that patients with OSA are also at increased risk of a variety of cardiovascular diseases including ischaemic heart disease (IHD) (Kiely 2000, Peker 2006), cerebrovascular disease (Shahar 2001, Yaggi 2005) and the metabolic syndrome (Coughlin 2004). To date, OSA has been shown to be an independent risk factor for hypertension (Stradling 2001, McNicholas 2007).

OSA can be successfully treated, most commonly with continuous positive airway pressure (CPAP) therapy which acts as a pneumatic splint holding the airway open during sleep. Observational studies suggest that the increased risk of cardiovascular disease (CVD) is reduced in patients treated with CPAP therapy (Marti 2002, Marin 2005) and numerous studies have shown a small but significant reduction in blood pressure in CPAP-treated patients (Bazzano 2007).

The mechanisms for the association of OSA and CVD are incompletely understood, but are likely to be multifactorial (McNicholas 2007). Evidence suggests that arterial stiffness and endothelial dysfunction may play a role in this and prior to starting this study there was limited evidence to suggest that CPAP therapy improved arterial stiffness and endothelial function. Those studies that did exist were however largely small and with one exception, non-randomised. In particular, the effect of CPAP on

arterial stiffness and endothelial function in OSA patients, without concomitant CVD or hypertension was unknown.

The data contained within this thesis were obtained from conducting a double-blind randomised placebo-controlled crossover trial of the effect of CPAP on arterial stiffness and endothelial function in patients with OSA. Additionally, a case-control study was undertaken examining vascular function in a subset of patients with OSAHS compared to well-matched controls.

Chapter 1 provides a comprehensive overview of OSA and its treatment. Chapter 2 outlines the existing evidence for, and potential mechanisms underlying the link between OSA and CVD in general, with particular focus on arterial stiffness and endothelial function. The methods employed in this study are described in Chapter 3. The results of the randomised controlled trial, case-control study and a comparison of the different methods of measuring arterial stiffness are presented in Chapters 4, 5 and 6. The discussion of the results of this study and conclusions are found in Chapter 7.

1.2 Historical perspectives on obstructive sleep apnoea (OSA)

OSA as we now know it to be, was first described from a pathophysiological point of view in the 1960s. However, there are case reports of the clinical syndrome dating back to the 19th Century (Lavie 1984a). Charles Dickens is often credited with the first description of a person with OSA in the character of Joe; an obese excessively sleepy boy in 'The Posthumous Papers of the Pickwick Club' in 1837. In 1918 Dr William Osler used the word 'Pickwickian' to describe overweight patients with obesity and hypersomnolence**.** This was expanded upon by Burwell in 1956, who described the association of obesity and alveolar hypoventilation leading to hypersomnolence and polycythaemia (Burwell 1956). However this condition was probably what we would now more correctly term obesity hypoventilation, with alveolar hypoventilation occurring during wakefulness and sleep, rather than upper airway collapse during sleep alone, leading to OSA (although, of course, the two can co-exist). It was not until 1965 that the link between nocturnal events and daytime symptoms were recognised initially by Gastaut (Gastaut 1965) and subsequently by Jung and Kuhlo (Jung 1965) who performed polysomnographic studies on so-called

'Pickwickian' patients. It was later appreciated that non-obese patients could also suffer nocturnal apnoeas and EDS and the term obstructive sleep apnoea syndrome was used in 1976 to describe this (Guilleminault 1976)**.** Following the appreciation that hypopnoeas were also important (Gould 1988), the syndrome became known as the obstructive sleep apnoea/hypopnoea syndrome (OSAHS).

1.3 Definition of obstructive sleep apnoea (OSA)

OSA refers to the physical condition whereby there is repetitive obstruction of the upper airway during sleep which may or may not result in EDS. If EDS is present, the condition is known as the obstructive sleep apnoea/hypopnoea syndrome (OSAHS).

Most people, if their sleep is studied, will have evidence of some respiratory events during sleep and the degree of respiratory disturbance is quantified using the apnoea/hypopnoea index (AHI). The AHI varies with age and gender (Riha 2009), and the level at which this is deemed to require treatment varies throughout the world. In a survey of European centres, an AHI of ≥15 was commonly used as the cut off for offering treatment with CPAP, with some centres using a lower cut off if there were associated co-morbidities or significant EDS and some using a higher cut off (Fietze 2011). The degree of respiratory disturbance is quantified using the apnoea/hypopnoea index (AHI). An apnoea is generally defined as a reduction in airflow of ≥90% from baseline for at least 10 seconds. The definition of a hypopnoea is less uniformly applied, but the then current 2007 American Academy of Sleep Medicine (AASM) manual defined it as a reduction in airflow of ≥30% for at least 10 seconds with an oxygen desaturation of ≥4% from baseline or a ≥50% reduction in airflow for at least 10 seconds with a ≥3% oxygen desaturation or associated arousal (Iber 2007). In the 2012 update of the AASM guidelines, the criteria for scoring a hypopnoea has been relaxed and is now defined as a reduction in airflow of ≥30% for at least 10 seconds associated with either a 3% desaturation *or* an arousal (Berry 2012). It is recognised that the degree of oxygen desaturation for any given reduction in airflow may vary from person to person, with greater desaturation seen in obese patients (Peppard 2009) or in those with underlying lung disease (Bradley 1985). Therefore, in lean patients without underlying lung disease,

the requirement for an oxygen desaturation may result in many arousals not being scored or included in the AHI, and the 2012 AASM update reflects this concern (Berry 2012). It is likely that the use of the new 2012 definition will result in a greater number of hypopnoeas being scored and hence an increase in the calculated AHI (BaHammam 2012).

Based upon the 1999 recommendations of the American Academy of Sleep Medicine (Sleep Medicine Task Force 1999), the severity of OSA, based upon the results of polysomnography (PSG), is often classified as follows (SIGN 2003):

AHI	Disease severity
5-15	Mild
15-30	Moderate
>30	Severe

The AHI increases with age (Bixler 1998, Young 2002b) and any cut-off point in making a diagnosis or grading severity is essentially arbitrary. However an AHI of 15 or greater is usually deemed in the UK (SIGN 2003) and Europe (Fietze 2011) to represent clinically significant disease.

Evidence of sleep disordered breathing in the absence of EDS is more common than the syndrome of OSAHS (Young 2002a) and is variably termed obstructive sleep apnoea (OSA), minimally symptomatic OSA or sleep disordered breathing in the literature. Confusingly, OSA and sleep disordered breathing can also be used to describe all patients with a predefined AHI, irrespective of daytime symptoms.

For clarity, within this thesis, the term obstructive sleep apnoea (OSA) refers to any patient with an AHI of ≥ 15 or greater, with obstructive sleep apnoea/hypopnoea syndrome (OSAHS) used to describe the subset of patients with an AHI ≥15 and evidence of EDS.

1.4 Normal sleep physiology

Normal sleep is a reversible physiological process which can be divided into two broad categories; rapid eye movement (REM) sleep and non-REM sleep. During this study, sleep staging at our centre followed the then standard Rechtschaffen and Kales

criteria (Rechtschaffen 1968). According to Rechtschaffen and Kales, non-REM sleep can be further subdivided (by electroencephalographic (EEG), electro-oculographic (EOG) and electromyographic (EMG) appearance during polysomnography) into stages I-IV with stages III and IV often referred to as slow wave sleep. REM sleep takes its name from the bursts of rapid eye movements that occur. These are associated with a general loss of skeletal muscle tone and it is during REM sleep that episodic dreaming occurs. Humans generally enter sleep through stage I or II and progress through the stages in a cyclical fashion, eventually entering REM after approximately 90 minutes of sleep. This process continues through the night with periods of REM occurring more frequently and lasting for longer as the night progresses. Sleep, recorded at polysomnography, can be represented as a hypnogram (shown below in Figure 1.1) with the sum of the component parts described as the sleep architecture.

Figure 1.1 Example of a normal hypnogram

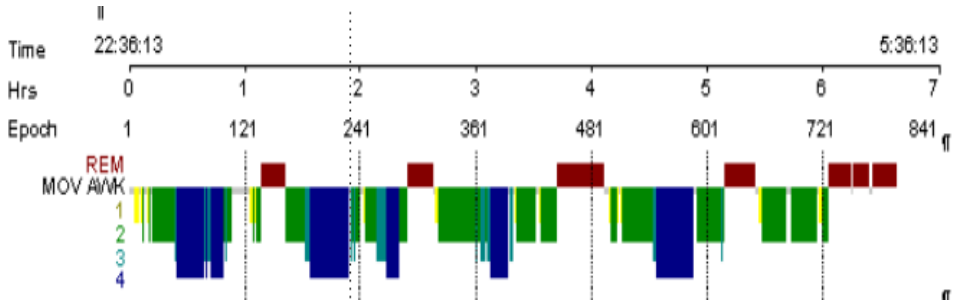

Figure 1.1 An example of a normal hypnogram, based upon the Rechtschaffen and Kales criteria used during this study (Rechtschaffen 1968). Hours in bed are represented by the black horizontal line at the top of the image. Periods of REM sleep are represented in red above the lower horizontal line and below this line are periods of non-REM sleep. Sleep stage I is represented in yellow, stage II in green, stage III in light blue and stage IV in dark blue. Any blank regions below the lower horizontal line represent periods of wakefulness.

Whilst similar in principle, the 2007 AASM guidelines recommended a change in the terminology used in sleep stage scoring (Iber 2007). REM sleep was renamed as Stage R and stages I and II renamed as Stages N1 and N2 respectively. Stage N3 now represents slow wave sleep and replaces stages III and IV in the Rechtschaffen and

Kales classification (Rechtschaffen 1968). In the 2007 classification, wakefulness is termed Stage W.

1.5 Pathophysiology of breathing during sleep

OSA is caused by repetitive obstruction of the upper airway during sleep, leading to the cessation or reduction of airflow despite continued respiratory efforts. Cessation of airflow due to this is known as an obstructive apnoea, with a reduction in airflow termed a hypopnoea. In contrast, a central apnoea represents a cessation in airflow without on-going respiratory effort. Central sleep apnoea (CSA) is associated with a diverse range of conditions including cardiac failure (where it is often termed Cheyne-Stokes Respiration) (Naughton 2012), cerebrovascular disease (Javaheri 2010) and less commonly may be idiopathic (Eckert 2007a). It can also be caused by high altitude (known as high–altitude periodic breathing) (Eckert 2007a) and by drugs, particularly opiates (Malhotra 2010). Whilst CSA may co-exist with OSA, they are separate entities and as such CSA will not be discussed further in this thesis. The site of the obstruction in OSA is the pharyngeal airway and this area is shown in the diagram below (Figure 1.2).

Figure 1.2 Sites of obstruction in the upper airway in OSA

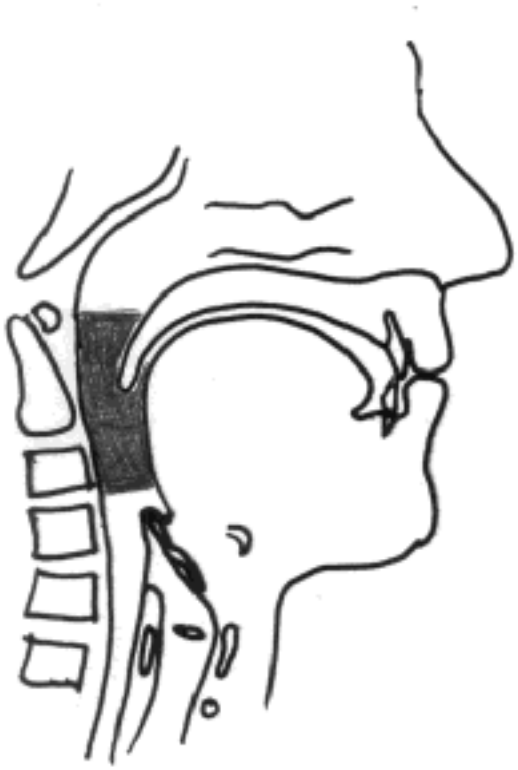

Figure 1.2 The shaded area in the figure denotes the sites where obstruction occurs. (*Figure reproduced with kind permission of Dr RL Riha)*

Obstruction may occur at multiple levels within the same airway (Bachar 2008), with the retropalatal and retroglossal regions most frequently implicated (Schellenberg 2000, Schwab 2003). Airway collapse occurs when negative pressure within the pharynx during inspiration exceeds the ability of the pharynx to remain patent (Remmers 1978).

Anatomical considerations affecting the upper airway clearly play a role, however the airway obstructions only occur during sleep and not when the patient is awake. This coupled with the often intermittent nature of respiratory events during sleep means that other factors including upper airway dilator muscle activity, lung volume, ventilatory control and sleep-state stability are likely to be important.

1.5.1 Anatomical considerations in obstructive sleep apnoea (OSA)

The size of the upper airway during wakefulness is smaller in patients with OSA (Eckert 2009) and many factors are known to affect this. Bony craniofacial abnormalities such as micrognathia, retrognathia or abnormalities of the mandible can all lead to a reduction in upper airway size. Airway length is also important, with longer airways more susceptible to collapse (Owens 2008) and airway length correlating with OSA severity (Segal 2008). The risk of developing OSA increases with the size of surrounding soft tissue structures, principally the lateral pharyngeal walls, tongue, uvula and tonsils (Schellenberg 2000, Schwab2003). Tonsillar and adenoidal hypertrophy is a leading cause of OSA in children (Marcus 2012). Airway trauma and subsequent inflammation due to snoring or recurrent obstructions may lead to further expansion of soft tissues (Paulson 2002). Increased inflammatory cell infiltrate has been reported in both the mucosa and musculature of the upper airway in patients with OSA compared to control subjects (Boyd 2004).

Fixed anatomical problems with the airway are common contributory factors in the non-obese patient with OSA (Nelson 1997). However, the commonest anatomical factor is probably obesity. Fat deposition in the anterior neck and surrounding soft tissues can compromise the airway and neck circumference has been correlated with disease severity (Davies 1990, Davies 1992). Even non-obese patients have been shown to have excess adipose tissue in the anterolateral region of the neck (Mortimore 1998).

Early cephalometric studies showed that only a relatively small proportion of the variance seen in AHI could be explained by upper airway anatomical variables, although this may be more important in younger, non-obese patients (Mayer 1996).

1.5.2 Upper airway dilator muscle activity in obstructive sleep apnoea (OSA)

During wakefulness, the upper airway dilator muscles counteract the effect of inspiration on the pharynx, keeping it patent. Dilator muscle activity is state dependent with reductions in both genioglossus (the largest of the dilator muscles) and tensor palatini activity during sleep in healthy subjects (Lo 2007, Mezzanotte 1996). As well as being state dependent, genioglossus activity can be modulated by

mechanoreceptors within the airway and chemoreceptors to protect against airway collapse (Stanchina 2002), with activity increasing following an obstructive breath (Jordan 2007). This protective reflex may however be diminished in patients with OSA (McGinlay 2008). The mechanisms leading to impaired dilator muscle activity during sleep in OSA are not clear, with congenital and acquired defects proposed. Evidence of disease progression has led to speculation that mechanical trauma to the upper airway due to snoring and repeated obstructive events may directly affect either the dilator muscles (Petrof 1996) or their innervation, however the importance of this in the pathogenesis of OSA is unclear (Eckert 2007b) and it may purely represent an epiphenomenon.

During the awake state, patients with OSA have higher genioglossus activity than control subjects (Mezzanotte 1992), and this may be a compensatory mechanism for an anatomically compromised airway (Owens 2008).

1.5.3 Lung volumes in obstructive sleep apnoea (OSA)

Upper airway size increases with increases in lung volume across the respiratory cycle (Eckert 2009) and animal work suggests that this is probably due to caudal traction on the upper airway, independent of upper airway dilator muscle activity (Van de Graaff 1988). A modest reduction in functional residual capacity (FRC) is seen during sleep (Hudgel 1984, Ballard 1990) and by experimentally increasing lung volumes in patients with OSA, Heinzer *et al* showed a reduction in the AHI (Heinzer 2006). Similarly, experimental increases and decreases in lung volume led respectively to decreases and increases in CPAP pressure requirements (Heinzer 2005). It is not yet clear what the relative contribution of lung volume is to the pathophysiology of OSA, but it is may be more important in patients who already have a compromised upper airway due to the factors outlined above. Lung volumes are reduced in obesity (Piper 2010) and this could be a further contributory factor in obese patients. A reduction in lung volume may be a sign of pre-existing lung disease, which may worsen the degree of oxygen desaturation following an obstructive event.

1.5.4 Ventilatory control in obstructive sleep apnoea (OSA)

During sleep, ventilation is largely controlled by negative feedback control, acting upon information from peripheral and central chemoreceptors. Sleep stage itself can also affect the depth and rate of respiration (Lydic 1987). The stability (or otherwise) of ventilation can be quantified as loop gain, i.e. the degree of ventilatory disturbance in relation to the degree of the provoking stimulus, with high loop gain indicating greater instability.

Evidence suggests that patients with OSA have ventilatory instability (Hudgel 1998, Younes 2001), with early observational studies demonstrating central apnoeas in patients with severe disease who had undergone a tracheostomy (thus removing any physical obstruction) (Eckert 2009). During a central apnoea, pharyngeal narrowing occurs, predisposing the airway to collapse and a subsequent obstructive event (Badr 1995). Furthermore a central apnoea may occur as the result of high loop gain following an obstructive event, hence predisposing to further obstruction and instability. Recent work has shown acetazolamide, a carbonic anhydrase inhibitor, to reduce both loop gain and the severity of OSA (Edwards 2012). However, the impact of ventilatory instability on disease pathophysiology and severity remains unclear and may be a result of the disease rather than a causative factor.

1.5.5 Sleep–state stability and obstructive sleep apnoea (OSA)

Arousal from sleep is a critical mechanism in preventing asphyxia following an obstructive event. However, this inevitably leads to disruption of normal sleep. Arousals can also contribute to ventilatory instability with hyperventilation commonly seen on awakening (Jordan 2004). However, not all obstructive events are terminated by an arousal (Younes 2004) and therefore, other mechanisms must exist to reopen the pharynx. In healthy subjects, dilator muscle activity has been shown to be responsive to a combination of changes in the partial pressures of carbon dioxide and upper airway resistance (Stanchina 2002). This is likely to also occur in patients with OSA (Berry 1997) and if the airway is re-opened in this way then arousal may be averted. Thus, the threshold at which arousal occurs may be important and this has been shown to vary from patient to patient (Younes 2007). This leads to the hypothesis that subjects with a lower arousal threshold may suffer more sleep

disruption and perpetuation of obstructive events than those in whom arousal occurs only if other mechanisms of airway re-opening are unsuccessful.

1.6 Natural history of obstructive sleep apnoea (OSA)

The natural history of OSA is unclear, with studies producing conflicting results. In a study of initially untreated patients, over half showed a subsequent increase in the AHI, independently of any weight gain (Pendlebury 1997). More recently, an increase in AHI over time was found largely to be due to concomitant weight gain (Berger 2009). Others have shown no change in AHI over at least five years, in the absence of significant weight gain (Sforza 1994). It is clear that weight gain leads to an increase in AHI (Peppard 2000a), and given the weight gain that often occurs with advancing years, OSA will inevitably progress in a proportion of patients.

1.7 Epidemiology of obstructive sleep apnoea (OSA)

1.7.1 Prevalence of obstructive sleep apnoea (OSA)

Obstructive sleep apnoea/hypopnoea syndrome (OSAHS) is usually quoted as affecting 2-4% of the middle aged population. This is based on information from the Wisconsin Sleep Cohort Study, published in the early 1990s. If symptoms of EDS are ignored, approximately 5 times as many subjects had polysomnographic evidence of at least mild OSA (Young 1993). These figures are confirmed in other studies of predominantly Caucasian populations (Stradling 1991, Bearpark 1995, Bixler 2001) with prevalence figures varying slightly depending on the definition of OSA used and the method of diagnosis. Studies of Asian and Indian populations suggest similar prevalence rates (Sutherland 2012). However, there is some evidence to suggest that African-Americans are at greater risk of developing OSA (Ruiter 2010). Other racial or ethnic differences have been reported but these may relate to greater levels of obesity or differing craniofacial morphology seen in those populations (Villaneuva 2005).

Recent reviews of accumulating epidemiological evidence suggest that the prevalence of OSA and OSAHS are increasing (Garvey 2015, Franklin 2015) and given the known association between OSA and obesity (Young 2005), this has been

linked to increasing levels of obesity in the population. Indeed a recent re-analysis of the original Wisconsin Sleep Cohort adjusting for current levels of obesity estimated that 34% of males and 17.4 % of females would have an AHI >5 and that 14% of males and 5% of females would have an AHI of >5 and evidence of EDS (Peppard 2013).

Given the known prevalence it is clear that much of the OSA within the population remains undiagnosed.

1.7.2 Risk factors for developing obstructive sleep apnoea (OSA)

The biggest risk factors for OSA are obesity, male sex and ageing. More than 40% of patients with OSA are obese (Young 2005) and obesity is thought to contribute to disease pathophysiology in a number of ways as described above. Additionally, obesity is independently associated with EDS (Bixler 2005, Slater 2013) and thus may make daytime symptoms associated with OSA appear worse. The BMI is not the most sensitive measure of risk in patients with OSA: neck circumference (Davies 1992) and measures of central obesity may correlate better with disease severity (Vgontzas 2000). Epidemiological studies show males are 2-3 times more likely to develop OSA than females (Punjabi 2008). This is likely to be due to a number of anatomical factors, including their predisposition to develop central (rather than peripheral) obesity (Lemieux 1993). Differences exist in upper airway structure and function between the sexes with the male upper airway larger, but more prone to collapse (Martin 1997, Mohsenin 2003). In females, the menopause is a risk factor for OSA, which may be attenuated by hormone replacement therapy (Bixler 2001), suggesting a hormonal aetiology for gender differences. Post-menopausal women however are still less likely to develop OSA than men. Even taking into account the difference in prevalence between the sexes, female patients are under-represented in sleep clinics and hence in studies. The reasons for this are unclear but it may reflect an increase in disease severity amongst males (O'Connor 2000). There is some evidence to suggest that females are less likely to report ‘classical’ symptoms of OSA than men and may be more likely to report other symptoms, such as fatigue or difficulty initiating sleep (Jordan 2003). This may contribute to a lower index of suspicion amongst patients and physicians.

The prevalence of OSA increases with age up to an age of between 55 and 65 years, after which it seems to plateau (Bixler 1998, Young 2002b). The increase is likely to be multifactorial and weight gain may be a significant factor. However, a number of studies suggest that in the older population, the reasons for developing OSA may be different to that seen in the (more commonly described) middle aged population (Edwards 2014). Physiological and anatomical changes predisposing the upper airway to collapse appear to increase with age (Malhotra 2006, Eikermann 2007, Martin 1997). The number of spontaneous arousals from sleep has also been shown to increase with age (Redline 2004), suggesting that the arousal threshold becomes lower with age, although this has not been a universal finding (Edwards 2010). Additionally, OSA may present differently in the elderly, with nocturia, falls and cognitive impairment seen more frequently than in the middle aged population (Launois 2007). These factors, along with the observation that OSA may have less effect on mortality in the elderly population (Lavie 2005) have led to speculation as to whether OSA in the elderly represents a separate entity (Launois 2007).

As discussed above, craniofacial abnormalities are a risk factor for OSA including congenital syndromes such as Down's syndrome and Pierre Robin syndrome among others (Kent 2010). Aside from these well characterised genetic syndromes there is evidence of a genetic basis for OSA from family and twin studies (Riha 2009). In addition, known risk factors for OSA such as obesity and craniofacial abnormalities may be heritable. It has been estimated that up to 40% of the variance in AHI is attributable to familial factors (Redline 2000). Given the complex pathophysiology of OSA, it is likely to be a polygenic condition interacting with environmental factors, particularly obesity. The lack of a single OSA phenotype makes identification of genetic influences more complex with interest so far focusing on genes involved in obesity, craniofacial morphology, ventilatory control and sleep regulation. Acquired conditions such as hypothyroidism and acromegaly increase the risk of developing OSA, as can pregnancy. Sedative drugs and alcohol can exacerbate OSA which may in part be due to relaxation of the upper airway dilator muscles. Cigarette smoking has also been associated with OSA (Wetter 1994), although the exact nature of the relationship remains unclear.

1.8 Clinical presentation of obstructive sleep apnoea (OSA)

Patients with OSA may present with nocturnal symptoms (usually reported by a bed partner), daytime symptoms (secondary to sleep disturbance) or both. Nocturnal symptoms include snoring, frequent awakenings, choking sensations resulting in awakening from sleep, apnoeas and enuresis. Most patients with OSA snore, but absence of snoring does not exclude a diagnosis. Patients may also report a dry mouth or headache in the morning and often awaken feeling unrefreshed. Daytime symptoms include excessive daytime sleepiness (EDS), fatigue, poor concentration, low mood and cognitive impairment. These symptoms can impact on patients' quality of life and on their ability to drive or work safely. Subjective scales can be used in order to try and determine the presence of EDS, the best-known and used of which is the Epworth Sleepiness Score (ESS) (see Appendix 1) (Johns 1991). Patients grade the likelihood of falling asleep in a variety of situations from 'would never doze' to 3, 'high chance of dozing'. A score of ≥11 out of a maximum of 24 is deemed to represent EDS, although this is not specific to OSA. The ESS has the advantage of being cheap and easy to administer and has been shown to reliably differentiate between normal and abnormal levels of sleepiness (Johns 1991, Johns 1992), and improves following successful treatment (McDaid 2009). However it does not correlate with disease severity, as measured during overnight sleep monitoring; neither does it closely correlate with more objective measures of sleepiness such as the modified sleep latency test (MSLT) or maintenance of wakefulness test (MWT) (Benbadis 1999, Johns 2000). For a variety of reasons, patients may under- or overestimate their symptoms and so a partner's perspective is useful and may correlate slightly better with objective measures of sleepiness (Kingshott 1995). The onset of OSA is usually insidious, occurring over years and so a high index of suspicion is required, particularly in patients presenting with vague symptoms of fatigue or mood disturbance. A detailed sleep history should always be obtained in these cases. Clinical examination in patients with OSA is often normal; however there are some salient features to be noted. A significant proportion of patients are obese and a BMI should be recorded: however, neck circumference and waist to hip ratio are probably more useful measurements in this condition than BMI

alone (Davies 1992, Vgontzas 2000)**.** Patients should be examined to exclude obvious craniofacial abnormalities and their oral cavities inspected.
Given the association of OSA with cardiovascular disease (McNicholas 2007) and hypertension in particular (Stradling 2001), a blood pressure should also be recorded.

1.9 Diagnosis of obstructive sleep apnoea (OSA)

A diagnosis of OSA is made based on the history and objective overnight monitoring of sleep to determine if there is any evidence of sleep disordered breathing. To fulfil the definition of OSHAS, patients additionally need to have symptoms of EDS and this can be determined using the Epworth Sleepiness Score (see section 1.8 above). Sleep can be monitored in a number of ways. The current 'gold standard' is considered to be overnight polysomnography (PSG) which provides the most comprehensive assessment of a nights' sleep. A PSG requires an in-patient stay and involves the recording of numerous sleep and respiratory variables. These routinely include electroencephalography (EEG), electro-oculography (EOG), electromyography (EMG), oxygen saturations, nasal airflow, snoring, thoraco-abdominal movement, and body position. The various sleep stages are scored (see section 1.4 for details) enabling the total sleep time to be determined. As described above in section 1.3, respiratory events during sleep, namely the number of apnoeas and hypopnoeas per hour can then be determined and expressed as the apnoea/hypopnoea index (AHI). A less labour intensive option is a limited sleep study (or polygraphy) which involves the monitoring of respiratory variables but without EEG monitoring. After appropriate instruction, patients can apply the equipment themselves at home, avoiding a hospital admission and the associated costs. A recent systematic review suggested that while the AHI determined at PSG and limited study were not directly comparable, limited studies were an acceptable means of diagnosing obstructive apnoea in patients with a high pre-test probability of the disease under the supervision of an appropriately qualified service (Fleetham 2011). Limited studies are felt to be less useful in those with other co-morbidities (Fleetham 2011, Collop 2007) and the lack of sleep staging information means that arousals cannot be scored and hence some hypopnoeas may be missed giving an underestimate of disease severity. In addition, the rate of data loss with limited

studies ranges from 3 to 18% (Flemons 2003), which if the facilities are available, require repeat investigation, usually with a full PSG. Single channel oximetry is used as a screening tool in some centres, but it is generally felt to be insufficient as the sole means of investigating OSA, missing approximately one third of cases in one series (Douglas 1992).

1.10 Consequences of obstructive sleep apnoea (OSA)

The 'immediate' consequences of OSA include excessive daytime sleepiness, cognitive impairment, subsequent impairment of quality of life and increased risk of road traffic (and probably) workplace accidents (George 2004, Ulfberg 2000, Lindberg 2001). Importantly, OSA is also associated with a significant increase in morbidity and mortality largely due to cardiovascular disease (McNicholas 2007) and as the main subject of this thesis, will be discussed in depth in the following chapter.

1.10.1 Excessive daytime sleepiness (EDS)

The presence of excessive daytime sleepiness (EDS) along with evidence of OSA is the hallmark of the obstructive sleep apnoea/ hypopnoea syndrome (OSAHS). EDS affects quality of life and is a significant risk factor for road traffic accidents (see section 1.9.3 below). The often insidious nature of OSA means that patients may not appreciate how abnormally sleep they are, with this only becoming apparent retrospectively following commencement of treatment (Engleman 1997). CPAP therapy improves EDS in patients with OSA, with the greatest effect seen in patients with more severe disease (McDaid 2009).

1.10.2 Cognitive impairment

OSA has been associated with cognitive impairment across a broad range of functions including attention, learning and executive function which is most significant in those with moderate or severe disease (Beebe 2003, Engleman 2004, Lal 2012). Proposed mechanisms for cognitive impairment include the impact of EDS, sleep fragmentation itself and intermittent hypoxia (Bucks 2013). Improvements in cognitive function have been shown with CPAP treatment, but this

is not a universal finding (Kielb 2012) and certainly is not as impressive as the improvements in EDS seen with CPAP.

1.10.3 Road traffic accidents

Patients with OSAHS are 2-4 times more likely to be involved in a road traffic accident (George 2004), with just over a third of patients in one study retrospectively admitting to driving impairment due to sleepiness prior to commencing therapy (Engleman 1997). Current UK Driver and Vehicle Licensing Agency (DVLA) guidelines require patients with OSAHS to report their condition and mandate that driving must cease until satisfactory control of symptoms is achieved; regulations are stricter for those holding a group 2 licence (DVLA 2013). The relationship between disease severity, as measured by the AHI, and the risk of accident is not clear (Turkington 2001), although this is not too surprising given the often multifactorial nature of road traffic accidents. Equally unclear is the relationship between reported sleepiness and driving risk, as for a variety of reasons, not least concerns regarding the potential loss of their driving licence, patients may under-report their symptoms (Engleman 1997). Worryingly, commercial drivers appear to be at even greater risk of developing OSA (Tregear 2009).

1.10.4 Quality of life

OSA is associated with significant impairment of health-related quality of life across a number of domains and not simply related to EDS or cardiovascular complications (Jenkinson 1997, Lacasse 2002). The degree of impairment does not appear to correlate with disease severity as determined objectively using the AHI (Flemons 2002). An OSA-specific questionnaire, the Calgary Sleep Apnea Quality of Life Index (SAQLI) has been validated for measuring changes in quality of life with treatment (Flemons 1998). Early studies using general (rather than disease-specific) measures of quality of life suggested that improvements were seen with CPAP treatment (Engleman 1999, Jenkinson 1999), however this was not confirmed in a more recent meta-analysis (Jing 2008) and further studies using disease-specific measures of quality of life are required.

1.11 Treatment of obstructive sleep apnoea (OSA)

The 'gold standard' treatment for OSA is continuous positive airway pressure (CPAP) therapy. It is not however a cure and only alleviates symptoms if satisfactory compliance is achieved. In milder disease, oral devices may play a role and are often better tolerated. Given the association with obesity, lifestyle modification resulting in weight reduction would seem an obvious treatment for OSA and is widely recommended by physicians and in guidelines. Over the years numerous surgical procedures for the upper airway have been proposed. However the role of these operations is usually limited to situations where there is a clearly defined anatomical abnormality that can be ameliorated, such as tonsillar hypertrophy. At present there is no role for drug treatment in OSA.

1.11.1 Continuous Positive Airway Pressure (CPAP) Therapy

Continuous positive airway pressure (CPAP) therapy was first described as a treatment for OSA over 30 years ago (Sullivan 1981) and remains standard therapy (SIGN 2003, NICE 2008). Positive pressure is delivered to the airway via a tight fitting nasal or oro-nasal mask, creating a pneumatic splint, holding open the upper airway and preventing both sleep fragmentation and oxygen desaturation. The mask is connected, via tubing, to a flow generator. To determine pressure requirements, a titration study (either performed manually with PSG monitoring or using an intelligent auto-setting CPAP device) is usually performed, with the aim of reducing the AHI to below 5. Numerous CPAP devices are available commercially and can either deliver a fixed pressure of air, or may be auto-titrating; meaning the device continually adjusts the pressure delivered during the night to keep the airway open. CPAP is an effective treatment for EDS, particularly in those with moderate to severe disease (McDaid 2009), and may improve quality of life and cognitive function. Observational studies showa reduction in road traffic accident risk in CPAP-treated patients (Tregear 2010). CPAP use is associated with a reduction in the cardiovascular morbidity and mortality associated with OSA (Marin 2005) and numerous studies have shown that CPAP is effective in reducing blood pressure in patients with OSA (Bazzano 2007). The effects of CPAP on the cardiovascular

system constitute one of the main themes of this thesis and this is discussed in detail in the following chapter.

CPAP therapy is not without side-effects and due to the tight-fitting nature of the mask, the positive pressure of air blowing through it and the noise associated with this, it may not be tolerated by patients (or their bed partners). The side effects include local skin irritation from the mask, conjunctivitis due to air leak and nasal congestion or dryness. CPAP is not a 'cure' for OSA and is only effective in reducing symptoms if it is worn regularly at night. Side effects, along with the intrusiveness and inconvenience of wearing the device on a nightly basis mean, that despite the proven efficacy of CPAP, there are significant issues with compliance. Early studies suggested that CPAP was rejected initially or within the first week of treatment by between 5 and 50% of patients with another significant proportion discontinuing CPAP in the subsequent three years (Engleman 2003). Amongst those who continue to use CPAP, it is not entirely clear what constitutes adequate use and this is variably defined in studies. The use of CPAP for four hours per night on 70% of nights is often quoted as being adequate (Sawyer 2011). A significant proportion of subjects in research studies are non-compliant using this standard (Weaver 2008) and 'real-life' compliance is likely to be even lower.

1.11.2 Oral appliances

Numerous oral appliances (also known as mandibular repositioning splints or mandibular advancement devices) are available (both custom made and commercially) and are worn at night, with the aim of advancing the mandible and thus increasing the size of the upper airway. Oral appliances have been the subject of fewer randomised controlled trials than CPAP, but there is evidence to support their use in the treatment of OSA at the milder end of the spectrum, with improvements seen in AHI and EDS (Mehta 2001, Gotsopoulos 2002, Barnes 2004), although these have generally been shown to be inferior to the improvements seen with CPAP (Barnes 2004, Lim 2009). In the recently published and largest randomised controlled trial to date, both CPAP and oral appliance led to significant improvements in the AHI (although the reduction with CPAP was greater) and ESS. Both treatments led to similar improvements in disease-specific quality of life and

driving simulator performance (Phillips 2013a). Oral appliances may also be effective in lowering blood pressure in patients with OSA, although most of the evidence is not from randomised controlled trials (Iftikhar 2013).
Despite their poorer efficacy, oral appliances are often more acceptable to patients than CPAP (Hoffstein 2007, Phillips 2013a), particularly in those with milder disease (Barnes 2004). Oral appliances are not without side effects, which can include a dry mouth, excessive salivation, local irritation and pain, and in the longer term can cause changes to the bone structure of the lower face and bite (Clark 2000, Roberston 2001). National guidelines (SIGN 2003, Kushida 2006) recommend the use of oral appliances in those with mild disease or in those whom CPAP cannot be tolerated.

1.11.3 Lifestyle modification

Given the clear association with obesity it would seem logical that weight reduction would be important in the management of OSA. More than 10 years ago, Peppard *et al* showed that in the general population a weight gain of 10% was associated with a 32% increase in the AHI, with a 26% reduction in AHI following a weight loss of 10% (Peppard 2000a). Intensive lifestyle counselling and a weight reduction programme has been shown to reduce the AHI to below 5 in around two-thirds of overweight patients with mild OSA (Tuomilehto 2009) and a very low calorie diet regime significantly reduced the AHI in obese patients with moderate to severe OSA, with the effects persisting at one year (Johansson 2009). Tuomilehto *et al* went on to show that if successful weight loss was maintained, then disease progression over 5 yr follow up was also reduced (Tuomilehto 2014). A recent meta-analysis identified 12 randomised controlled studies of the effect of weight loss, exercise, or other lifestyle modification upon sleep apnoea severity and concluded that intensive lifestyle interventions that included caloric restriction +/- exercise regimes were effective at reducing indices of OSA severity (Thomasouli 2013). A subsequent meta-analysis reports similar findings, but noted that despite improvements in indices of OSA severity only a minority of patients were effectively ‘cured’ of OSA (Mitchell 2014). The effectiveness of weight loss for OSA is an area of increasing interest with a recent RCT showing greater improvements in CRP, insulin resistance and serum triglyceride levels in patients with OSA randomised to weight loss alone

when compared to CPAP therapy. Similar reductions in blood pressure were seen in both the weight loss and CPAP groups (Chirinos 2014). Further research is needed to determine the effectiveness of weight loss strategies in the longer term. Current Scottish Intercollegiate Guidelines Network (SIGN) guidelines recommend advising overweight patients to lose weight, but in recognition of the fact that this is rarely achieved, warn that this should not delay the institution of proven therapies such as CPAP (SIGN 2003). More recently however it has also been suggested that there is a bi-directional relationship between obesity and OSA, with OSA perhaps perpetuating the overweight state (Ong 2013). Advice is usually also given to patients to improve sleep hygiene and to avoid contributory factors such as alcohol, sedative drugs and cigarettes.

1.11.4 Surgery

The first surgical treatment for OSA was tracheostomy which, while effective (Guilleminault 1981), is invasive and carries significant side effects and is now only recommended in severe cases where all other treatments have failed (SIGN 2003). Subsequently, numerous upper airway procedures have been used to treat OSA, including uvulopalatopharyngoplasty (UPPP), laser-assisted uvulopalatoplasty, maxillo-mandibular osteotomy and palatal implants. There is a lack of randomised controlled trial evidence regarding the efficacy of surgery in adults (Caples 2010). As such surgery is not recommended (SIGN 2003) and may actually make future treatment with CPAP more difficult. The exceptions to this are cases in which there are obstructing lesions affecting the upper airway, including adenotonsillectomy in children. Evidence from non-randomised controlled studies suggests that adenotonsillectomy is effective in the treatment of paediatric OSA (Redline 2011). More recently the results of a multicentre randomised controlled trial comparing early adenotonsillectomy with watchful waiting in paediatric patients with OSA have been published (Marcus 2013). No differences were noted in the primary outcome measures of attention or executive function as measured by the Developmental Neuropsychological Assessment. However in children undergoing adenotonsillectomy, greater improvements were seen in the secondary outcomes of polysomnographic findings, symptoms, quality of life and behaviour at 7 months.

Bariatric surgery for the control of morbid obesity may be effective in reducing the AHI (Greenberg 2009), but a recent study showed that although it resulted in a greater weight loss than calorie restriction and exercise, the effect upon the AHI was not statistically greater (Dixon 2012).

1.11.5 Drug treatment

Given the challenges of the above treatments, pharmacotherapy would be an appealing option and numerous drugs have been investigated including respiratory stimulants, promoters of upper airway dilatation and REM sleep suppressants (Lin 2012). Despite this, drug therapy currently plays no significant role in the treatment of OSA. An exception to this may be the treatment of an underlying condition such as hypothyroidism, although there is no good evidence that this is an effective treatment for OSA. Adjunctive therapies such as the use of intra-nasal steroids in concomitant rhinitis may be useful (Kiely 2004), and stimulants such as modafinil have been shown to reduce sleepiness that persists despite CPAP use (Pack 2001, Black 2005). The latter is not a universal finding and may actually reduce CPAP compliance (Kingshott 2001, Lin 2012). Due to concerns regarding side effects and potential illicit use, modafinil is no longer licensed for the treatment of residual sleepiness in OSA in Scotland (SMC 2010).

Given the often multifactorial aetiology of OSA, it seems unlikely that a single drug treatment would be effective for in all cases. More work is clearly required in this area, not least in determining the phenotypes that may benefit from specific treatments.

1.12 Concluding remarks

OSA is a common and underdiagnosed condition that carries with it a significant symptom burden, and when it is associated with EDS it is termed OSAHS .The aetiology of OSA is often multifactorial, but obesity is an important risk factor. CPAP therapy is an effective treatment for obstructive events and EDS, however despite its efficacy, it is not always tolerated by patients. Over the last three decades it has become increasingly clear that untreated OSA is associated with increased cardiovascular morbidity and mortality and the evidence for this, along with the

potential pathophysiological mechanisms linking OSA and CVD, will be discussed in depth in Chapter 2.

Chapter 2: Obstructive sleep apnoea and cardiovascular disease

2.1 Introduction

Cardiovascular disease (CVD) is common, as is OSA, and the two conditions share a number of risk factors including increasing age, male gender and obesity (Kiely 2000, Leung 2001). Untreated moderate to severe OSA is associated with greater cardiovascular morbidity and mortality (Marin 2005). OSA is an independent risk factor for hypertension and has been associated with most other types of CVD (Stradling 2001, McNicholas 2007, Parati 2013). Numerous mechanisms for the development of CVD in OSA have been proposed, namely intermittent hypoxia (IH), repetitive arousals/sleep fragmentation and intra-thoracic pressure swings (McNicholas 2007). These variously lead to activation of the sympathetic nervous system, systematic inflammation and oxidative stress; the common end point of which may be increased arterial stiffness and endothelial dysfunction. CPAP therapy may reduce CVD risk (Marti 2002, Marin 2005) and has been shown to reduce blood pressure in patients with OSA (Bazzano 2007). Prior to starting this study, evidence from small studies suggested that CPAP therapy could reduce arterial stiffness (Kitihara 2006) and improve endothelial function (Ip 2004). In this chapter, the available evidence for the association between OSA and CVD prior to the start of this study in 2007 will be discussed, along with the potential pathophysiological mechanisms behind such an association. More recent work will be discussed along with the findings of this study in Chapter 7.

2.2 Cardiovascular disease (CVD)

CVD is the leading cause of death globally (WHO 2002) and encompasses a broad spectrum of diseases affecting the cardiovascular system including, but not exclusively, hypertension, ischaemic heart disease (IHD), cerebrovascular disease and congestive cardiac failure. Hypertension is a risk factor for other CVDs, particularly IHD and cerebrovascular disease (Kannel 2000). CVD has traditionally been viewed as a disease of the developed world, however the incidence is also increasing in the developing world where it has been linked to increasing urbanisation, dietary changes and increases in smoking rates, obesity and diabetes

(Celermajer 2012). Most CVD is the result of vascular dysfunction due to hypertension or atheromatous disease and numerous risk factors have been identified. Common risk factors for CVD include obesity, lack of physical activity, smoking alcohol use, hypertension, hypercholesterolaemia and type II diabetes mellitus. In the UK, mortality from CVD has fallen in recent years, but despite public health campaigns aimed at lifestyle modification and advances in the treatment of CVD, the disease burden remains high (Department of Health 2013). In view of this, there has been much interest in the identification of preclinical CVD and the prediction of which patients are at risk of developing clinically overt CVD so that therapeutic intervention can be offered earlier. Along with interest in circulating biomarkers (van Holten 2013), arterial stiffness and endothelial function (see sections 2.3 and 2.4 below) have been established as measures of vascular 'health'. Evidence of increased arterial stiffness and endothelial dysfunction may be seen in the absence of overt CVD or hypertension (Celermajer 1992, Reddy 1994, Laurent 2006, Lane 2006), but both have been associated with subsequent CVD and indeed a poorer prognosis from pre-existing CVD (Laurent 2006, Perticone 2001, Yeboah 2007).

2.3 Arterial stiffness

Arterial stiffness, essentially a measure of vascular ageing, refers to a reduction in the normal expansion and contraction of arteries in response to changes in pressure and can be measured non-invasively in a number of ways at a variety of sites along the vascular tree. A consequence of increased arterial stiffness is increased propagation velocity of blood flow through the arterial tree. Arterial stiffness has been shown to be an independent predictor of CVD in both the general population and in high risk groups (Laurent 2006).

The structure and function of arteries changes along the arterial tree. Elastic fibres predominate within the thoracic aorta providing a cushioning function (Safar 2003). There has been much interest in measurement of arterial stiffness in this region as this is the arterial segment that the left ventricle 'sees', with greater stiffness here leading to increased cardiac afterload and impaired coronary blood flow (Weber 2004). Stiffness in the thoracic aorta is primarily determined by the relative contributions of the major scaffolding proteins within the extra-cellular matrix

(ECM), namely collagen and elastin (Laurent 2006, Safar 2003). Collagen and elastin are subject to a constant process of production and degradation, which in normality retains the *status quo*. Any process leading to elastin damage or an increase in the relative collagen content leads to increased arterial stiffness (Alvolio 1998, Zieman 2005). Factors known to increase relative collagen content within the vascular ECM include; ageing (London 2004, Sawabe 2010), systemic inflammation and increased pressure within the vessel lumen (i.e. systemic hypertension) (Zieman 2005, Tomiyama 2010). Arterial calcification, such as that seen with ageing (Dunmore-Buyze 2002) and renal disease (Guerin 2000), is probably also important in promoting stiffening (McEniery 2009, Cecelja 2012). Arterial stiffening can also occur in conjunction with the intimal lipid and inflammatory cell deposition seen in atherosclerosis. However, it is unclear if atherosclerosis alone is sufficient to cause arterial stiffening (Zieman 2005, Quinn 2012). Histological examination of intima from stiffened vessels confirms increased collagen content, degraded elastin fibres, abnormal endothelial cells, inflammatory cell infiltrate and increased vascular smooth muscle cells (Zieman 2005).

Collagen and smooth muscle fibres predominating within the arterial wall of peripheral arteries and stiffness here is primarily modulated by vasomotor tone as influenced by endothelial function (See section 2.4 below), the sympathetic nervous system, the renin aldosterone system and vessel wall hypertrophy. In health, arterial stiffness increases along the arterial tree, although in later life or in CVD disease, the central vessels may become stiffer than those more peripherally (Safar 2003).

Prior to commencing this study there was emerging evidence that OSA was associated with increased arterial stiffness and this is discussed in section 2.11 below.

2.4 Endothelial dysfunction

The endothelium is a single layer of cells lining the vascular intima throughout the entire vascular tree and forms the interface between circulating blood and the vessel wall. In health the endothelium has a number of important roles including; the regulation of vascular tone, haemostasis, cellular adhesion, vascular inflammation and smooth muscle cell proliferation (Deanfield 2007). In response to a variety of

physical and chemical signals, the endothelium can produce a wide range of vasoactive factors, of which the most studied is probably nitric oxide (NO), the main mediator of vascular function (Hayward 2002, Tomiyama 2009, Marti 2012). NO is produced by endothelial NO synthetase (eNOS) in response to endogenous signalling molecules such as acetylcholine and bradykinin, drugs such as beta-2 agonists (Hayward 2002, Wilkinson 2001), ischaemia, temperature change and mechanical factors including shear stress (Marti 2012). On release, NO acts upon vascular smooth muscle cells, activating guanylate cyclase and leading to cyclic guanosine monophosphate (GMP)-mediated vasodilatation (Marti 2012). Under normal conditions, shear stress is primarily responsible for NO release, enabling tissue perfusion to adapt to changes in cardiac output (Deanfield 2007). Along with vasodilation, NO also acts to prevent atheroma formation (Wilkinson 2002) by inhibiting vascular smooth muscle proliferation, platelet aggregation, leucocyte adhesion to endothelial cells and nuclear transcription of cell adhesion molecules (Tomiyama 2009). Integral to this inhibition is the s-nitrosylation of cysteine residues within a wide variety of key regulator proteins including the transcription factor NFκB (Deanfield 2007).

Prostacyclin released by the endothelium also vasodilates. The endothelium can also effect vasoconstriction through the release of prostanoids and endothelin, along with the conversion of angiotensin I to angiotensin II on the endothelial surface (Deanfield 2007). The obligatory role of the endothelium in vasodilatation provides a means of quantifying endothelial function, with impaired vasodilatation following the application of an appropriate stimulus (Anderson 1999, Wilkinson 2002) and is termed endothelium-dependent vasodilation.

Endothelial dysfunction (usually regarded as impaired endothelium-dependent vasodilation) represents a preclinical vascular abnormality which occurs prior to atheroma formation (Celermajer 1992, Reddy 1994) and reflects an activation of the vascular endothelium that can be induced by a variety of stimuli (Deanfield 2007). Pathological stimulation of the endothelium leads to a switch to redox signalling and mitochondrial release of ROS which react with cysteine groups in the same key regulator proteins leading to increased transcription and protein activation. Under normal conditions NO is produced by eNOS, however following endothelial

activation eNOS can also generate ROS, thus setting up a vicious cycle (Deanfield 2007, Feng 2012). Numerous risk factors for CVD, including age (Taddei 1995), hypertension (Perticone 2001), diabetes (Clarkson 1996), smoking (Celermajer 1993), hypercholesterolaemia (Creager 1990, Chowienczyk 1992) and obesity (Arcaro 1999, Suh 2005) are associated with endothelial dysfunction**.** Interventions aimed at reducing these risk factors, such as the use of antihypertensives, smoking cessation, lipid lowering drugs and physical exercise have been shown to improve endothelial function, suggesting endothelial dysfunction plays an important pathophysiological role in the development of CVD (Vita 2002). Indeed, studies have shown endothelial dysfunction to be an independent predictor of cardiovascular morbidity and mortality (Perticone 2001, Heitzer 2001, Heitzer 2005, Yeboah 2007). Prior to commencing this study, evidence of a link between OSA and endothelial dysfunction was beginning to emerge and this is discussed in section 2.12 below. Endothelium-independent vasodilation refers to the vasodilatatory response to exogenous NO (such as GTN, used in this study). This is a vascular smooth muscle response and in the presence of exogenous NO, is not dependent on NO release from an intact endothelium. GTN is converted to NO within the smooth muscle cell, activating guanylate cyclase and leading to cyclic guanosine monophosphate (GMP) accumulation, effecting vasodilation (Adams 1998). Whilst many studies have demonstrated impaired endothelium-dependent vasodilation in CVD and in subjects with cardiovascular risk factors (see above), fewer studies have reported a statistically significant impairment in endothelium-independent vasodilation in similar patients (Schachinger 1995, Adams 1998). In a large study of adults without overt CVD or hypertension, endothelium-independent vasodilation was significantly correlated with endothelium dependent vasodilation ($r=0.41$; $p<0.001$) (Adams 1995) and in another study was shown in coronary vessels to be predictive of cardiovascular events (Schachinger 2000). Potential mechanisms for impaired endothelium-independent vasodilation are smooth muscle cell dysfunction, including altered responsiveness to any of the stimulators of guanylate cyclase within the cell and mechanical factors preventing vessel wall dilatation, such as vessel wall fibrosis (Adams 1998, Schachinger 1995). More recently, it has been speculated that damage to vascular smooth muscle requires a longer exposure to a given risk factor than is

required for damage to the endothelium, which theoretically may explain why impairment of endothelium-dependent vasodilation is more commonly seen (Hoyos 2015).

Endothelial function can be considered in both the microvasculature and microvasculature, although a distinction between the two is often not made in the literature. Both have been shown to correlate with coronary artery endothelial function (Anderson 1995, Bonetti 2004) but macrovascular and microvascular endothelial function are only weakly correlated with each other (Hamburg 2011) and this may reflect their different physiological roles of conduit and resistance arteries (Flammer 2012). It has been suggested that macrovascular endothelial impairment may be more important in patients with pre-existing CVD whereas microvascular endothelial dyfunction may be important in detecting pre-clinical CVD (Flammer 2012, Yannoutsos 2014).

2.5 Evidence for a link between OSA and cardiovascular disease

Evidence of an association between OSA and cardiovascular disease (CVD) began to emerge in the 1980s. Early studies reported associations between snoring, cardiovascular risk factors (Jennum 1993a, Jennum 1993b) and a variety of CVDs (Parish 1990). With a high incidence of undiagnosed OSA found amongst snorers (Block 1987) however, and work suggesting that snoring alone (in the absence of OSA) was not associated with an increased risk of hypertension (Hoffstein 1988), snoring was deemed likely to be a confounder (Waller 1989). The focus switched to examining the relationship between OSA and CVD. A later longitudinal study with follow up over ten years did not show simple snorers to be at increased cardiovascular risk when compared to healthy non-snorers (Marin 2005).

Initial observational studies reported that patients with OSA who were not treated with either tracheostomy or CPAP therapy had mortality rates of between 11 and 13% at five years, largely due to vascular disease (Parish 1990). Subsequently the population based Sleep Heart Health Study, reported the (self-reported) prevalence of CVD to be 1.42 times greater in those with an AHI in the upper quartile of the study, as compared to those in the lower quartile. This, despite the fact that the median AHI in the upper quartile was only 19 (Shahar 2001). In a study of patients referred to a

sleep laboratory, free from known CVD at baseline, subjects found to have OSA had a five-fold increase in the incidence of CVD after seven years of follow up when compared to those without OSA, independently of age, BMI, blood pressure or smoking status. This figure rose to 11-fold in subjects who were deemed to be incompletely treated (Peker 2002). The association between OSA and CVD is further evidenced by studies showing that patients with untreated OSA are at greater risk of death due to CVD than those receiving effective treatment (Marti 2002, Marin 2005, Doherty 2005, Martinez-Garcia 2012a). In the largest prospective observational study to date, patients with severe untreated OSA had an approximately three-fold increase in both fatal and non-fatal cardiovascular events after ten years, after adjustment for potential confounders (Marin 2005). A significant source of bias in this study was the possibility that patients who declined CPAP treatment differed from those who accepted treatment in other unmeasurable ways, i.e. compliance with other treatments and risk taking behaviour.

Marti *et al* compared the mortality in patients with severe OSAHS who were considered to be treated following weight loss, surgery or CPAP therapy with untreated patients from the same centre. To reduce bias, patients who declined treatment with CPAP after it became available at the study centre in 1988 were excluded. Overall mortality was significantly higher in the untreated group than in those treated, particularly in patients under 50 years of age. In treated patients, mortality was not significantly different than in the general population (Marti 2002).

Doherty *et al* followed 168 patients with OSAHS who had been commenced on CPAP therapy at least five years previously. Approximately one third of the original cohort were intolerant of CPAP at the time of follow-up and outcomes in this group were compared to the group still using CPAP therapy. Although there was no difference in the development of CVD morbidity between the two groups, death due to CVD was significantly higher in the CPAP intolerant group (14.8% vs. 1.9%; p=0.009) (Doherty 2005). More recently, in a large prospective observational study, Martinez-Garcia *et al* showed that in subjects over 65 years of age, moderate to severe OSA was associated with increased cardiovascular mortality (hazard ratio of 2.25). Cardiovascular mortality was significantly lower (hazard ratio 0.93) in those

receiving effective CPAP therapy (defined as CPAP use ≥4 hours per night) (Martinez-Garcia 2012a).

CVD is a broad term, encompassing a number of diseases including, but not exclusively, hypertension, IHD, cerebrovascular disease and congestive cardiac failure and is also closely linked with the metabolic syndrome. OSA is established as an independent risk factor for hypertension (Stradling 2001) and this is discussed in section 2.6 below. Evidence for individual associations between OSA and IHD, cerebrovascular disease and congestive cardiac failure are examined separately below (See sections 2.7, 2.8 and 2.9 below). There is a significant amount of evidence that OSA is associated with CVD, but with the exception of hypertension, it has not been possible to conclusively demonstrate that OSA is an independent risk factor for CVD. This lack of evidence has led in the past to concern that the adverse cardiovascular effects of OSA have been overemphasised (Wright 1997). However, more recent evidence particularly with respect to hypertension would contradict this view.

2.6 OSA and hypertension

Hypertension is a major, but potentially modifiable, risk factor for CVD and OSA and hypertension commonly co-exist. Indeed it has been estimated that 40% of patients with OSA have hypertension and a similar percentage of patients with hypertension have OSA (Phillips B 2005, Parati 2013). However, as for CVD in general, OSA and diurnal hypertension have a number of shared risk factors, most notably obesity, increasing age and gender, which in the past have confounded the issue of OSA as an independent risk factor for hypertension (Stradling 2001). Several large scale epidemiological studies have sought to control for known confounders (Grote 1999, Lavie 2000, Nieto 2000, Peppard 2000) and studies have subsequently shown CPAP therapy to reduce blood pressure in patients with OSA (Bazzano 2007). In light of these studies and prior to the commencement of our study, OSA had been established as an independent risk factor for hypertension (Stradling 2001, Buyse 2007, McNicholas 2007) and had been recognised in European (Mancia 2007) and American (Chobanian 2003) guidelines as a cause of secondary hypertension. Given the prevalence of OSA in patients with hypertension, it is likely that OSA is

responsible for a proportion of what was previously deemed essential hypertension. OSA is particularly recognised as a cause of treatment-resistant hypertension (Grote 2000, Logan 2001).

2.6.1 Hypertension – an overview

At the time of starting this study, hypertension was defined by the then current National Institute for Health and Care Excellence (NICE) guidelines as a clinic blood pressure of greater than 140/90 mmHg on at least two separate measurements (NICE 2006). However, the first sign of hypertension may be the loss of the normal nocturnal dip (approximately 20 mmHg) in blood pressure (Phillips B 2005). Hypertension is usually asymptomatic but is estimated to be responsible for 4.5 % of worldwide disease burden (Whitworth 2003) and has a prevalence of over 30% in the adult population in Scotland (Scarborough 2010). Following many epidemiological studies, including notably a wealth of information gained from studying over 5000 inhabitants of Framingham, Massachussets since 1948, hypertension is a well-established risk factor for IHD, cerebrovascular disease, cardiac failure and peripheral arterial disease (Kannel 2000), accounting for around a third of all atherosclerotic events (Kannel 1996). Between the ages of 40 and 69 years, every sustained 20 mmHg increase in systolic blood pressure (or 10 mmHg increase in diastolic blood pressure) leads to a two-fold increase in cardiovascular mortality down to a blood pressure as low as 115/75 mmHg (Lewington 2002). A full discussion of the treatment of hypertension is outwith the scope of this thesis. However, in general terms, unless an underlying cause is identified, initial treatment involves lifestyle modification including weight loss, increased exercise and a reduction in salt and alcohol intake. Pharmacotherapy is the next step and numerous classes of drugs are available and are often used in combination. Following the recognition of OSA as a cause of hypertension there have been numerous studies examining the effect of CPAP therapy upon blood pressure (Bazzano 2007) and these are discussed in more detail below (See section 2.6.4 below).

2.6.2 Blood pressure and sleep

Blood pressure is normally lower during sleep in both the normotensive and hypertensive populations (Bristow 1969, Millar-Craig 1978, Pickering 1982), so called 'dipping', with a gradual fall in blood pressure from the onset of sleep with a prompt rise on wakening (Bristow 1969). The exception to this is during REM sleep, which is accompanied by increased sympathetic activity and fluctuations in blood pressure (Phillips B 2005). It has long been established that OSA causes a rise in mean nocturnal blood pressure (Davies 1993a, Leung 2001) due to the recurrent acute elevations in blood pressure associated with each obstructive event. This has been demonstrated in response to intermittent hypoxia (Leuenberger 1995) and transient arousals from sleep (Davies 1993b), such as that seen in OSA.

A significant proportion of patients with OSA do not experience a nocturnal drop in blood pressure (so called 'non-dippers') and in other populations, this lack of a nocturnal drop in blood pressure, has been shown to be associated with increased cardiovascular risk and a poorer prognosis (Parati 2006).

2.6.3 OSA – an independent risk factor for hypertension

There has long been an appreciation of an association between OSA and hypertension. Early case series reported an increased frequency of hypertension in patients with OSA (Guilleminault 1976, Stradling 2001), followed by reports of a high prevalence of OSA among patients attending hypertension clinics compared to non-hypertensive controls (Lavie 1984b, Kales 1984). Although subsequent studies seemed to support a causative role for OSA in hypertension, they were largely subject to the same criticisms, namely that they failed to account for the fact that OSA and hypertension share a number of important risk factors including, most notably, obesity (and particularly upper body obesity that may not be well accounted for by measuring BMI alone), age and alcohol intake (Stradling 2001). These risk factors are difficult to disentangle or indeed account for, in study design. For example, patients with OSA and excessive daytime sleepiness are likely to lead a more sedentary lifestyle than subjects without OSA, this is in itself a risk factor for hypertension (before even considering the effect of a sedentary lifestyle on obesity) and could certainly confound any study of the relationship between the two

conditions. These factors, along with other confounders such as the way in which blood pressure was measured and indeed in the way that OSA was diagnosed, along with the inclusion of patients taking antihypertensive therapy led to varying opinions as to the relationship between OSA and hypertension (Wright 1997).

Evidence for a causal relationship between OSA and hypertension was derived from animal studies as early as 1992, with a rise in diurnal blood pressure seen in rats exposed to periods of intermittent hypoxia (Fletcher 1992). Brooks *et al* were able to simulate OSA in tracheostomised dogs by intermittently occluding the tracheostomy tube during sleep over several months (Brooks 1997). This led to a rise in diurnal blood pressure which subsequently resolved after the removal of the simulated OSA. Following the publication of four large well-conducted studies (Grote 1999, Lavie 2000, Nieto 2000, Peppard 2000) between 1999 and 2000, there has been a general acceptance of OSA as an independent risk factor for hypertension (Stradling 2001, McNicholas 2007). The cross-sectional community based Sleep Heart Health study (Nieto 2000) examined blood pressure and sleep disordered breathing in 6132 middle aged and older subjects. Blood pressure and the prevalence of hypertension increased significantly with increased AHI. Subjects with an AHI of ≥30 had an odds ratio of 1.37 for hypertension compared to those with an AHI of <1.5 after adjusting for confounders including BMI, neck circumference, waist-to-hip ratio, alcohol use and smoking. Again, after adjustment for confounders, subjects who spent ≥12% of sleep time with oxygen saturations of less than 90% had an odds ratio of 1.45 for hypertension when compared to subjects who spent <0.05% of sleep time with oxygen saturations of less than 90%, after adjusting for the same confounders. In the prospective, population based Wisconsin Sleep Cohort, a dose response relationship between the severity of OSA and presence of hypertension (defined as a blood pressure of ≥140/90 mmHg or the use of anti-hypertensive medication) four years later was demonstrated (n=709). After adjusting for BMI, neck and waist circumference, age, sex, alcohol use and smoking, the odds ratio for hypertension was 2.89 for subjects with an AHI >15. Interestingly, the odds ratio for hypertension was raised at 1.42 in those with an AHI generally considered to be within the normal range (AHI 0.1-4.9) (Peppard 2000). Similarly, in two studies of patients referred to a sleep clinic (Grote 1999, Lavie 2000), the severity of OSA (as measured by the

RDI and AHI respectively) was independently and linearly associated with blood pressure, despite adjustment for confounders. When hypertension was defined as ≥160/95 mmHg, rather than ≥140/90 mmHg, the odds ratio was higher still for each category of OSA severity (Grote 1999). Additionally the relationship between OSA and hypertension was found to be greater in those aged ≤50 years (Grote 1999). Several studies have reported that the association between OSA and hypertension is greater in younger patients (Bixler 2000, Grote 2000) and indeed in the Sleep Heart Health Study, the association between the AHI and blood pressure was lost when patients aged ≥65 years were examined separately. An association did however remain between blood pressure and the percentage of sleep time with oxygen saturations below 90% (Nieto 2000).

2.6.4 The effect of CPAP therapy on blood pressure in OSA

Good evidence, from randomised controlled trials that regular CPAP use reduces blood pressure in OSA followed the initial observational studies on OSA and hypertension. Over ten years ago, Faccenda *et al* showed a small reduction in 24-hour diastolic blood pressure with CPAP compared to a tablet placebo. A greater reduction, along with a reduction in 24-hour systolic blood pressure was seen in those with the most severe disease (Faccenda 2001). A subsequent study confirmed this finding, with a small reduction in mean 24-hour blood pressure (2.5mmHg reduction) seen with CPAP compared to sham CPAP (Pepperell 2002). Numerous studies followed and subsequent meta-analyses reported significant, albeit small (around 2-3mmHg) reductions in blood pressure with CPAP therapy (Bazzano 2007, Haentjens 2007, Schein 2014) with the greatest reductions in those with more severe OSA, higher BMI and higher baseline blood pressures (Bazzano 2007). In more recent meta-analyses of the effect of CPAP on blood pressure in patients with OSA and resistant hypertension a greater reduction in blood pressure was seen (Iftikhar 2014, Liu 2015), suggesting that it is this group of patients who have the most to gain from CPAP in terms of blood pressure reduction. A reduction in blood pressure with CPAP therapy has not however been a universal finding, with studies in patients with mild OSA (Barnes 2002), patients with OSA in the absence of EDS (Barbe 2001, Robinson 2006) and a study of patients with OSA and treated hypertension (Campos-

Rodriguez 2006) finding no change in blood pressure following CPAP therapy. This remains an area of interest as it may provide further insights into the pathophysiology of the association between OSA and hypertension.

2.7 OSA and Ischaemic heart disease (IHD)

More than 20 years ago Hung *et al* reported an increased prevalence of OSA in patients following an acute myocardial infarction compared to an age-matched control group, which persisted after adjustment for other cardiovascular risk factors (Hung 1990). Subsequent epidemiological studies confirmed that OSA was over-represented in patient populations with IHD (Mooe 1996a, Mooe 1996b, Peker 1999). In the population based, Sleep Heart Health Study there was a modest increase in self-reported coronary heart disease in the upper quartile of AHI compared to the lower quartile (1.27 (95 % CI 0.99–1.62) (Shahar 2001). Patients with OSA were found to be at high risk of future coronary heart disease, using Framingham-derived risk prediction methods (Kiely 2000) and after 7 years of follow-up, patients with OSA, free from CVD at baseline, were significantly more likely to develop coronary artery disease than snorers without OSA (16.2% vs. 5.4%; p=0.003) (Peker 2006).

In patients with known coronary artery disease, the presence of co-existing OSA indicated a poor prognosis with a relative risk of 1.62 (1.09- 2.41: p=0.017) of the composite end point (death, cerebrovascular event or myocardial infarction) in subjects with an AHI ≥10 (Mooe 2001). Increased cardiovascular mortality at five years was also reported by Peker *et al* in a relatively small group of patients hospitalized with CVD who had an RDI of ≥10. They also found the respiratory disturbance index (RDI) to be an independent predictor of cardiovascular mortality (Peker 2000). In relatively small numbers of patients, effective CPAP therapy has been shown to attenuate this increased risk of coronary artery disease (Peker 2006). Additionally, in a prospective non-randomised study, patients with OSA and coronary artery disease who received effective CPAP therapy experienced a hazard ratio of 0.24 (95% CI 0.09-0.62; p<0.01) for a composite cardiovascular end-point including cardiovascular death, acute coronary syndrome, hospitalisation for heart failure, or need for coronary revascularisation (Milleron 2004).

2.8 OSA and cerebrovascular disease

OSA is common in patients following a cerebrovascular accident (CVA) and indeed is much commoner than central sleep apnoea (CSA) (Good 1996, Kaneko 2003). Several studies have shown a similar prevalence of OSA in patients following a transient ischaemic attack (TIA) (Bassetti 1999, Parra 2000) suggesting that OSA predates, rather than is a consequence of, the CVA (Leung 2001, Hamilton 2004). This has not been a universal finding however, with McArdle *et al* finding OSA to be no more common in patients with TIA than in matched controls (McArdle 2003). No clear pattern of CVA, in terms of location or severity has been associated with OSA. This, along with the finding that although there may be a reduction in central apnoeic events, the severity of OSA remained constant three months after a CVA, further suggests that OSA is a risk factor for cerebrovascular disease (Parra 2000).

In a large population-based study, the odds ratio for CVA were raised in the highest AHI quartile compared to the lowest quartile (1.58 [95% CI 1.02-2.46]) (Shahar 2001). Subsequently Yaggi *et al* showed that, after adjustment for known confounders (including hypertension), the presence of OSA was associated with a hazard ratio of 1.97 for the composite end point of CVA or all-cause mortality. They also demonstrated a dose-response effect, with those with the most severe OSA being at greatest risk (Yaggi 2005). This finding was made, despite the fact that a significant number of patients were receiving treatment for OSA. A greater than two-fold increased stroke risk has been shown in elderly subjects with an AHI ≥30 after adjustment for known confounders (Munoz 2006). In the population based Wisconsin Sleep Cohort, an AHI ≥20 was associated with a four-fold risk of prevalent CVA, independently of confounders. In the prospective arm of the study, the odds ratio of having a first CVA during the four year follow- up was similarly high. However this finding failed to remain statistically significant after adjustment for age, sex and BMI (Arzt 2005).

The concomitant presence of OSA and CVA leads to a poorer prognosis, both in terms of mortality (Parra 2004) and functional recovery (Good 1996, Kaneko 2003a). There is evidence from non-randomised studies to suggest that commencing CPAP treatment in patients with OSA following a CVA reduces subsequent CVA risk (Martinez-Garcia 2005, Martinez- Garcia 2012b) and in moderate to severe OSA,

may reduce all-cause mortality (Martinez-Garcia 2009). CPAP therapy may improve wellbeing (Wessendorf 2001) and in one randomised study reduced depressive symptoms (Sandberg 2001), but had no effect in another study (Hsu 2006). In a randomised study, subjects found to have OSA (AHI ≥20) after a first ischaemic stroke who received CPAP therapy, were more likely to have had a neurological improvement at one month. They also had a longer time interval before the incidence of subsequent cardiovascular events, but after 18 months follow-up there was no difference in cardiovascular mortality in those randomised to receive CPAP therapy (Parra 2011). Further follow-up of these patients at five years however, did show a reduction in cardiovascular mortality in those treated with CPAP (Parra 2014).
It is unclear whether CPAP therapy in OSA is beneficial in terms of primary prevention of cerebrovascular disease due to the lack of prospective, randomised studies (See section 2.10 below).

2.9 OSA and congestive cardiac failure (CCF)

OSA is variably reported to be present in between 11 and 53% of patients with congestive cardiac failure (CCF) (McNicholas 2007), a higher proportion than the 2-4% prevalence (Young 1993) in the general population. CSA is commoner still (Javaheri 1998), but further discussion of this is beyond the scope of this thesis.
In the Sleep Heart Health Study, the odds ratio for self-reported heart failure between those in the highest and lowest AHI quartiles was 2.38 (95% CI 1.22-4.62) independently of other known risk factors (Shahar 2001). As above, OSA is an independent risk factor for hypertension (Stradling 2001), itself a risk factor for CCF. It has also been postulated that the negative intra-thoracic pressure seen during obstructive events may, along with the acute haemodynamic impact of such events, exert direct deleterious effects on the left ventricle (Hamilton 2004). CCF itself may predispose to the development of OSA, with the periodic breathing commonly seen in CCF making the pharyngeal wall more vulnerable to collapse with the subsequent development of obstructive apnoea (Shamsuzzaman 2003). Additionally fluid redistribution at night from dependant regions such as the lower limbs to the upper body and neck may predispose to airway collapse (Chiu 2006, Shiota 2007).

Animal studies show that dogs exposed to intermittent nocturnal apnoeas over several months had a decrease in left ventricular ejection fraction (LVEF) (Parker 1999). Following myocardial infarction and subsequent percutaneous coronary intervention PCI, the presence of OSA inhibits left ventricular systolic recovery (Nakashima 2006). There is little evidence regarding the impact of OSA on mortality in CCF patients. In a study of patients with severe CCF, referred to a specialist centre, the presence of concomitant OSA did not increase mortality after a median of 52 months follow-up (Roebuck 2004). However, some of the patients with OSA did receive CPAP therapy during the follow-up period.

Two randomised studies have shown CPAP to improve LVEF after one and three months of CPAP therapy respectively, in patients with CCF and concomitant OSA (Kaneko 2003b, Mansfield 2004). The study by Kaneko was small, with only 12 patients randomised to receive CPAP therapy, but also demonstrated an improvement in blood pressure (Kaneko 2003). However more recently Smith *et al*, using a placebo controlled cross over study design, did not show any improvement in LVEF after six weeks of CPAP therapy (Smith 2007). Therefore, whilst CPAP therapy may improve symptoms of OSA in patients with CCF, it is not clear whether it is beneficial in terms of measures of left ventricular function or mortality. A confounding issue relates to the observation that patients with OSA and CCF are much less likely to report EDS than patients with OSA alone (Kaneko 2003, Arzt 2006). The reasons for this are unclear, but it is interesting to note that for any given AHI, patients with OSA and CCF are likely to have a lower BMI than patients with OSA alone (Arzt 2006). The consequence of patients being less sleepy prior to commencing CPAP therapy is that they may well be less compliant with CPAP therapy due to a lack of a perceived benefit. The absence of EDS may also mean they are less likely to be investigated for possible OSA in the first instance. Additionally, CPAP therapy has previously been shown to be ineffective in reducing blood pressure in OSA patients without EDS (Barbe 2001, Robinson 2006). The mechanism for this is unclear but clearly this may also be a factor in non-sleepy CCF patients.

2.10 Difficulties in establishing OSA as in independent risk factor for CVD

Significant difficulties exist in trying to determine whether OSA is an independent risk factor for CVD. OSA and CVD share numerous risk factors, the most important of which are likely to be increasing age, male gender and obesity (Kiely 2000, Leung 2001, McNicholas 2007), particularly upper body obesity (Davies 1990, Ben–Noun 2003). The number of potential shared risk factors makes accounting for these in study design, and hence demonstrating OSA as an independent risk factor, very difficult. Equally, OSA is now known to be an independent risk factor for hypertension (Stradling 2001), which is itself a major risk factor for most other CVD (Kannel 2000). Therefore, teasing out any extra risk from OSA alone is likely to be difficult and would require, among other things, very careful blood pressure matching of subjects.

A further significant issue is the clear association between OSA and the metabolic syndrome. The metabolic syndrome is essentially a clustering of cardiovascular risk factors and in 2001 the diagnosis was defined as comprising three of the following: increased waist circumference, high blood pressure, increased fasting glucose, increased triglycerides and decreased high density lipoprotein (HDL) cholesterol (Cleeman 2001). Obesity (Young 1993, Young 2005), hypertension (Stradling 2001), insulin resistance (Ip 2002, Punjabi 2002) and dyslipidaemia (Ip 2000, Kiely 2000) have all individually been reported in association with OSA, leading to the suggestion from some that OSA could be considered a component of the metabolic syndrome rather than a separate disease entity (Vgontzas 2005). Coughlin *et al* were the first to examine the prevalence of all the component parts of the metabolic syndrome in patients with OSA (Coughlin 2004). The prevalence of the metabolic syndrome in patients with OSA was 87%. After adjustment for known confounders including BMI, patients with OSA were nine times more likely to fulfil the diagnosis of the metabolic syndrome than subjects without OSA. The exact mechanism behind this association is unclear, but hormonal control of sleep and metabolism are linked (McNicholas 2007) and in healthy subjects, acute hypoxia induces glucose intolerance (Oltmanns 2004). Further evidence of an association comes from studies showing that CPAP therapy is effective at improving individual components of the

syndrome, namely blood pressure (Stradling 2001) and HDL cholesterol (Borgel 2006). The effect of CPAP therapy on insulin sensitivity is not clear, with some studies showing an improvement whilst other report no change (Punjabi 2005). This association between OSA and metabolic syndrome makes teasing out the relative contribution of OSA to CVD risk extremely difficult**.**

CVD typically develops over many years and as such, long term follow up studies are required. Much of the evidence for an association between OSA and CVD comes from studies showing that patients treated with CPAP therapy suffer less CVD morbidity and mortality (Marti 2002, Marin 2005, Doherty 2005). Ideally, these would be randomised controlled studies; however given the already proven benefits of CPAP therapy in terms of OSA symptom control (Engleman 1999, McDaid 2009), it would be unethical to withhold CPAP therapy for prolonged periods of time.

2.11 OSA and arterial stiffness

Prior to starting this study, evidence was beginning to emerge that OSA was associated with increased arterial stiffness. Arterial stiffness, as measured by the augmentation index (AIx), is transiently increased during obstructive events in patients with OSA, in the absence of changes in non-invasively measured blood pressure (Jelic 2002). Philips *et al* subsequently showed in 57 otherwise healthy patients referred with suspected OSA that the AIx correlated with OSA severity (Phillips C 2005). Arterial stiffness, as measured by brachial-ankle pulse wave velocity, was shown to be higher in patients with OSA (some of whom also had CVD) (Nagahama 2004, Shiina 2006) and aortic distensibility was shown to be lower in a small group of patients with OSA (free from CVD) (Kasikcioglu 2005), as compared to control groups. Furthermore, in a case-control study of 30 subjects with OSA in the absence of CVD, Drager *et al* showed that aortic pulse wave velocity (PWV) was elevated even in patients with mild to moderate disease. They also found PWV to correlate significantly with OSA severity (Drager 2005). A subsequent small (n=17) non-randomised study of the effect of CPAP on arterial stiffness showed reductions in brachial-ankle pulse wave velocity after two months of treatment, raising the possibility that arterial stiffness may be reversed, or at least attenuated by CPAP (Kitihara 2006). Since starting this study, there has been an explosion of work

in this area, and this will be discussed along with the findings of this study in Chapter 7.

2.12 OSA and endothelial dysfunction

Almost 20 years ago, Carlson *et al* reported impaired endothelial function (both endothelium-dependent and -independent vasodilatation) in subjects with OSA compared to control subjects (Carlson 1996). Endothelium–dependent vasodilatation was subsequently shown to be impaired in OSA in a number of studies (Kato 2000, Duchna 2000, Imadojemu 2002, Ip 2004, Oflaz 2006), even in those with only mild OSA (Duchna 2006). These studies were all small (number of patients with OSA ranging from 8 to 30) and reported a number of different measures of endothelium-dependent vasodilatation including flow-mediated dilatation (Kato 2000, Ip 2004, Olfaz 2006, El Solh 2007), hand vein compliance (Duchna 2000,Duchna 2006) and venous occlusion plethysmography (Carlson 1996, Imadojemu 2002). None of these subsequent studies confirmed the impairment of endothelium-independent vasodilatation reported by Carlson *et al* (Carlson 1996). In a small study of male patients with OSA, without known CVD, endothelium-dependent vasodilatation was negatively correlated with the degree of nocturnal hypoxaemia (Kraiczi 2001). A subsequent larger study reported flow mediated-dilatation to be negatively correlated with the AHI, but on sub-group analysis by sex, this only remained true in females, with no correlation in the male population (Faulx 2004). A large population based study appeared to confirm these findings, with endothelial dysfunction correlating with measures of OSA severity including the AHI and the percentage of sleep time with oxygen saturations below 90% (Nieto 2004). BMI was, however, a significant confounder in this study and on multiple regression analysis the association weakened, with only the hypoxaemia index remaining significant ($p=0.041$). Proposed circulating markers of endothelial dysfunction were shown to be altered in patients with OSA, including soluble adhesion molecules (Ohga 1999), nitric oxide (Lavie 2003) and vascular endothelial growth factor (Lavie 2002) with higher numbers of circulating apoptotic endothelial cells noted in patients with OSA (El Solh 2007).

On the basis of these studies, and prior to commencing this study, several small studies had shown an improvement in endothelial function after a period of CPAP treatment (Duchna 2000, Imadojemu 2002,Ip 2004, Duchna 2005, Duchna 2006, Lattimore 2006, El Sohl 2007) ranging from two weeks (Imadojemu 2002) to six months (Duchna 2005) and in one case in patients with mild OSA (AHI 6.7 +/-3.8) (Duchna 2006). Only one of these was a randomised controlled trial and this additionally showed that in a subset of patients in whom CPAP therapy was then withdrawn for a week, endothelial function returned to baseline levels (Ip 2004). As with arterial stiffness, interest in the association between OSA and endothelial dysfunction has grown considerably since starting the study and this will be discussed, along with the findings of this study in Chapter 7.

2.13 Mechanisms of cardiovascular damage in OSA

2.13.1 Introduction

The immediate physiological effects of OSA have been well documented for some time and include intermittent hypoxia, repeated arousals/sleep fragmentation and intra-thoracic pressure swings leading to activation of the sympathetic nervous system. Any causal link between OSA and CVD is likely to be multifactorial (McNicholas 2007), but prior to starting this study, it was felt that intermittent hypoxia might play the most important role (Lavie 2003, Foster 2007). The precise mechanisms underlying the association between OSA and CVD were (and indeed remain) incompletely understood, but the important biological consequences of the abnormal physiology seen in OSA are likely to include activation of the sympathetic nervous system, systemic inflammation and oxidative stress along with the metabolic dysregulation commonly seen in OSA. Increased arterial stiffness and endothelial dysfunction are known to be precursors of overt CVD (Celermajer 1992, Reddy 1994, Laurent 2006, Lane 2006) and as such may represent the final common pathway for damage to the cardiovascular system from OSA. This is represented schematically in Figure 2.1 below

Figure 2.1 Mechanisms of cardiovascular damage in OSA

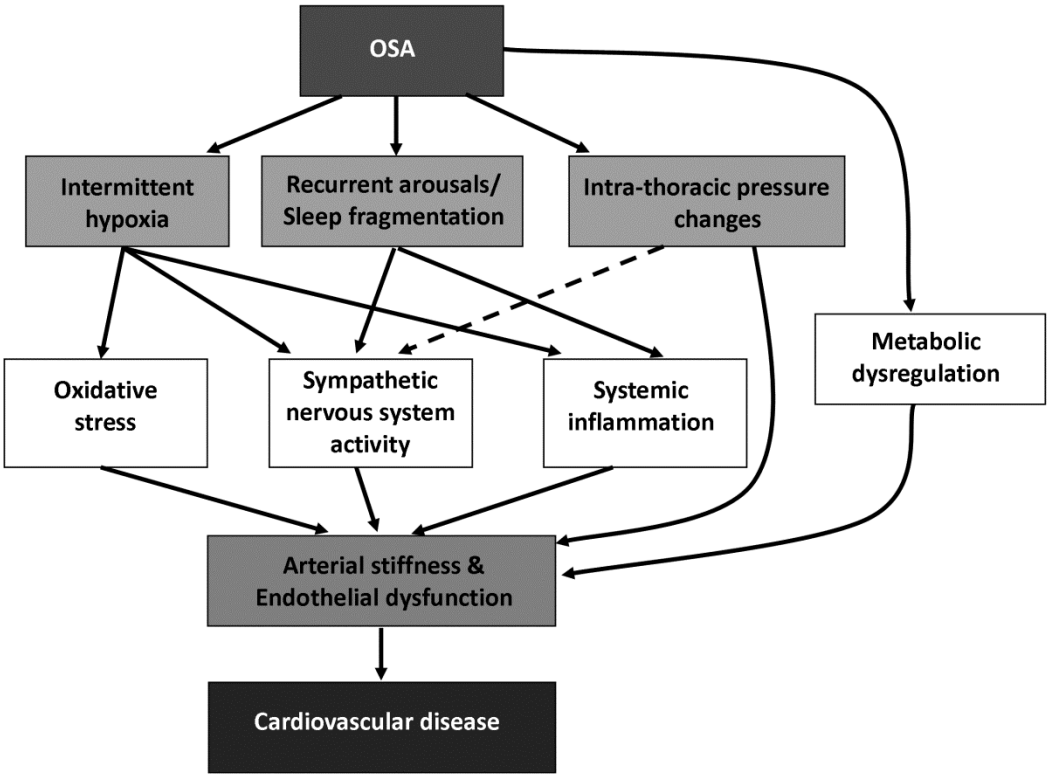

Figure 2.1 Diagram of the pathophysiological mechanisms linking OSA and cardiovascular disease. The broken line represents a *possible* link between intra-thoracic pressure changes and activation of the sympathetic nervous system.

In the sections below, the available evidence for the potential pathophysiological mechanisms linking OSA and CVD at the time of starting this study will be laid out, with more recent studies discussed along with the findings of this study in Chapter 7.

2.13.2 Intermittent hypoxia (IH)

Unique to OSA, intermittent hypoxia (IH) refers to the repetitive episodes of desaturation and subsequent rapidly occurring re-oxygenation which often follow obstructive events. These episodes have been likened to ischaemia-reperfusion injuries (Ryan 2005) and unlike the well described adaptive changes associated with sustained hypoxia; the molecular response to IH is incompletely understood. A number of deleterious consequences have been described, including activation of the

sympathetic nervous system, systemic inflammation and oxidative stress, and in animal models, exposure to chronic IH leads to sustained arterial hypertension (Fletcher 1992, Greenberg 1999).

2.13.2.1 Intermittent hypoxia (IH) and sympathetic nervous system activity

It is well recognised that the recurrent apnoeas associated with OSA lead to an acute increase in blood pressure that is preceded by increased sympathetic nerve activity (SNA) (Hedner 1988, Katraggada 1997). However, patients with OSA also have evidence of persistently elevated SNA as evidenced by increased urinary catecholamines (Fletcher 1987, Dimsdale 1995) and by directly measured muscle SNA (Carlson 1993, Somers 1995, Narkiewicz 1998a). Evidence for the association between intermittent hypoxia (IH), increased SNA and hypertension initially came from animal studies (Prabhakar 2005). Rats exposed to 30 days of IH, achieved by intermittently altering the fraction of inspired oxygen, developed significant dose-dependent increases in arterial blood pressure compared to those kept at normoxia. The rise in blood pressure however, was not apparent in rats exposed to IH in whom the sympathetic nervous system had been inhibited, suggesting a crucial role for SNA in the development of hypertension (Lesske 1997, Bao 1997). Similarly, in dogs with a tracheostomy inserted to enable intermittent nocturnal airway obstruction, an elevation in day time blood pressure was seen. In the same dogs, subsequently subjected to sleep fragmentation under a separate protocol, but without airway obstruction, no increase in daytime blood pressure was seen (Brooks 1997), again suggesting an important role for IH. Furthermore, animals exposed to chronic IH show an increased sympathetic response to subsequent hypoxia (Greenberg 1999). Healthy humans exposed to sustained or intermittent hypoxia over a 20 minute period show evidence of increased muscle SNA, which persists for at least 20 minutes (Morgan 1995, Xie 2000) and maybe beyond three hours (Cutler2004) after the cessation of hypoxia. During voluntary breath holds and the Mueller manoeuvres in healthy humans, arterial blood pressure and SNA increase (Morgan 1993, Katragadda 1997). This increase can be abolished by ganglionic blockade of the autonomic nervous system with trimethaphan, again suggesting that an intact

sympathetic nervous system is required (Katragadda 1997). CPAP therapy has been shown to reduce urinary catecholamines (Hedner 1995), plasma noradrenaline (Heitmann 2004) and directly measured SNA (Narkiewicz 1999). CPAP however attenuates all of the immediate physiological effects of OSA and so this does not itself directly implicate IH. Further evidence for IH as an important cause of increased SNA comes from studies in patients with OSA in whom the increase in SNA was attenuated by the provision of 100% oxygen (Leuenberger 1995, Narkiewicz 1998b).

2.13.2.2 Intermittent hypoxia (IH) and systemic inflammation

Systemic inflammation is now recognised as playing a central role in the initiation and progression of atherosclerosis (Faxon 2004). C-reactive protein (CRP) is a well-recognised marker of systemic inflammation and predicts cardiovascular risk in healthy subjects (Ridker 2001) and along with tumour necrosis factor α (TNFα), predicts future cardiovascular events in patients with underlying CVD (Lindahl 2000, Ridker 2000). Chronic hypoxia is associated with an elevated CRP (Hartmann 2000) and initial studies in patients with OSA, both with (Yokoe 2003), and without (Shamsuzzaman 2002) CVD, showed the CRP to be elevated and related to the severity of OSA, independently of the BMI (Shamsuzzaman 2002, Yokoe 2003). Interleukin (IL)-6, a pro-inflammatory cytokine involved in the synthesis of acute phase proteins including CRP, has also been shown to be elevated in OSA (Yokoe 2003, Ciftci 2004) and both CRP and Il-6 fell with CPAP therapy (Yokoe 2003). This finding has not however been replicated in more recent studies. In a large study, Guiileminault *et al* did not find the CRP to be elevated in patients with OSA (Guilleminault 2004). Philips *et al* showed no change in CRP after withdrawal of CPAP therapy, despite confirmation of OSA recurrence and evidence of increased sympathetic activity (Phillips 2007).

Further evidence for the role of IH in systemic inflammation comes from a cell culture model of IH. The molecular effects of chronic hypoxia are well defined and via the transcription factor hypoxia-inducible factor -1 (HIF-1), mediate an adaptive response directed at increasing oxygen delivery to the tissues (Semenza 2004). HeLa cells exposed to IH however appear to preferentially up-regulate pro-inflammatory

pathways via nuclear factor-κB (NFκB) (Ryan 2005). Furthermore, Ryan *et al* showed that TNFα (part of the NFκB -mediated inflammatory cascade) was increased in patients with OSA as compared to control subjects and decreased following 6-weeks of CPAP therapy (Ryan 2005). Other studies have reported similar findings (Minoguchi 2004, Ryan 2006), along with increased levels of other NFκB-mediated inflammatory markers such as Il-6 (Yokoe 2003, Minoguchi 2005), Il-8 (Dyugovskaya 2003, Ryan 2006) and intercellular adhesion molecule (ICAM)-1 (Ohga 1999). There is evidence from human studies suggesting that the increases in a number of the above markers of inflammation are dose-dependent (Shamsuzzaman 2002, Yokoe 2003, Minoguchi 2005), often using the AHI as a measure of severity. This however, does not necessarily imply that hypoxia is the main, or only driver of inflammation, as intra-thoracic pressure changes and arousals also increase with the AHI. In an animal model, where IH can be simulated by altering the fraction of inspired oxygen without inducing airway obstruction or arousal, NFκB activation has also been reported (Greenberg 2006) lending further weight to the theory that IH causes systemic inflammation. Whilst NFκB seems to be preferentially activated in IH, there is evidence to suggest that, as with chronic hypoxia, there is activation of HIF-1 pathways in patients with severe OSA-related hypoxaemia (Schulz 2002, Winnicki 2004). This is largely an adaptive pathway (Semenza 2004), however HIF-1 may also have an inflammatory function (Cramer 2003).

NFκB also mediates the expression of both tissue factor and Factor VIII, important components in the extrinsic and intrinsic coagulation cascades, respectively (Greenberg 2006). There is evidence that patients with OSA have increased blood viscosity, fibrinogen and platelet activation (McNicholas 2007). This, along with numerous studies pointing to hypercoagulability as a risk factor for CVD in the general population (von Känel 2003) has led to speculation that this may be a factor in the pathogenesis of CVD in OSA (Leung 2001, McNicholas 2007).

2.13.2.3 Intermittent hypoxia (IH) and oxidative stress

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capabilities of a biological system. Under normal conditions, ROS have an essential signalling role. However, due to their reactive nature, when present in excess, ROS can directly interact with cellular components, leading to cellular dysfunction (Dröge 2002). Additionally, superoxide -a common ROS- reacts with nitric oxide (NO), diminishing the availability of NO to the endothelium (Beckman 1996), which may contribute to endothelial dysfunction (Lavie 2003). Cell culture work suggests that IH leads to increased ROS production (Peng 2003) and animal models of IH have demonstrated an increase in ROS and oxidation products (Xu 2004, Chen 2005). Markers of oxidative stress have been shown to be increased in patients with OSA (Schulz 2000, Dyugovskaya 2002). This has not however been a universal finding (Svatikova 2005, Suzuki 2006), with further work required to elucidate the importance of oxidative stress in the mechanism of cardiovascular damage in OSA.

2.13.3 Sleep fragmentation/arousals

Not all obstructive events are associated with significant oxygen desaturation, particularly in young, otherwise healthy patients, in whom arousals may be more important from a pathophysiological point of view. Obstructive events are commonly associated with a degree of arousal from sleep, which serves to activate the upper airway dilator muscle, permitting resumption of unobstructed breathing (Shamsuzzaman 2003). Repeated arousals lead to sleep fragmentation, sleep deprivation and excessive daytime sleepiness, the latter being the hallmark of OSAHS. During non-REM sleep, in healthy human subjects, arousal stimuli (such as a loud noise) are accompanied by bursts of SNA (Somers 1993a). In snorers, with polysomnographic evidence of frequent arousals but not OSA, hypertension was much commoner than in snorers with less frequent arousals (Lofaso 1996). In dogs, spontaneous arousals led to an increase in heart rate that could be significantly attenuated by sympathetic blockade, suggesting a role for arousals in SNA (Horner 1995). This is however somewhat at odds with subsequent work in dogs exposed to sleep fragmentation, where no increase in blood pressure was seen (Brooks 1997)

and further studies are required in this area. Significantly fragmented sleep can lead to sleep deprivation and experimental sleep deprivation is known to affect glucose metabolism (Knutson 2007). Short sleep duration has been linked to obesity (Chaput 2007, Taheri 2004) and experimental sleep restriction has been shown to be associated with raised inflammatory markers (Vgontzas 2004, Irwin 2006). Irwin *et al* found evidence of raised SNA, as measured by increased plasma catecholamines, in sleep deprivation (Irwin 1999). These studies reflect sleep deprivation rather than sleep fragmentation *per se* and the role of arousal in CVD remains unclear.

2.13.4 Intra-thoracic pressure changes

OSA leads to repetitive forced inspiration against an obstructed (or partially obstructed) airway, generating significant intra-thoracic pressure swings, with negative pressures as low as -80mmHg reported (Shiomi 1991). Negative intra-thoracic pressure results acutely in increased left ventricular transmural pressure, which in turn leads to increased afterload (Buda 1979, Virolainen 1995) and increased myocardial oxygen demand (Bradley 2001), culminating in decreased stroke volume (Stoohs 1992). It remains unclear whether these acute physiological changes result in ventricular hypertrophy and cardiac remodelling over time.
Intra-thoracic pressure swings also increase the pressure gradient across the aortic wall (Magder 1983) and this may exert shear stress upon the aortic walls. In animal studies, aortic diameter was acutely increased during episodes of negative intra-thoracic pressure (Peters 1988 a and b).This along with the observation, albeit in a small study, that OSA is common in patients with Marfan's syndrome (Cistulli 1993) and in patients presenting with dissection of the thoracic aorta (Sampol 2003) has led to the suggestion that negative intra-thoracic pressure may contribute to aortic dilatation and dissection (Cistulli 1997, Sampol 2003). More recently there has been increased interest in this area and this will be discussed in Chapter 7.
It has also been postulated that intra-thoracic pressure swings may contribute to the increased sympathetic activity seen in OSA (Somers 1993b, McNicholas 2007). The autonomic response to the Mueller manoeuvre is complex, with sympathetic activity initially falling and subsequently increasing to more than twice the baseline levels. A similar magnitude of increase in sympathetic activity was seen in voluntary apnoea

however, albeit without the initial drop in sympathetic activity (Somers 1993b). Similar studies have shown an increase in sympathetic activity in both the Mueller manoeuvre and voluntary apnoea (Morgan 1993, Katragadda 1997). In one (Morgan 1993), this was significantly attenuated by the application of supplemental oxygen, perhaps suggesting that intra-thoracic pressure change alone may not be a significant factor in the pathophysiology of increased sympathetic activity although further work is required.

2.14 Impact of the acute pathophysiological consequences of OSA on vascular function

In health, the vascular endothelium is primarily responsible for the regulation of vascular tone and haemostasis and has a number of roles in preventing vascular stiffening and atherosclerosis (see Sections 2.3 and 2.4). Endothelial dysfunction is widely recognised as a precursor to overt CVD (Celermajer 1992, Reddy 1994) and most CVD risk factors are known to adversely affect endothelial function (Deanfield 2007). Accumulating evidence suggests that impairment of endothelial function and a reduced capability for repair are important mechanisms linking OSA and CVD (Kohler 2010).

Endothelial dysfunction actually reflects an activation of the vascular endothelium that can be induced by a variety of stimuli. This activation is mediated by a change in intracellular signalling mechanisms and forms part of the acute physiological response to infection, but is considered abnormal if induced by pathological stimuli such as those encountered in OSA (Deanfield 2007). Under normal circumstances, the endothelium remains quiescent due to the inhibitory effects of nitric oxide (NO) upon inflammation, cellular proliferation and thrombosis. Integral to this inhibition is the s-nitrosylation of cysteine residues within a wide variety of key regulator proteins including the transcription factor NFκB. Pathological stimulation of the endothelium leads to a switch to redox signalling and mitochondrial release of ROS which react with cysteine groups in the same key regulator proteins leading to increased transcription and protein activation. Under normal conditions NO is produced by endothelial NO synthetase (eNOS), however following endothelial activation eNOS can also generate ROS, thus setting up a vicious cycle (Deanfield 2007, Feng 2010).

The acute pathophysiological consequences of OSA, namely intermittent hypoxia (IH), sleep fragmentation/arousals and intra-thoracic pressure changes, have all been proposed as causes of endothelial dysfunction, although the mechanisms are not fully elucidated (Kohler 2010, Feng 2012, Hoyos 2015). Of these, intermittent hypoxia (IH) has been the most studied (Feng 2012) and has been linked to endothelial dysfunction through oxidative stress, systemic inflammation and increased sympathetic nervous system activity (SNA).

Oxidative stress secondary to IH increases the production of reactive oxygen species (ROS) (Peng 2003, Xu 2004, Chen 2005) which have been implicated in endothelial dysfunction in a number of ways. Oxidative stress acts to inhibit the phosphorylation of eNOS (Tanaka 2005) and may also promote the breakdown of NO (Deanfield 2005) leading to a reduction in NO bioavailability. Additionally the oxidation of a crucial eNOS co-factor (tetrahydrobiopterin) leads eNOS to preferentially produce ROS, rather than NO (Deanfield 2007, Feng 2010). This further compounds the reduction in NO bioavailability and resultant endothelial dysfunction and drives increased oxidative stress. It is also suggested that ROS may upregulate the endothelial production of adhesion molecules which may then promote the migration of circulating leukocytes into the subendothelial space (Deanfield 2007) leading to structural changes within the endothelium.

IH is associated with systemic inflammation with preferential activation of NFκB pathways (Ryan 2005). Evidence for inflammation of the vascular endothelium comes from the upregulation of COX-2 and inducible nitric oxide synthetase (iNOS) in endothelial cells harvested from patients with OSA compared to control subjects (Jelic 2008). NFκB stimulates production of circulating adhesion molecules (Kohler 2010), which as described above can lead to structural changes within the endothelium.

As described in section 2.13.2.1, IH leads to increased sympathetic nervous system activation (SNA) which leads to acute increases in blood pressure and sustained arterial hypertension, both of which have been linked to arterial stiffness and endothelial dysfunction (Kohler 2010).

Other proposed mechanisms linking IH with endothelial dysfunction include the promotion of endothelial cell apoptosis (Feng 2012) and the release of vasoactive

substances such as endothelin-1 and angiotensin II, leading to vasoconstriction (Kohler 2010).

Similar to IH, arousals are associated with increased SNA (Somers 1993a) and acute increases in blood pressure which may exert shear stress upon the vasculature causing endothelial damage. OSA generates significant intra-thoracic pressure swings (Shiomi 1991) and there is evidence from animal (Peters1988 a and b) and human (Stöwhas 2011) studies to suggest that the resulting shear stress has a direct damaging effect on vasculature.

Repeated exposure to endothelial damage (such as that seen in OSA) can overwhelm the endogenous protective mechanisms within endothelial cells leading to endothelial dysfunction and endothelial cells damage (Deanfield 2007). Markers of such damage include the presence of circulating endothelial microparticles (EMPs) released from activated or apoptotic endothelial cells which can themselves have a direct effect upon endothelial function (Ayers 2013). Some studies have shown circulating EMPs to be increased in OSA (Ayers 2013, Trzepizur 2014), but this has not been a universal finding (Feng 2012). When injected into mice, EMPs from patients with OSA have however been shown to impair endothelial function (Priou 2010).

Following endothelial damage, repair can occur by replication of local mature endothelial cells, however in the presence of on-going risk factors this is unlikely to be sufficient to maintain endothelial integrity. Circulating endothelial progenitor cells (EPCs) provide an alternative repair mechanism and can be mobilised from the bone marrow via a process that is in part NO-dependent (Deanfield 2007). Several studies have shown EPCs to be reduced in OSA, but again this has not been a universal finding (Feng 2012).

As described above the known pathophysiological effects of OSA may directly impair endothelial function thorough a variety of mechanisms, some of which may be self- propagating and furthermore may impair endothelial repair processes.

2.15 Overview of Chapter 2

The discussion above demonstrates that there is a strong association between OSA and CVD, but with the exception of hypertension, there is **insufficient evidence to conclusively state that OSA is an independent risk factor for CVD**. OSA appears to be associated with increased arterial stiffness and impaired endothelial function, although the evidence for this was limited prior to starting this study. The potential pathophysiological mechanisms of any association are likely to be complex and remain incompletely understood, particularly in subjects without evidence of CVD at the time of OSA diagnosis. CPAP therapy has been shown to lower blood pressure and may attenuate cardiovascular risk, but it is not clear which groups of patients are most likely to benefit from this.

2.16 Aims of study

Many of the studies discussed above included patients with concomitant cardiovascular disease and/or hypertension. Additionally, with respect to the effect of CPAP on arterial stiffness and endothelial function there was a paucity of placebo-controlled randomised trial data prior to commencing this study.
With this in mind, the main aims of this study were:

1. To determine whether CPAP therapy has an effect upon measures of arterial stiffness and endothelial function in patients with OSA, in the **absence of known cardiovascular disease or hypertension**.
2. To compare arterial stiffness and endothelial function in a subset of 20 patients with OSAHS (defined as OSA plus EDS) and a group of well-matched control subjects.

Chapter 3: Methods

3.1 Introduction

As discussed in the previous chapter, the aim of this study was to determine whether CPAP had any effect upon measures of arterial stiffness and endothelial function in patients with OSA, in the absence of known cardiovascular disease (CVD). This was undertaken within the context of a double-blind randomised placebo-controlled crossover trial. Additionally, arterial stiffness and endothelial function in a subset of 20 patients with OSAHS (defined as OSA plus EDS) were compared with a group of well-matched control subjects.

Subjects were recruited for the study between March 2007 and February 2009. The study was approved by the Lothian Local Research Ethics Committee and written informed consent was obtained from all subjects. The study is registered with the International Standard Randomized Controlled Trial Number Register (ISRCTN48783995).

3.2 Study design

3.2.1 The randomised placebo-controlled trial

Eligible subjects (see Table 3.1 below for inclusion and exclusion criteria) underwent vascular assessments at baseline and were then randomised to receive either CPAP (S8 Autoset TM, ResMed, Abingdon, UK) or sham CPAP for 12 weeks before crossing into the second arm of the study for a further 12 weeks. Vascular assessments comprising measurements of arterial stiffness, endothelial function along with blood pressure were repeated after each arm of the study (see Figure 3.1 below). The primary end point of the study was aortic distensibility with secondary endpoints of pulse wave velocity, augmentation index and endothelial function (as measured by the effects of salbutamol and GTN on the augmentation index). Anthropometric measurements and an ESS were repeated at each visit.

Figure 3.1 Flowchart of study design (randomised placebo-controlled trial)

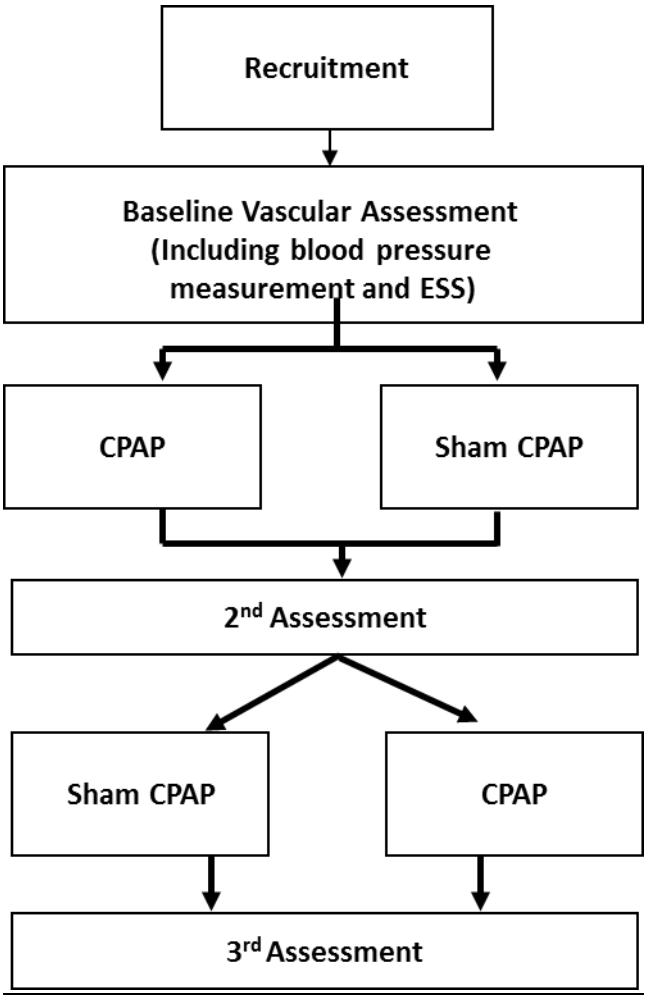

Figure 3.1 Flowchart of the study design. Anthropometric data, blood pressure measurements and ESS were also recorded at each of the three vascular assessments.

Randomisation was performed by a single researcher (MV) who was not involved in the measurement of vascular outcomes, by means of a computer generated balanced block. Both the subjects and the researchers undertaking the vascular assessments (AJ and MC) were blinded to treatment allocation. Unblinding occurred after all of the patients had completed the study and all trial data had been entered into a database and the database locked.

3.2.2 The case-control study

Twenty of the patients recruited to the randomised controlled trial were matched with 20 healthy controls (see section 3.4 below for inclusion and exclusion criteria). On the grounds that patients who report EDS are usually deemed to have more severe disease, it was decided *a priori* to match only patients who fulfilled the criteria for OSAHS (i.e. AHI $\geq$15 and ESS $\geq$11). On initial screening of control subjects their height and weight was ascertained to ensure that, assuming they remained eligible after polysomnography, they would be a suitable match for a subject with OSAHS. Controls were matched on a one-to one basis for sex, age (+/- 5 years) and BMI (+/- 10%). This was done individually for each control subject by examining their sex, age and BMI details against the baseline demographics of patients with OSAHS. Given the prevalence of undiagnosed OSA in the general population, it was anticipated that recruitment of matched (particularly BMI-matched) controls without polysomnographic evidence of OSA may be challenging. On this basis a pragmatic decision to match 20 subjects one-to-one with control subjects was made and this was not based upon a formal power calculation.

Control subjects underwent vascular assessments (along with ESS and anthropometric measurements) on one occasion and this was compared to baseline measurements for the matched patients. Subjects and controls were matched on a one-to-one basis for age (+/-5 years), sex and BMI (+/-10%).

3.3 Recruitment of subjects with obstructive sleep apnoea

Consecutive subjects aged 18 years or over attending the Department of Sleep Medicine at the Royal Infirmary of Edinburgh were approached if they met the criteria below (Table 3.1).

Table 3.1 Inclusion and exclusion criteria for subjects with OSA recruited to the randomised controlled trial

Inclusion criteria
Obstructive sleep apnoea diagnosed as an AHI ≥15 on overnight polysomnography
Exclusion criteria
History of cardiovascular disease (including hypertension) History of cerebrovascular disease History of diabetes mellitus (type I or type II) History of respiratory failure Use of anti-hypertensive medications Previous CPAP use History of sleepiness when driving Professional drivers Contra-indications to MRI scanning (e.g. implanted metal) Inter-current illness

Potentially eligible subjects were given or posted a patient information sheet and a reply slip to denote interest. For patients indicating interest in participating, a detailed past medical history (including a smoking history) was taken, medical records examined and a fasting venous glucose sample was taken to ensure the inclusion/exclusion criteria were met. Of the 1698 patients commenced on CPAP during the study period, 166 were considered eligible to participate (prior to commencing CPAP). Of the 166 patients, 75 declined to participate or did not reply, with a further 38 subsequently found to be ineligible. In total, 53 patients entered the study (see Figure 4.1 for CONSORT diagram).

Patients entering the study provided written consent, and a copy of this, along with a patient information sheet was sent to their general practitioner. Subjects and controls were reimbursed for travelling expenses associated with their participation in the study.

3.4 Recruitment of healthy controls

In order to recruit 20 healthy controls, advertisements were placed around the hospital and on the hospital and university intranet requesting volunteers for the study. The advert did not refer to obstructive sleep apnoea. As a result of this 73 responses were received, of whom 12 were eligible and could be matched to patients with obstructive sleep apnoea/hypopnoea syndrome (OSAHS).

Following further ethics committee approval, and with the assistance of Dr Kelly McGorm at the Scottish Primary Care Research Network (SPCRN), several local GP practices were approached to help with control recruitment. One GP practice, Inchpark Surgery in Edinburgh, agreed to assist and a practice database search was carried out for men aged between 30 and 55 who had no history of cardiovascular disease (including hypertension), cerebrovascular disease, diabetes mellitus or OSA**.** Information sheets and reply slips were posted out to all of the potentially eligible subjects. Two hundred and fifty-six letters were mailed out inviting recipients to consider volunteering as control subjects. We received 25 positive responses, following which a detailed medical history (including a smoking history) was taken and a fasting venous glucose sample was taken to exclude diabetes mellitus. Of the 25, eight were eligible and could be matched to patients with OSAHS.

Controls were subject to the same exclusion criteria as patients with obstructive sleep apnoea (see above). To determine eligibility, an ESS was completed (Appendix I) and all subjects underwent overnight polysomnography within the Department of Sleep Medicine. In order to participate further, controls were required to have an ESS of $\leq$10 and an AHI of <10. The decision to use this AHI cut off was a pragmatic a priori decision based upon the confirmed asymptomatic nature of the control subjects.

3.5 Polysomnography (PSG)

All patients and controls underwent attended 16-channel in-patient overnight polysomnography (PSG) (Compumedics Ltd., Abbotsford, Australia) performed in single rooms within the Department of Sleep Medicine. In patients recruited to the randomised control study the median time between PSG and the first vascular assessment was 53 days (31-185). This was principally due to delays in the reporting

of routine PSGs within the department of Sleep Medicine at the time this study was conducted. It was ascertained that all subjects remained symptomatic on the day of the initial vascular assessment.

Polysomnography at this centre consists of continuously recorded electroencephalography (EEG), electrooculography (EOG), electromyography (EMG) of submentalis and tibialis muscles, oronasal flow, oxygen saturations, respiratory effort (thoracic and abdominal), snoring and body position. Information was recorded and stored using a computerised system (Compumedics Ltd) and later scored manually by trained sleep physiologists working within the department. Sleep was categorised by stage (stage I, II, III, IV, REM sleep or awake) in 30 second epochs using the then standard Rechtschaffen and Kales criteria (Rechtschaffen 1968) enabling a calculation of total sleep time.

Respiratory events were scored manually and categorised as apnoeas or hypopnoeas. An apnoea was defined as a reduction in airflow of ≥90% from baseline for at least 10 seconds and a hypopnoeas as a reduction in airflow of ≥30% for at least 10 seconds with an oxygen desaturation of ≥4% from baseline or a ≥50% reduction in airflow for at least 10 seconds with a ≥3% oxygen desaturation or associated arousal (Iber 2007). The apnoea/hypopnoea index (AHI) was then calculated as number of events per hour of sleep.

3.6 Epworth Sleepiness Score (ESS)

Excessive daytime sleepiness (EDS) was defined as a score of ≥11 on the Epworth Sleepiness Score (ESS) (Johns 1991). A copy of the ESS is found in Appendix 1. Subjects with OSA completed an ESS at baseline and at the end of each limb, with controls completing an ESS at baseline only as they were assessed only once.

3.7 Anthropometric data

Anthropometric measurements were made in all participants at baseline and after each limb of the study to ensure there had been no significant change in weight. Weight was measured using electronic scales (Seca 704, SECA, Birmingham, UK) and height was measured with a stadiometer (Bodycare, Warwickshire, UK) enabling calculation of the BMI (kg/m^2). Using a cloth tape measure, neck circumference was

measured at the level of the crico-thyroid membrane, with waist and hip measurements made at the level of the umbilicus and iliac crests respectively.

3.8 Serum glucose and cholesterol

Fasting glucose and cholesterol measurements were taken in the morning and processed by the clinical biochemistry laboratory at the Royal Infirmary of Edinburgh. Serum glucose (mmol/L) was determined using a commercial kit (Olympus Life Science Research Europa GmbH, Munich, Germany) on an Olympus AU460 analyser (Olympus Analyzers, Center Valley, PA, USA). Glucose is oxidised by glucose oxidase into gluconic acid and hydrogen peroxide which, in the presence of peroxidase, reacts with 4-aminoantipyrine and hydroxybenzoic acid, forming a red compound. Colour intensity monitored at 500nm is proportional to the concentration of glucose in the sample.

Serum cholesterol (mmol/L) was measured using a commercial kit (Alpha laboratories Ltd., Eastleigh, UK) adapted for use on a Cobas Fara centrifugal analyser (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Cholesterol is hydrolysed into free cholesterol and fatty acids by cholesterol esterase. Free cholesterol is oxidized into H_2O_2 and cholestene-3-one in the reaction catalysed by cholesterol oxidase. Peroxidase catalyses the reaction between H_2O_2, 4-aminoantipyrine and hydroxybenzoate to produce a red colour which is monitored at 505 nm.

3.9 Continuous Positive Airway Pressure (CPAP) Therapy

3.9.1 Pressure titration and treatment

Optimum CPAP pressures were determined during an overnight study using an automated CPAP titration device (Spirit^{TM}, ResMEd, Abingdon, UK) within the Department of Sleep Medicine. During titration the pressure required to abolish apnoeas and hypopnoeas is recorded. The CPAP unit is usually then set at the pressure that which has been shown to abolish 95% of respiratory events. Mask fitting was undertaken by MV or specialist nurses within the department. Following randomisation, CPAP units (S8 Autoset^{TM}, ResMed, Abingdon, UK) were set up

either at the prescribed pressure or as sham units (see section 3.9.3 below) by MV to maintain blinding of the chief investigator (AJ). Contact telephone numbers were provided to patients with details of whom to contact should they experience any problems.

3.9.2 Choice of placebo

There has been much debate over the years regarding the ideal placebo for trials involving CPAP (Karlawish 2001, Brown 2011)**.** The Declaration of Helsinki permits the use of a placebo 'Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm' (WMA 2008). Evidence to date shows that patients with OSAHS are at increased risk of cardiovascular disease. Although CPAP is clearly effective in improving sleep quality and reducing daytime symptoms (McDaid 2009), its effect on cardiovascular disease is not yet proven beyond doubt. Thus, within the confines of a randomised controlled study, the use of a placebo is considered to be justified (Brown 2011).

Randomising patients to receive no treatment is clearly an option, but this prevents the blinding of patients and researchers and may conceal a placebo-effect due to therapeutic CPAP. Both sham (or sub-therapeutic) CPAP (Smith 2007, Cross 2008) and oral placebos (Faccenda 2001, McArdle 2001) have been used successfully in the past in studies at this centre. Oral placebos were initially favoured in part due to (subsequently unfounded) concerns regarding the safety of sham CPAP (Douglas 2002), but this requires patients to believe in the possibility of an effective drug treatment for OSA. Also an oral placebo does not replicate any of the potential *mystique* associated with the provision of a CPAP machine and the experience of wearing a mask. Sham CPAP has been shown to have a placebo-effect upon the ESS (Jenkinson 1999) but little (Rodway 2010) or no (Jenkinson 1999, Farre 1999) effect on respiratory events during sleep. For these reasons, sham CPAP is currently the placebo of choice and was used in this trial.

3.9.3 Sham CPAP

Sham CPAP was delivered using the same machine, circuit and mask as therapeutic CPAP (S8 Autoset^{TM}, ResMed, Abingdon, UK) and appeared almost identical. Sub-therapeutic pressure was achieved by setting the delivered airway pressure to a minimum and adding a flow-restricting connector to the machine outlet along with the addition of extra holes to the mask allowing air to escape and to prevent re-breathing. This delivers a CPAP pressure at the mask of around 1cm H_2O and has been used successfully in two previous placebo-controlled studies at this centre (Smith 2007, Cross 2008), a landmark study examining the effect of CPAP upon blood pressure (Pepperell 2002) and in the recent study of the effect of CPAP withdrawal (Kohler 2011). This method was chosen as pressures around 1cm H20 have previously been shown to be ineffective in treating OSA (Jenkinson 1999).

3.9.4 Compliance Monitoring

Studies show that patients significantly over-report CPAP compliance, as do CPAP machines that simply record the amount of time for which the machine has been switched on (Sawyer 2011). In this study, compliance (with both CPAP and sham) was recorded by the CPAP machine as the time spent at pressure (i.e. the time spent actually wearing the mask) every night during the study. After each limb, this information was downloaded giving a very accurate measure of average nightly usage over each 12 week period.

3.10 Assessment of vascular function

3.10.1 Introduction

In this study we non-invasively examined a number of measures of arterial stiffness along with endothelial function. The three measures of arterial stiffness employed were aortic distensibility, measured directly during cardiac magnetic resonance imaging (MRI), along with aortic pulse wave velocity (sometimes referred to as carotid-femoral pulse wave velocity and hereafter referred to as PWV in this thesis) and augmentation index (AIx) measured by applanation tonometry. These are

measures of local aortic stiffness, regional stiffness and wave reflection respectively, giving a comprehensive assessment of arterial stiffness throughout the arterial tree. All vascular studies were conducted at the same time of day (early morning) beginning with the cardiovascular MRI. In line with the Expert Consensus Document on Arterial Stiffness (Laurent 2006), subjects were asked to fast overnight prior to attending and to abstain from smoking, alcohol and caffeine for at least 10 hours. The analysis of the MRI images was performed throughout by MC (Department of Medical Physics, Royal Infirmary of Edinburgh) and the remainder of the vascular assessments were undertaken by AJ. Both AJ and MC were blind to patient treatment arm throughout.

3.10.2 Arterial pressure waveforms and pulse wave analysis (PWA)

The arterial pressure waveform is generated by contraction of the left ventricle and changes as it travels along the arterial tree (see Figure 3.2 below). In keeping with the propagative model of the arterial tree, the waveform is a composite of the forward pressure wave and the reflected wave from the periphery arising from sites of impedance mismatch (Laurent 2006).

Figure 3.2 Schematic diagrams of the radial and corresponding aortic pressure waveforms

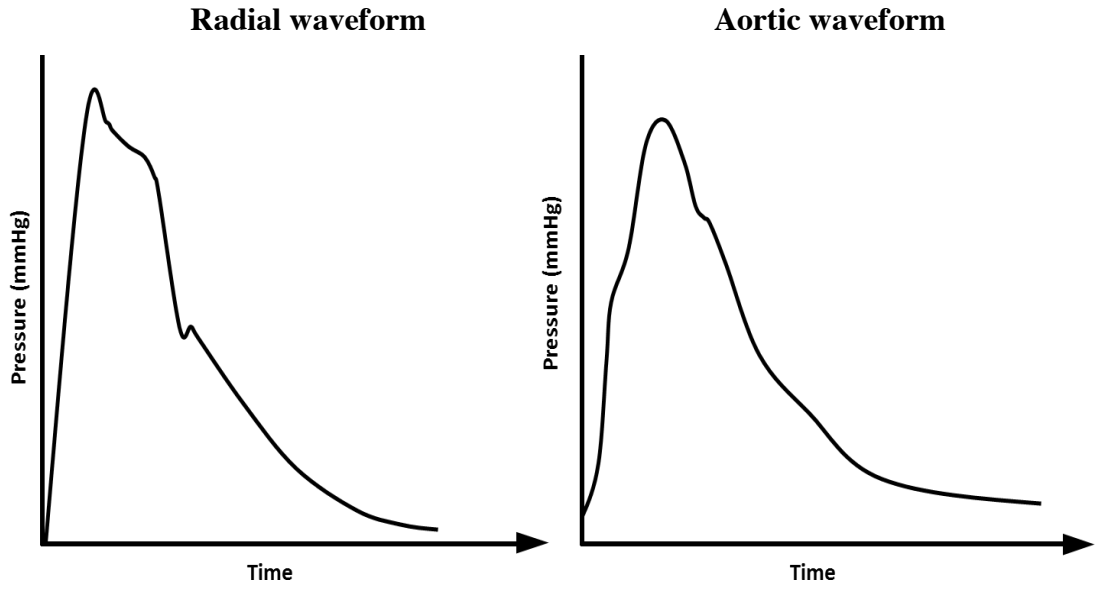

Figure 3.2 Waveform obtained at the radial pulse and the corresponding aortic waveform

In young healthy subjects the reflected wave returns during diastole aiding coronary perfusion. As arteries stiffen, the reflected wave returns earlier in the cardiac cycle increasing central pressure and cardiac afterload (Weber 2004, Laurent 2006). This is shown in Figure 3.3 below.

Figure 3.3 Schematic diagrams of aortic pressure waveforms from 'normal' and stiffened aortas

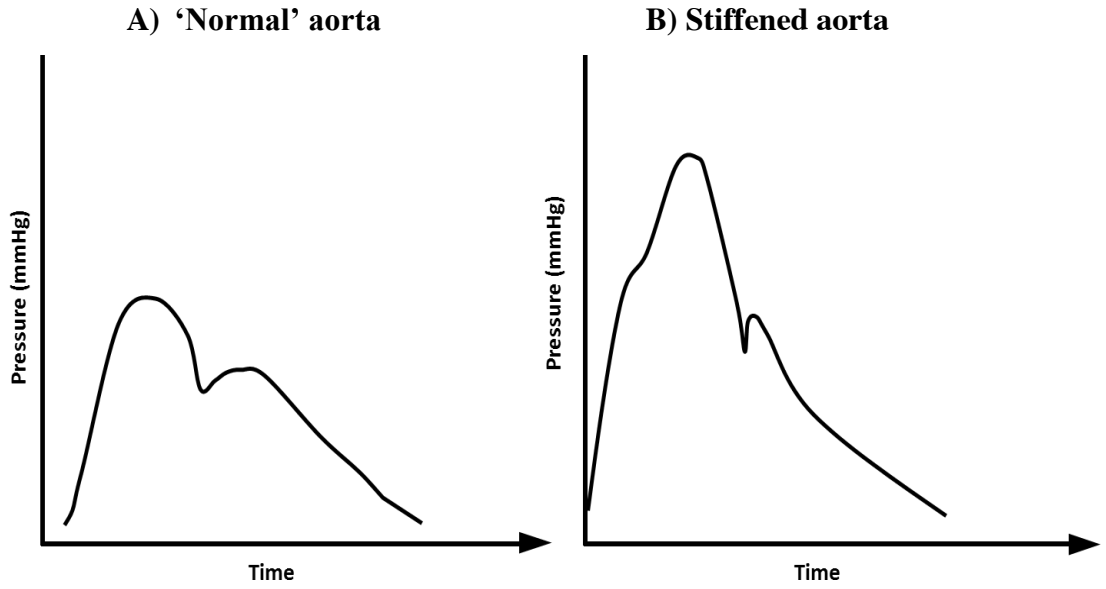

Figure 3.3 Schematic diagrams of waveforms from 'normal' (A) and stiffened (B) aortas. In (A) the reflected wave returns in diastole. In (B) the reflected wave arrives earlier in the cardiac cycle.

Whilst there has long been interest in the arterial pressure waveform, it is only since the development of highly sensitive micromanometer-tipped probes that accurate non-invasive recordings of the waveform have been possible using a technique called applanation tonometry. By using a micromanometer-tipped probe to flatten but not occlude a peripheral pulse, the circumferential pressures are equalised permitting a recording of the waveform similar to that obtained invasively (O'Rourke 2001). Once recorded, a generalised transfer function can be applied to the peripheral waveform giving the corresponding central waveform. The generalised transfer function corrects for pressure wave amplification in the upper limb and has been validated in a number of patient populations and haemodynamic states (Karamanoglu 1993, Chen 1997, Pauca 2001, Sharman 2006, Payne 2010). The derived central waveform can then be analysed, known as pulse wave analysis (PWA), allowing the calculation of a number of central parameters including the augmentation index (AIx) which was measured in this study (see Figure 3.4 below).

Figure 3.4 Annotated aortic pressure waveform

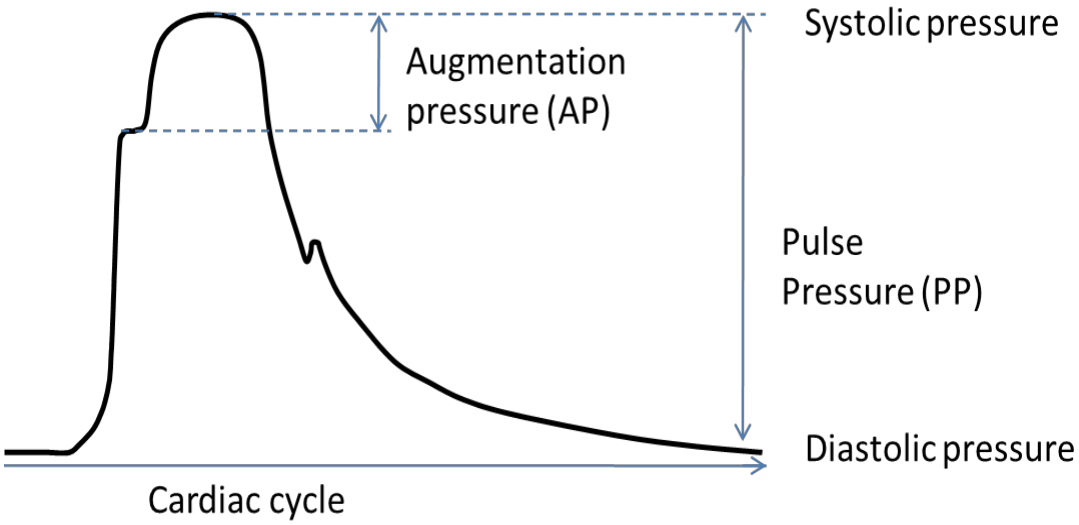

Figure 3.4 Aortic pressure waveform annotated with the components required to calculate the augmentation index (AIx). The augmentation pressure (AP) is the contribution that the reflected wave makes to systolic pressure. The AIx is calculated as (AP/PP) x 100 and is expressed as a percentage

3.11 Measurement of arterial stiffness

3.11.1 Aortic distensibility

Aortic distensibility was measured using cardiovascular MRI with all studies performed on the same 1.5 Tesla MRI (Philips Medical Systems, Best, Netherlands) within the Department of Radiology at the Royal Infirmary of Edinburgh. Studies were conducted in the supine position with images acquired during a breath-hold. Sagittal oblique images of the aorta were used to define planes perpendicular to the aortic axis for ECG-gated steady state free precession imaging with full R-R coverage in the ascending and descending aorta at the level of the right pulmonary artery and at the level of the diaphragm.

MRI parameters were repetition time (TR) 3.2 ms, echo time (TE) 1.6 ms, in-plane resolution 1.5mm, slice thickness 8mm and temporal resolution 22 ms.

Non-invasive blood pressure measurements (S/5^{TM} monitor, Datex-Ohmeda, Helsinki, Finland) were taken immediately before and after the acquisition of each

image with the mean of the two readings used to calculate the pulse pressure. The minimum and maximum aortic cross-sectional areas during the cardiac cycle were subsequently identified by MC for all subjects using an automated edge-finding algorithm (EasyVision, Philips Medical Systems) and used to determine distensibility at each of the sites according to the formula below (Resnick 1997): Aortic distensibility = $(A_{max}-A_{min})/(A_{min} \times \text{Pulse pressure})$ where A_{max} and A_{min} are the maximum and minimum aortic areas during the cardiac cycle.

3.11.1.1 Reproducibility of aortic distensibility measurements

Repeated aortic distensibility measurements were undertaken at each of the three levels in five patients during the study. These measurements were obtained under the same conditions and during the same MRI session as the initial measurements by MC. The intraclass correlation coefficient (ICC) across all three sites (15 repeat measurements in total) was 0.85 (95% CI 0.60-0.95); $p<0.001$. The number of repeat measurements that could be performed was limited by the availability of the MRI scanner.

3.11.2 Aortic pulse wave velocity (PWV)

Aortic pulse wave velocity (PWV) records the speed at which the systolic pressure wave reaches the peripheries. PWV is a measure of regional aortic stiffness along the aortic and aorto-iliac pathways, (Laurent 2006) with PWV increasing with arterial stiffness. PWV is reproducible (Wilkinson 1998) and considered to be the 'gold standard' measurement of arterial stiffness (Laurent 2006). PWV is an independent predictor of cardiovascular outcome in both high risk groups (Blacher 1999a, Blacher 1999b) and the general population (Willum-Hansen 2006).

All studies were carried out in a temperature-controlled room after at least 30 minutes rest and in accordance with the Expert Consensus Document on Arterial Stiffness (Laurent 2006) by a single operator (AJ). PWV was measured with the subject in the supine position by the sequential acquisition of carotid and femoral pressure waveforms obtained during applanation tonometry at the respective pulses using a hand-held micromanometer (Millar Instruments, Houston Tx) connected to the SphygmoCor® system (version7, AtCor Medical, Sydney, Australia).

Waveforms were gated to the R-wave of a simultaneously recorded electrocardiogram and collected directly onto a laptop computer. Straight line distances between the sternal notch and each of the measurement sites (carotid and femoral) were determined using a tape measure and the path length was calculated as the difference between the two distances.

After approximately 10 waveforms had been acquired at each peripheral site, the SphygmoCor® software identified the foot of each pulse waveform by means of the intersecting tangent algorithm (Chiu 1991). The mean time from the peak of the QRS complex of each ECG waveform and the onset of the corresponding waveform at the pulse site was then determined giving a mean time difference for both the carotid and femoral pulse sites. The mean difference in time between the two peripheral sites (Δt) was then used to calculate PWV as shown in the equation below.

$$PWV = \frac{\text{distance travelled}}{\Delta t} \text{ m/s}$$

Real-time quality control information is provided by the SphygmoCor® software and readings were only accepted for analysis if they met the suggested quality control standards (AtCor). Results with a standard deviation of 15% or greater were excluded as were those with a standard deviation of 10-15% if the onset of the waveform was not clearly identified (McAllister 2007). Where possible, three technically acceptable PWV readings were taken at each visit and the mean used for analysis. An example of a PWV report generated by the SphygmoCor® software is included in Appendix 1.

3.11.2.1 Reproducibility of PWV measurements

In order to assess the reproducibility of PWV measurements in this study, the first two consecutive PWV readings taken at the baseline visits of all subjects and controls were examined. As will be reported later, it was not possible to obtain any PWV readings in four participants, and of the 69 remaining participants, 66 had at least two measurements of PWV taken at baseline. Therefore the ICC calculation is

based upon 66 pairs of measurements. The ICC was 0.95 (95% CI 0.92-0.97); $p<0.001$.

3.11.3 Augmentation index (AIx)

The augmentation index (AIx) is the difference between the first and second systolic peaks of the central waveform expressed as a percentage of the pulse pressure (see Figure 3.4 above). The first systolic peak represents left ventricular ejection whilst the second is due to wave reflection from the periphery.

AIx is a reproducible measurement (Wilkinson 1998) of wave reflection and indirectly of stiffness throughout the entire arterial tree. Immediately after PWV measurements were taken, with the patient still in the supine position, applanation tonometry was performed by a single operator (AJ) at the radial artery using an automated tonometer (CBM 7000, Colin Corp., Komaki City, Japan) connected to the SphygmoCor® system. In order to obtain steady waveforms, a splint was first applied to the subject's wrist keeping it in a supine, extended position throughout. The tonometer was then attached to the wrist allowing continual radial waveform acquisition for determination of the AIx and subsequently for assessment of endothelial function (see section 3.12 below). Radial waveforms were calibrated to non-invasively obtained oscillometric brachial blood pressure measurements (CBM 7000, Colin Corp., Komaki City, Japan).

For each measurement of AIx, approximately ten sequential radial waveforms were averaged and a generalised transfer function (Chen 1997) was applied by the SphygmoCor® software giving a 'mean' central waveform.

From this, the AIx was determined as follows:

Augmentation Index (AIx) = (AP/PP) x100 and is expressed as a percentage.

Heart rate has been shown to influence AIx (Wilkinson 2002a) and so all values were corrected by the integral software to a heart rate of 75 beats per minute. Real-time quality control information is provided by the SphygmoCor® software and readings with greater than 10% variability in pulse height or in the diastolic portion of the

compiled radial waveforms were excluded. Where possible, five technically acceptable measurements of AIx were taken approximately three minutes apart at each visit and the mean used for analysis. An example of a PWA report generated by the SphygmoCor® software is included in Appendix 1.

3.11.3.1 Reproducibility of augmentation index (AIx) measurements

To assess the reproducibility of AIx measurements in this study, baseline measures of AIx taken for subjects and controls were examined. 100% of participants had at least two baseline AIx results and therefore an ICC calculation for 73 pairs of AIx readings was 0.96 (95%CI 0.94-0.98); $p<0.001$. Fifty of the participants had five baseline AIx measurements and an ICC based upon this data was 0.96 (95% CI 0.95-0.98); $p<0.001$.

3.12 Assessment of endothelial function

Vasomotor endothelial function was assessed non-invasively by measuring the endothelium-dependent effect of salbutamol (a β2-agonist) and endothelium-independent effect of glyceryl trinitrate (GTN) on the AIx (Wilkinson 2002b, Hayward 2002). Endothelium-dependent vasomotion is the most widely used clinical end-point in the assessment of endothelial function (Deanfield 2007) and relies on the release of nitric oxide (NO) from an intact endothelium given the appropriate physiological or pharmacological stimuli. NO acts as a vasodilator for vascular smooth muscle and is synthesised from L-arginine by endothelial nitric oxide synthetase (eNOS) (Förstermann 2006). β2 agonists, such as salbutamol, are known to exert an endothelium-dependent effect upon the vasculature with substantial inhibition of β2 agonist-mediated vasodilatation in the presence of NG-monomethyl-L-arginine, a substrate analogue inhibitor of NO synthetase (Dawes 1997, Wilkinson 2002b). The endothelium-dependent response is often compared to the response to direct stimulators of vascular smooth muscle GTN, known as the endothelium-independent response.

Endothelium-dependent changes in AIx measured using this non-invasive technique are reproducible, give an assessment of global endothelial function (Wilkinson 2002b, Hayward 2002) and correlate with invasively measured changes in forearm

blood flow in response to acetylcholine during venous occlusive plethysmography (Wilkinson 2002b). Using this technique, reductions in endothelium-dependent vasodilatation have been described in patients with coronary artery disease (Hayward 2002), hypercholesterolaemia (Wilkinson 2002b) and in a group of patients (many of whom also had underlying cardiovascular disease) with minimally symptomatic OSA (Kohler 2008).

Throughout this assessment, the calculation of AIx (corrected to a heart rate of 75 bpm) and the technical acceptability of the measurements remained the same as described above (see section 3.11.3). Immediately after baseline recordings of AIx were obtained as detailed in section 3.11.3 above, and under the same conditions, a 500µg tablet of GTN was given sub-lingually for three minutes and then removed. Based on previous research experience at this centre, the AIx was then recorded every minute for 10 minutes and then every 5 minutes for a further 15 minutes. The haemodynamic effects of GTN have previously been shown to persist for 25 minutes (Greig 2005) and therefore, 30 minutes after the administration of GTN, 400µg of inhaled salbutamol was given as two puffs (i.e. 2x200µg) via a spacer device. The AIx was subsequently recorded every minute for 10 minutes and then every 5 minutes for a further 10 minutes. The response to each of the drugs was defined *a priori* as the maximum change in AIx after drug administration (Wilkinson 2002b, McEniery 2006).

3.13 Measurement of blood pressure

Office blood pressure and 24-hour ambulatory blood pressure were measured at baseline and at the end of each limb of the study (see Figure 3.1 above).

3.13.1 Office blood pressure

Office blood pressure was measured using the validated Omron 705IT (HEM 759-E) oscillometric device (Omron, Milton Keynes, UK) (Coleman 2006). Two measurements were taken after at least 30 minutes' rest in the supine position on each occasion with the mean being used for analysis. Mean arterial pressure (MAP) was calculated in mmHg using the formula below:

MAP (mmHg) = 1/3 x (systolic BP-diastolic BP) + diastolic BP.

3.13.2 Ambulatory blood pressure measurement (ABPM)

Ambulatory blood pressure was measured over a 24-hour period using a SpaceLabs 90207 (Spacelabs Medical Ltd, Hertford, UK), a validated (O'Brien 2001) lightweight battery powered device. The device consists of a microprocessor weighing 347g connected by tubing to a cuff wrapped around the upper arm. The monitor utilises the oscillometric method of blood pressure determination and bleeds pressure in discrete (approximately 4 mmHg) steps.

On each occasion, prior to fitting, the device was initialised, enabling calibration of the 'real-time' clock and the assignment of a study number to the data collection. Four different cuff sizes are available for adults and the appropriate size was determined by first measuring the circumference of the upper limb. The cuff was applied to the non-dominant arm with the centre of the inflatable bladder directly over the brachial artery. The microprocessor was carried in a pouch attached to a belt around the waist. After fitting, the accuracy of the device was checked by performing a measurement during which the cuff was connected, via a T-tube splitter to both the device and a mercury sphygmomanometer, whilst auscultating the pulse at the brachial artery.

The device was programmed to take a blood pressure measurement every 30 min during the daytime (0700 - 2159), heralded by a beep. On hearing the beep, participants were urged, if safe to do so, to relax their arm at their side whilst the measurement was taken. During the night (2200 - 0659), in order to minimise the effect upon sleep, readings were taken every hour and were not accompanied by a beep. The cuff initially inflated to 170mmHg and subsequently to 30mmHg above the previous reading. If a reading was unsuccessful for any reason, the device was programmed to attempt one further measurement approximately one minute later. If both readings were unsuccessful no further attempts were made until the next scheduled measurement. Participants were asked to continue their normal daily activities whilst wearing the device and to only to remove the device for the purposes of bathing. Instructions on how to reposition the device after bathing were provided.

Once returned, raw data and summary statistics were downloaded from the device via a PC interface. Mean ABPM was deemed suitable for further analysis if it was based upon at least 12 daytime and 6 nocturnal measurements. The devices were serviced regularly throughout the study by the Department of Medical Physics.

3.14 Data analysis

All data were entered into a database and locked before unblinding of treatment order occurred. Data were subsequently transferred to SPSS version 19 (IBM) for statistical analysis. Depending upon the distribution of the data, differences between treatment arms were analysed using paired t-tests or Wilcoxon signed rank tests. Breslow and Day emphasised the importance of using matched analyses when examining matched data in their text on the analysis of case-control studies (Breslow 1980). This approach has subsequently been reinforced (Swinscow 1997, Niven 2012), with recommendations to use the statistical tests outlined above. Repeated measures analysis of variance (ANOVA) was used to look for evidence of an order effect. Differences between patients with OSAHS and control subjects were analysed using paired t-tests or Wilcoxon signed rank tests depending on the distribution of the data, with differences in categorical data assessed using McNemar's test.

The unpaired t-test, Mann Whitney U test and chi-squared test were used to analyse baseline differences between; 1) the subset of patients with OSAHS who were matched in the case-control study and the 43 patients who completed the randomised controlled trial, 2) those who completed the randomised controlled trial and those who dropped out and 3) patients with and without EDS. In the case-control study, one way ANOVA was used to look for differences in AIx and changes in AIx after salbutamol according to smoking history.

Depending on the distribution of the data, either Spearman's rank correlation coefficient or Pearson correlation coefficient were used to examine the relationships between measures of vascular function and OSA severity. A stepwise linear regression analysis was performed with variables entered into the model if the initial significance of their correlation with the dependent variable was ≤ 0.05.

Reproducibility of vascular measurements was assessed by determining the intra-class correlation coefficient (ICC). In all analyses a p value of <0.05 was considered

statistically significant. Data are reported as mean (standard deviation) or median (interquartile range) depending on the distribution of the data. Unless stated otherwise, results are expressed as mean (standard deviation) throughout.

The primary analysis of the randomised controlled trial was a modified intention-to-treat analysis. An *a priori* decision was made not to impute missing data, however results for all subjects who attended for all three vascular assessments were analysed, irrespective of their compliance with CPAP (or sham CPAP) therapy. A sensitivity analysis of this approach in which baseline values were carried forward in patients who dropped out of the study was subsequently performed and is reported in Section 4.7

A sample size of 40 patients was determined on the basis of pilot data, to provide a 90% chance of detecting a 10% difference in mean aortic distensibility (the primary endpoint of this study at a significance level of 5%. The aim had therefore been to recruit 60 patients into the randomised control trial to allow for dropouts. The pilot data was the result of a study that compared MRI-determined aortic distensibility in patients with OSA and in control subjects (RL Riha-unpublished data).

Chapter 4: Results of the Randomised Controlled Trial

4.1 Recruitment and Demographics

Of 1698 patients commenced on CPAP in the Department of Sleep Medicine during the study period (2007-2009), 166 were considered to be potentially eligible to participate. Seventy-five of these patients either did not respond to the invitation or declined to participate and a further 38 were subsequently found to be ineligible on the basis of the inclusion and exclusion criteria of the trial. In total, 53 patients entered the study, ten of whom subsequently dropped out, leaving 43 patients completing both limbs of the study and attending all three vascular assessments (see Figure 4.1). Of these 43, 24 had EDS and 19 did not. The mean age of patients completing the study was 46 (9) years and 65% of the participants were male. Baseline characteristics for those completing the study are shown below in Table 4.1 below. The median AHI was 31 (IQR 20-41), indicative of moderate to severe disease severity. However, the 4% desaturation rate was relatively low at 9.3 (12.3) per hour, the mean minimum oxygen saturations were relatively high at 86.1% (5.1) and the mean percentage of sleep time spent with oxygen saturations below 90% was low at 4.4% (13.2). These patients did not experience significant nocturnal intermittent hypoxia.

There were no changes in smoking status during the duration of the study. A small but statistically significant increase in BMI was noted in the six months between the first and third vascular assessment visits [29.9 (27.3-31.6) kg/m^2 vs. 30.1 (27.4-33.3) kg/m^2; $p<0.001$].

Figure 4.1 Study Flowchart

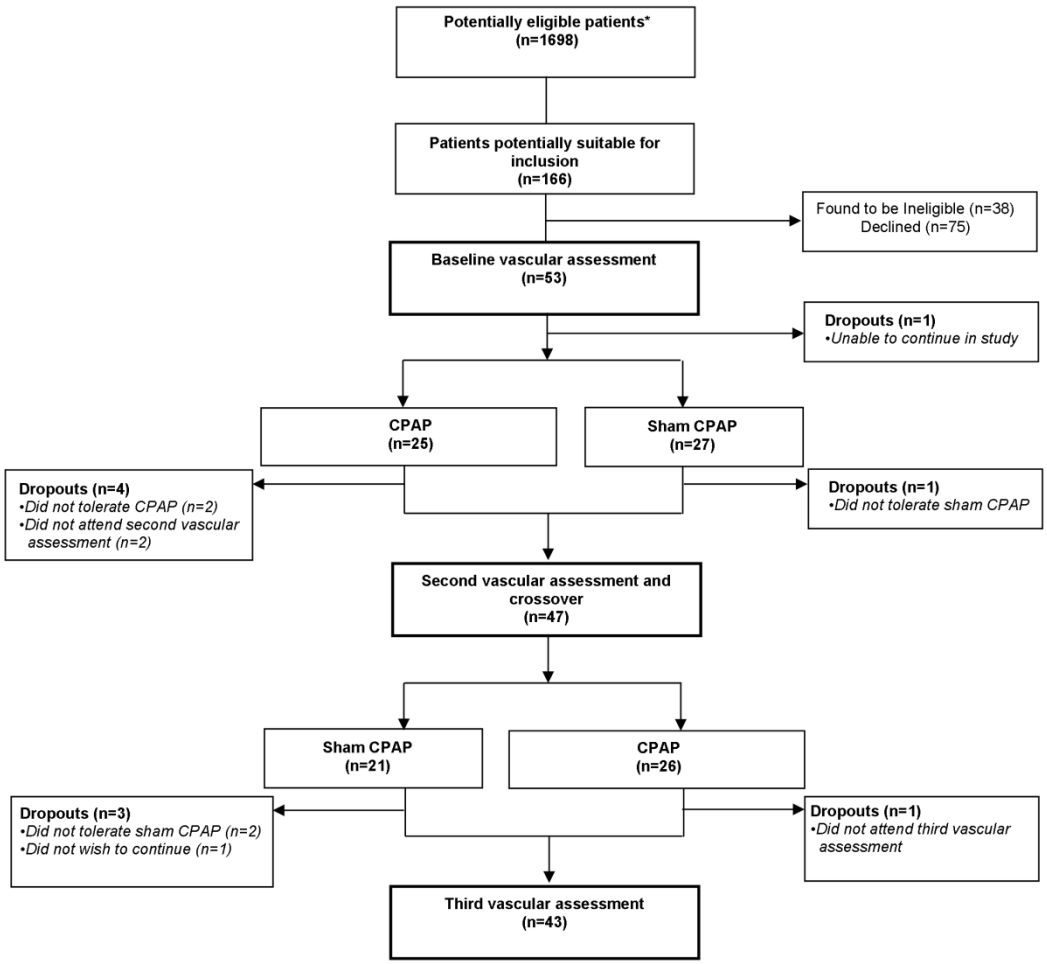

Figure 4.1 CONSORT diagram for the randomised controlled trial

*Number of patients diagnosed with OSA and commenced on CPAP therapy within the Department of Sleep Medicine during the study period

Table 4.1 Baseline characteristics of subjects completing the study (n=43)

Age (years)	46 (9)
Sex	28 male
BMI (kg/m^2)*	29.9 (27.3-31.6)
Neck circumference (cm)*	40 (37-41.5)
Waist to hip ratio	0.95 (0.07)
Systolic blood pressure (mmHg)	128 (13)
Diastolic blood pressure (mmHg)	76 (9)
MAP (mmHg)	93 (10)
Fasting glucose (mmol/L)	4.9 (0.4)
Total cholesterol (mmol/L)	5.3 (1.0)
Current smokers	23 %
Ex-smokers	23 %
Cigarette pack year history (amongst smokers and ex-smokers)	11.4 (8.0)
On no regular medication	56 %
Antidepressant medication	16 %
Inhaled asthma medication	14 %
Proton pump inhibitor medication	12 %
Thyroxine medication	7 %
Orlistat medication	2 %
Non-steroidal anti inflammatory medication	2 %
Apnoea/hypopnoea index (AHI)*	31 (20-41)
4% O_2 desaturation rate/hour	9.3 (12.3)
Minimum O_2 saturation (%)	86.1 (5.1)
TST90 (%)	4.4 (13.2)
Epworth sleepiness score (ESS)*	13 (6-15)

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: body mass index (BMI); mean arterial blood pressure (MAP), percentage of sleep time spent with oxygen saturations of less than 90% (TST90)

Although it was not a formal exclusion criteria, none of the patients recruited to the study were receiving statin therapy.

The ten subjects who did not complete the study were not significantly different from those who did (n=43) in terms of demographic data or baseline vascular function (see Table 4.2 below). Of those who dropped out, two received CPAP in the first limb and were unable to tolerate it, three were unable to tolerate sham CPAP (two of whom had already received therapeutic CPAP in the first limb) and a further five could not continue in the study due to other commitments.

Table 4.2 Demographic and baseline vascular function in subjects completing the study and those who dropped out

	Subjects who dropped out of the study (n=10)	Subjects who completed the study (n=43)	p value
Age (years)	46 (9)	46 (9)	1.0
Sex	70% male	65 % male	0.54
BMI (kg/m^2)*	30.6 (28.4-34.0)	29.9 (27.3-31.6)	0.54
Neck circumference (cm)*	41.5 (36.8-45.1)	40 (37.0-41.5)	0.16
Waist to hip ratio	0.97 (0.05)	0.95 (0.07)	0.34
Systolic blood pressure (mmHg)	131 (14)	128 (13)	0.51
Diastolic blood pressure (mmHg)	77 (7)	76 (9)	0.55
MAP (mmHg)	95 (9)	93 (10)	0.51
Fasting glucose (mmol/L)	4.7 (0.4)	4.9 (0.4)	0.07
Total cholesterol (mmol/L)	5.3 (0.8)	5.3 (1.0)	0.92
Current smokers	40 %	23 %	0.43
Ex-smokers	40 %	23 %	0.43
Apnoea/hypopnoea index (AHI)*	30 (24-37)	31 (20-41)	0.53
Epworth sleepiness score (ESS) *	12 (9.5-16.5)	13 (6-15)	0.34
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	5.2 (2.3)	5.0 (1.9)	0.89
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)	4.4 (3.3-6.7)	4.8 (3.3-5.4)	0.53
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	7.5 (3.1)	6·5 (1·9)	0.38
PWV (m/s)	7.8 (1.7)	7.6 (1.5)	0.75
AIx (%)	18.3 (8.2)	17.1 (11.6)	0.73
Change in AIx (%) following salbutamol	-3.0 (-8- -1.5)	-4.5 (-6 - -3)	0.50
Change in AIx (%) following GTN	-13.9 (3.6)	-14.0 (4.6)	0.94

Results presented as mean (standard deviation) unless otherwise stated. *Median (Interquartile range). Abbreviations: aortic distensibility (AoD); body mass index (BMI); mean arterial blood pressure (MAP).

4.2 Baseline blood pressure measurements

Baseline office blood pressure measurements are reported above (Table 4.1). Of the 43 patients completing the study, 38 had technically adequate 24 hour ambulatory blood pressure measurements (ABPM) at baseline, giving a median 24 hour systolic BP of 116 (113-124) mmHg, mean diastolic BP of 73 (8) mmHg and a median MAP of 87 (83-94 mmHg).

Office blood pressure measurements were available for all subjects who entered the study at baseline and at each subsequent visit whereas the dataset of ABPM readings is incomplete. At baseline the reasons for missing ABPM data were as follows; equipment failure in one subject, three subjects removing the ABPM machine overnight due to concerns regarding sleep disruption and one subject who declined an ABPM monitor at the time it was being applied. The ability to repeat inadequate ABPM recordings was limited due to the availability of ABPM devices and patients' other commitments. In view of the above, office blood pressure measurements are presented first throughout this chapter with ABPM results shown subsequently.

Office and ambulatory blood pressure measurements were significantly correlated for systolic ($r=0.59$; $p<0.001$), diastolic ($r=0.60$; $p<0.001$) and MAP readings ($r=0.59$; $p<0.001$) at baseline.

4.3 Baseline vascular function

Baseline measurements of arterial stiffness and endothelial function are given in Table 4.3 below. The images obtained during MRI scanning were of insufficient quality to determine aortic distensibility in the ascending and descending aorta in eight subjects and at the level of the diaphragm in one subject at baseline. For reasons of body habitus it was not possible to measure PWV in three subjects at baseline.

Table 4.3 Baseline vascular function in subjects completing the study (n=43)

AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=35)	5.0 (1.9)
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=35)*	4.8 (3.3-5.4)
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=42)	6.5 (1.9)
PWV (m/s) (n=40)	7.6 (1.5)
AIx (%) (n= 43)	17.1 (11.6)
Change in AIx (%) following Salbutamol (endothelium-dependent) (n=43)*	-4.5 (-6 - -3)
Change in AIx (%) following GTN (n=43) (endothelium-independent) (n=43)	-14.0 (4.6)

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); glyceryl trinitrate (GTN)

4.4 Baseline measurements in sub-groups with and without excessive daytime sleepiness (EDS)

EDS (defined as an ESS ≥11) was not a requirement for recruitment to the study. Of the 43 subjects who completed the study, 24 had EDS and 19 did not. Those without EDS were older [50 (9) vs. 43 (7) years; p=0.005], but otherwise they were similar in terms of demographics and baseline blood pressure (Table 4.4).

Table 4.4 Baseline characteristics of subjects *with* and *without* EDS (defined as ESS ≥11)

	Subjects with EDS (n=24)	Subjects without EDS (n=19)	p value
Age (years)	43 (7)	50 (9)	**0.005**
Sex	58% male	74% male	0.29
BMI (kg/m^2)*	30.7 (27.4-35.6)	29.5 (27.1-30.4)	0.08
Neck circumference (cm)*	40.3 (36.3-41.5)	39.5 (38.0-41.0)	0.81
Waist to hip ratio	0.94 (0.07)	0.96 (0.07)	0.37
Systolic blood pressure (mmHg)	127 (14)	129 (12)	0.62
Diastolic blood pressure (mmHg)	76 (10)	75 (9)	0.73
MAP (mmHg)	93 (11)	93 (10)	0.99
ABPM systolic blood pressure (mmHg)	116 (114-124)	116 (111-124)	0.70
ABPM diastolic blood pressure (mmHg)	73 (8)	72 (8)	0.84
ABPM MAP (mmHg)	87 (83-94)	87 (83-93)	0.87
Fasting glucose (mmol/L)	4.9 (0.5)	4.9 (0.3)	0.73
Total cholesterol (mmol/L)	5.3 (1.0)	5.3 (1.0)	0.81
Current smokers	33%	11%	0.15
Apnoea/hypopnoea index*	32 (19-41)	31 (20-49)	0.83
4% O_2 desaturation rate/hour	7.6 (11.0)	11.3 (13.8)	0.33
Minimum O_2 saturation (%)	86.4 (6.5)	85.8 (2.6)	0.71
TST90	2.7 (5.0)	6.2 (19.0)	0.39
Epworth sleepiness score *	15 (14-18)	6 (4-8)	**<0.001**

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); body mass index (BMI); mean arterial blood pressure (MAP); percentage of sleep time spent with oxygen saturations of less than 90% (TST90).

Sub-group analysis showed subjects with EDS to have increased distensibility in the ascending aorta [5.8 (1.9) vs. 4.3 (1.6); p=0.02] with a non-significant trend towards increased distensibility at the level of the diaphragm [7.0 (1.9) vs. 5.8 (1.9); p=0.06] than those without EDS (see Table 4.5). As noted above in Table 4.4, subjects without EDS were significantly older and as shown below (Section 4.19), distensibility in the ascending aorta correlated well with age in the study group overall, which may explain this finding. There were no other differences in arterial stiffness or endothelial function between the two groups at baseline (see Table 4.5).

Table 4.5 Baseline vascular function in subjects *with* and *without* EDS (defined as ESS ≥11)

	Subjects with EDS (n=24)	Subjects without EDS (n=19)	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	5.8 (1.9)	4.3 (1.6)	**0.019**
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)*	4.6 (3.3-5.9)	4.8 (3.2-5.3)	0.63
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	7.0 (1.9)	5.8 (1.9)	0.06
PWV (m/s)	7.5 (1.2)	7.9 (1.8)	0.45
AIx (%)	18.8 (11.2)	15.1 (12.1)	0.31
Change in AIx (%) following salbutamol *	-4 (-6- -2)	-5.5 (-6- -4)	0.21
Change in AIx (%) following GTN	-14 7 (4.8)	-13.0 (4.1)	0.24

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); excessive daytime somnolence (EDS); glyceryl trinitrate (GTN)

4.5 Compliance with continuous positive airway pressure (CPAP) therapy

There was a difference in CPAP usage between the two limbs of the study with subjects using CPAP for an average of 3.0 hrs per night (range 0-8.1hrs) and sham CPAP for an average of 2.0 hrs per night (range 0-5.8hrs); p=0.001. Compliance was very accurately measured as time at pressure throughout the entire study period (see

section 3.9.4). As discussed in Chapter 1, there is no clear cut definition of adequate compliance but four hours per night on 70% of nights is often quoted as being sufficient (Sawyer 2011). In this group, 17 patients (40%) used CPAP for four hours or more on average every night throughout the 12 week period. CPAP usage in this sub-group was 5.9 hrs per night (range 4-8.1hrs).

CPAP usage amongst those who entered the CPAP limb first did not differ significantly from those who entered the sham limb first (p=0.09). Somewhat surprisingly, sham CPAP usage was also unaffected by the order in which the patients received CPAP and sham CPAP (p=0.83). Compliance with CPAP correlated well with compliance with sham CPAP during the study, r=0.62; p<0.001. There was no correlation between CPAP compliance and severity of OSA as measured by the AHI(r= 0.23; p=0.134). CPAP compliance in this study was negatively correlated with EDS as measured by the ESS (r= -0.39; p=0.009), suggesting that those patients who were subjectively sleepier had poorer CPAP compliance. CPAP compliance in subjects with an ESS ≥11 appeared lower than in those without EDS (ESS <11), but this was not statistically significant (p=0.06).

4.6 Results of the Randomised Controlled Trial

Across the group as a whole (n=43), the ESS fell more on CPAP than on sham CPAP [7 (4-11) vs. 10 (5-13); p<0·001]. In those with EDS at baseline, the ESS was 10 (7-13) on CPAP compared to 13 (11-15) on sham CPAP (p=0.002). Those without EDS at baseline also had a lower ESS on CPAP than on sham CPAP [4 (2-6) vs. 5 (2-8); p=0.04]. Using repeated measures ANOVA, no significant treatment order effects were seen for any of the measured vascular variables detailed below.

4.6.1 Arterial Stiffness

The images obtained during MRI scanning were of insufficient quality to make comparisons of aortic distensibility in the ascending aorta in five subjects, the descending aorta in four subjects and at the level of the diaphragm in three subjects. Additionally one patient who underwent MRI scanning at baseline subsequently reported claustrophobia and did not undergo MRI scanning at the second and third vascular assessments. For reasons of body habitus it was not possible to obtain PWV

measurements in three subjects at the second and third vascular assessment (the same three subjects in whom baseline PWV measurements were not possible). In one subject it was not possible to obtain a radial pulse waveform of sufficient quality (See Chapter 3, section 3.11.3) to measure the AIx at the second vascular assessment. Twelve weeks of CPAP therapy did not alter aortic distensibility or PWV. There was a non-significant trend towards a lower AIx on CPAP than on sham CPAP (see Table 4.6).

Table 4.6 Arterial stiffness following CPAP and sham CPAP treatment periods

	CPAP	Sham CPAP	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=37)	4.9 (1.7)	5.1 (1.9)	0.44
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=38)*	4.6 (3.9-5.7)	4.5 (3.7-5.1)	0.22
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=39)	6.8 (1.9)	6.9 (2.3)	0.80
PWV (m/s) (n=40)	7.5 (1.2)	7.6 (1.4)	0.29
AIx (%) (n=42)	15.5 (11.9)	16.6 (11.7)	0.08

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); continuous positive airway pressure (CPAP)

4.6.2 Endothelial Function

As reported above (Section 4.6.1), it was not possible to obtain a radial pulse waveform of sufficient quality to measure the AIx, and hence the effect of salbutamol or GTN on the AIx at the second vascular assessment in one subject. Twelve weeks of CPAP therapy did not affect endothelial function, with no difference in endothelium-dependent on endothelium-independent change in AIx seen (see Table 4.7 below).

Table 4.7 Endothelial function following CPAP and sham CPAP treatment periods

	CPAP	Sham CPAP	p value
Change in AIx (%) following salbutamol (n=42)* (endothelium-dependent)	-5 (-8 - -3)	-4 (-6.8 - -2.5)	0·50
Change in AIx (%) following GTN (n=42) (endothelium-independent)	-14.1 (5.1)	-15.3 (4.6)	0·13

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: augmentation index (AIx); continuous positive airway pressure (CPAP); glyceryl trinitrate (GTN)

4.6.3 Sub-group analysis of patients using CPAP for ≥4 hours per night.

Seventeen patients had a CPAP compliance of ≥4 hours per night and vascular function results were analysed separately for these patients. Twelve weeks of CPAP therapy did not lead to any change in arterial stiffness or endothelial function in these patients as shown in Table 4.8.

Table 4.8 Vascular function following CPAP and sham CPAP treatment periods in patients using CPAP for ≥4 hours per night.

	CPAP	Sham CPAP	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=15)	4.9 (1.5)	5.1 (1.9)	0.59
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=15)*	4.2 (3.3-4.9)	4.3 (3.4-5.1)	0.63
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=15)	6.5 (2.0)	6.7 (2.4)	0.75
PWV (m/s) (n=16)	7.9 (1.6)	8.1 (1.7)	0.12
AIx (%) (n= 17)	12.5 (12.5)	13.6 (11.1)	0.28
Change in AIx (%) following salbutamol (endothelium-dependent) (n=17)*	-5.5 (-7- -3)	3.5 (-5.5- -2.5)	0.37
Change in AIx (%) following GTN (endothelium-independent) (n=17)	-12.5 (4.37)	-14.9 (4.6)	0.08

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); continuous positive airway pressure (CPAP); glyceryl trinitrate (GTN)

4.6.4 Sub-group analysis of patients *with* and *without* excessive daytime sleepiness (EDS)

As in the group overall, in patients with EDS there was a non-significant trend towards a lower AIx on CPAP than on sham [16.5 (11.4)% vs. 18.2 (11.2)%; p=0.058]. Aside from this there were no differences in arterial stiffness between the CPAP or sham CPAP limb in either sub-group as shown in Tables 4.9 and 4.10 below.

Table 4.9 Vascular function following CPAP and sham CPAP treatment periods in patients *with* EDS (n=24)

	CPAP	Sham CPAP	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=20)	5.5 (1.7)	6.0 (1.9)	0.12
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=21)*	4.7 (3.9-6.3)	4.7 (3.8-5.5)	0.69
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=22)	7.3 (1.9)	7.2 (2.3)	0.89
PWV (m/s) (n=22)	7.4 (1.1)	7.5 (1.3)	0.31
AIx (%) (n=23)	16.5 (11.4)	18.2 (11.2)	0.058
Change in AIx (%) following salbutamol (n=23)* (endothelium-dependent)	-4.8 (-7.6- -3)	-4.3 (-7- -3.4)	0.59
Change in AIx (%) following GTN (n=23) (endothelium-independent)	-14 6 (5.1)	-16.2 (3.8)	0.14

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); continuous positive airway pressure (CPAP); glyceryl trinitrate (GTN)

Table 4.10 Vascular function following CPAP and sham CPAP treatment periods in patients *without* EDS (n=19)

	CPAP	Sham CPAP	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=17)	4.2 (1.4)	4.0 (1.1)	0.38
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=17)*	4.3 (3.8-5.5)	4.3 (3.5-4.7)	0.12
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=17)	6.2 (1.7)	6.5 (2.3)	0.54
PWV (m/s) (n=18)	7.6 (1.4)	7.8 (1.5)	0.32
AIx (%) (n=19)	14.3 (12.7)	14.7 (12.3)	0.68
Change in AIx (%) following salbutamol (endothelium-dependent) (n=19)*	-5.5 (-8- -1.5)	-4 (-6.5- -2)	0.72
Change in AIx (%) following GTN (endothelium-independent) (n=19)	-13.4 (5.2)	-14.1 (5.4)	0.56

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); continuous positive airway pressure (CPAP); glyceryl trinitrate (GTN)

4.6.5 Blood pressure (office and ABPM)

Office systolic blood pressure was slightly lower on CPAP than on sham CPAP as shown in Table 4.11 below.

Table 4.11 Office blood pressure measurements following CPAP and sham CPAP treatment periods (n=43)

	CPAP	Sham CPAP	p value
Systolic blood pressure (mmHg)	126 (12)	129 (14)	**0·03**
Diastolic blood pressure (mmHg)	77 (8)	77 (8)	0·90
MAP (mmHg)	94 (8)	94 (9)	0·29

Results presented as mean (standard deviation)

Abbreviations: continuous positive airway pressure (CPAP); mean arterial blood pressure (MAP)

Thirty-three of the 43 patients completing the study had adequate ABPM readings in both the CPAP and sham CPAP limbs. Missing data was due in two cases to patients refusing the ABPM monitor, in six cases patients removed the cuff for significant periods of time resulting in insufficient data for analysis and in two cases was due to equipment failure. No differences in systolic, diastolic or mean arterial pressures were seen (Table 4.12).

Table 4.12 ABPM blood pressure measurements following CPAP and sham CPAP treatment periods (n=33)

	CPAP	Sham CPAP	p value
ABPM systolic blood pressure (mmHg)*	119 (113-127)	117 (111-127)	0.32
ABPM diastolic blood pressure (mmHg)	73 (8)	72 (8)	0.54
ABPM MAP (mmHg)*	88 (85-95)	88 (83-96)	0.28

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); mean arterial blood pressure (MAP)

When daytime (0700 – 2159) and night time (2200 – 0659) ABPM readings were analysed separately, no differences in systolic, diastolic or mean arterial pressures (MAP) between the CPAP and sham CPAP limbs were seen (see Table 4.13 below).

Table 4.13 Daytime and night time ABPM results following CPAP and sham CPAP treatment periods

	CPAP	Sham CPAP	p value
DAYTIME (0700-2159)			
ABPM systolic blood pressure (mmHg)*	124 (116-132)	123 (115-128)	0.10
ABPM diastolic blood pressure (mmHg)	78 (9)	77 (9)	0.23
ABPM MAP (mmHg)*	94 (88-101)	94 (87-98)	0.11
NIGHT TIME (2200-0659)			
ABPM systolic blood pressure (mmHg)*	106 (103-117)	106 (102-117)	0.79
ABPM diastolic blood pressure (mmHg)	64 (8)	65 (8)	0.58
ABPM MAP (mmHg)*	78 (74-85)	78 (74-83)	0.39

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); mean arterial blood pressure (MAP)

4.6.5.1 Sub-group analyses

4.6.5.1.1 Subjects with and without EDS

When sub-groups with and without EDS were examined separately there was no significant difference between office blood pressures in the CPAP and sham CPAP treatment periods (Tables 4.14 and 4.15). A non-significant trend towards a lower systolic blood pressure was noted in the group without EDS.

Table 4.14 Office blood pressure measurements following CPAP and sham CPAP treatment periods in patients *with* EDS (n=24)

	CPAP	Sham CPAP	p value
Systolic blood pressure (mmHg)	125 (12)	128 (13)	0.16
Diastolic blood pressure (mmHg)	78 (8)	77 (8)	0.39
MAP (mmHg)	94 (8)	94 (9)	0.82

Results presented as mean (standard deviation)

Abbreviations: continuous positive airway pressure (CPAP); excessive daytime somnolence (EDS); mean arterial blood pressure (MAP)

Table 4.15 Office blood pressure measurements following CPAP and sham CPAP treatment periods in patients *without* EDS (n=19)

	CPAP	Sham CPAP	p value
Systolic blood pressure (mmHg)	127 (12)	130 (14)	0.09
Diastolic blood pressure (mmHg)	77 (9)	77 (9)	0.56
MAP (mmHg)	93 (9)	95 (10)	0.21

Results presented as mean (standard deviation)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); excessive daytime somnolence (EDS); mean arterial blood pressure (MAP)

Complete ABPM data was available for 18 patients with EDS and 15 patients without EDS. There were no significant differences in ABPM readings between the CPAP and sham CPAP treatment periods in either of these groups as shown in Tables 4.16 and 4.17 below.

Table 4.16 ABPM measurements following CPAP and sham CPAP treatment periods in patients *with* EDS (n=18)

	CPAP	Sham CPAP	p value
ABPM systolic blood pressure (mmHg)*	118 (113-128)	118 (112-127)	0.53
ABPM diastolic blood pressure (mmHg)	73 (9)	73 (8)	0.50
ABPM MAP (mmHg)*	88 (84-96)	88 (83-96)	0.46

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); excessive daytime somnolence (EDS); mean arterial blood pressure (MAP)

Table 4.17 ABPM measurements following CPAP and sham CPAP treatment periods in patients *without* EDS (n=15)

	CPAP	Sham CPAP	p value
ABPM systolic blood pressure (mmHg)*	119 (113-125)	116 (107-127)	0.41
ABPM diastolic blood pressure (mmHg)	73 (8)	72 (8)	0.84
ABPM MAP (mmHg)*	88 (86-92)	88 (81-93)	0.42

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); excessive daytime somnolence (EDS); mean arterial blood pressure (MAP)

4.6.5.1.2 Subjects using CPAP for ≥4 hours per night

In those patients using CPAP for ≥4 hours per night there was no difference in office blood pressure (n=17) or ABPM readings (n=14) between the CPAP and sham CPAP treatment periods (see Table 4.18).

Table 4.18 Office blood pressure and ABPM measurements following CPAP and sham CPAP treatment periods in patients using CPAP ≥4 hours per night

	CPAP	Sham CPAP	p value
Systolic blood pressure (mmHg)	126 (10)	129 (11)	0.11
Diastolic blood pressure (mmHg)	77 (8)	78 (8)	0.49
MAP (mmHg)	93 (8)	95 (9)	0.24
ABPM systolic blood pressure (mmHg)*	122 (114-128)	124 (107-129)	0.48
ABPM diastolic blood pressure (mmHg)	76 (10)	75 (9)	0.29
ABPM MAP (mmHg)*	90 (85-97)	90 (81-98)	0.34

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: ambulatory blood pressure measurement (ABPM); continuous positive airway pressure (CPAP); mean arterial blood pressure (MAP)

4.6.6 Association between OSA severity and vascular function

Amongst the 53 patients with OSA who entered the randomised controlled trial there was no significant correlation found between any of the measures of arterial stiffness or endothelial function and the AHI. All measures of arterial stiffness correlated with age and all, except for AIx, also correlated with office blood pressure measurements (Table 4.19).

Table 4.19 Association between arterial stiffness, office blood pressure and age amongst patients entering the study (n=53)

	Age	Systolic BP (office)	Diastolic BP (office)	MAP (office)
AoD Ascending Aorta ($mmHg^{-1}$ $x10^{-3}$)	**-0.59** (p<0.001)	**-0.51** (p<0.001)	-0.29 (p=0.06)	**-0.41** (p=0.007)
AoD Descending Aorta ($mmHg^{-1}$ $x10^{-3}$)	**-0.42** (p=0.004)	**-0.36** (p=0.018)	-0.16 (p=0.30)	**-0.25** (p=0.11)
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	**-0.43** (p=0.002)	**-0.49** (p<0.001)	**-0.33** (p=0.018)	**-0.42** (p=0.002)
PWV (m/s)	**0.47** p=0.001)	**0.46** (p=0.001)	**0.46** (p=0.001)	**0.49** (p<0.001)
AIx (%)	**0.67** (p=0.001)	0.27 (p=0.054)	0.23 (p=0.101)	0.26 (p=0.06)

Table 4.19 Values represent correlation coefficients (r).
Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); blood pressure (BP); mean arterial pressure (MAP)

Aortic distensibility in the ascending aorta was negatively correlated with systolic ABPM (r = -0.36; p=0.026) and at the level of the diaphragm with systolic ABPM (r = -0.48; p=0.001), diastolic ABPM (r = -0.31; p=0.043) and MAP ABPM (r = -0.36; p=0.016). PWV was correlated with systolic ABPM (r = 0.46; p=0.002), diastolic ABPM (r = 0.31; p= 0.045) and MAP ABPM (r = 0.42; p=0.016)
There was no significant correlation between any measures of arterial stiffness and BMI. A weak trend was noted between PWV and fasting glucose (r = 0.26; p=0.08) and fasting cholesterol (r = 0.27; p=0.06) along with a weak trend between aortic distensibility at the level of the diaphragm and fasting glucose (r = -0.25; p=0.08). Endothelial function did not correlate with age, blood pressure (office or ABPM), BMI or fasting levels of glucose or cholesterol in this group of patients.

4.7 Sensitivity analyses

Sensitivity analyses were performed for the main outcome measures of the study in which baseline values were carried forward in patients who dropped out of the study. These results of these analyses (see Table 4.20 below) were very similar to those in the primary analyses (see Table 4.6, 4.7 and 4.14).

Table 4.20 Sensitivity analysis. Measured vascular parameters following CPAP and sham CPAP treatment periods using baseline values carried forward for subjects who did not attend for all three vascular assessments.

	CPAP	Sham CPAP	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	4.9 (1.8)	5.1 (1.9)	0.39
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)*	4.5 (3.8-6.1)	4.4 (3.5-5.3)	0.23
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	7.1 (2.1)	6.9 (2.3)	0.55
PWV (m/s)	7.6 (1.3)	7.7 (1.4)	0.15
AIx (%)	16.1 (11.2)	17.0 (11)	0.08
Change in AIx (%) following salbutamol*	-4.5 (-8 - -2.6)	-4 (-6.5 - -2.5)	0.60
Change in AIx (%) following GTN	-14.1 (4.9)	15.1 (4.5)	0.12
Systolic blood pressure (mmHg)	127 (12)	129 (13)	0.03
Diastolic blood pressure (mmHg)	77 (8)	77 (8)	0.90
MAP (mmHg)	94 (9)	95 (9)	0.29

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); continuous positive airway pressure (CPAP); glyceryl trinitrate (GTN); mean arterial pressure (MAP)

4.8 Summary of results presented in Chapter 4

In this double-blind randomised placebo-controlled crossover trial of patients with OSA but without overt CVD, 12 weeks of CPAP had no significant effect upon any measure of arterial stiffness or endothelial function. A trend towards a lower AIx was seen with CPAP; however this was not statistically significant. In keeping with previous findings (Bazzano 2007), office systolic blood pressure was lower on CPAP than on sham CPAP. CPAP compliance was sub-optimal; however sub-group analysis of those with CPAP usage of ≥4 hours per night also showed CPAP to have no significant effect upon arterial stiffness or endothelial function. Sub-group analysis of patients with EDS showed a non-significant trend towards a lower AIx on CPAP, but no other improvements in arterial stiffness or endothelial function were seen with CPAP in this group. In the group of patients with OSA, no correlation was noted between arterial stiffness or endothelial function and the AHI.

These results and those reported in Chapter 5 will be discussed in detail along with the potential limitations of the study in Chapter 7.

Chapter 5: Results of the Case–Control Study

5.1 Recruitment and Demographics

Ninety-eight positive responses were received from potential control subjects following the advertisements and the mail-out from the GP surgery (see Figure 5.1). Of these, 20 eligible control subjects were matched for age, sex and BMI on a one-to-one basis, with a subset of 20 patients with OSAHS (defined as OSA plus EDS) recruited to the randomised controlled study (RCT).

The 20 patients that we were able to match with control subjects did not differ in terms of demographic information from the 43 patients who completed the RCT, other than having a higher Epworth Sleepiness Score (ESS) (Table 5.1). Excessive daytime sleepiness (EDS), defined as an ESS ≥11, was a requirement for patients recruited to the case-control study (see Section 3.2.2), but not the RCT.

There was no difference in measured vascular parameters at baseline between the 20 patients in the case-control study and the 43 patients completing the RCT (Table 5.2).

Figure 5.1 Flowchart detailing recruitment of control subjects to the case control study

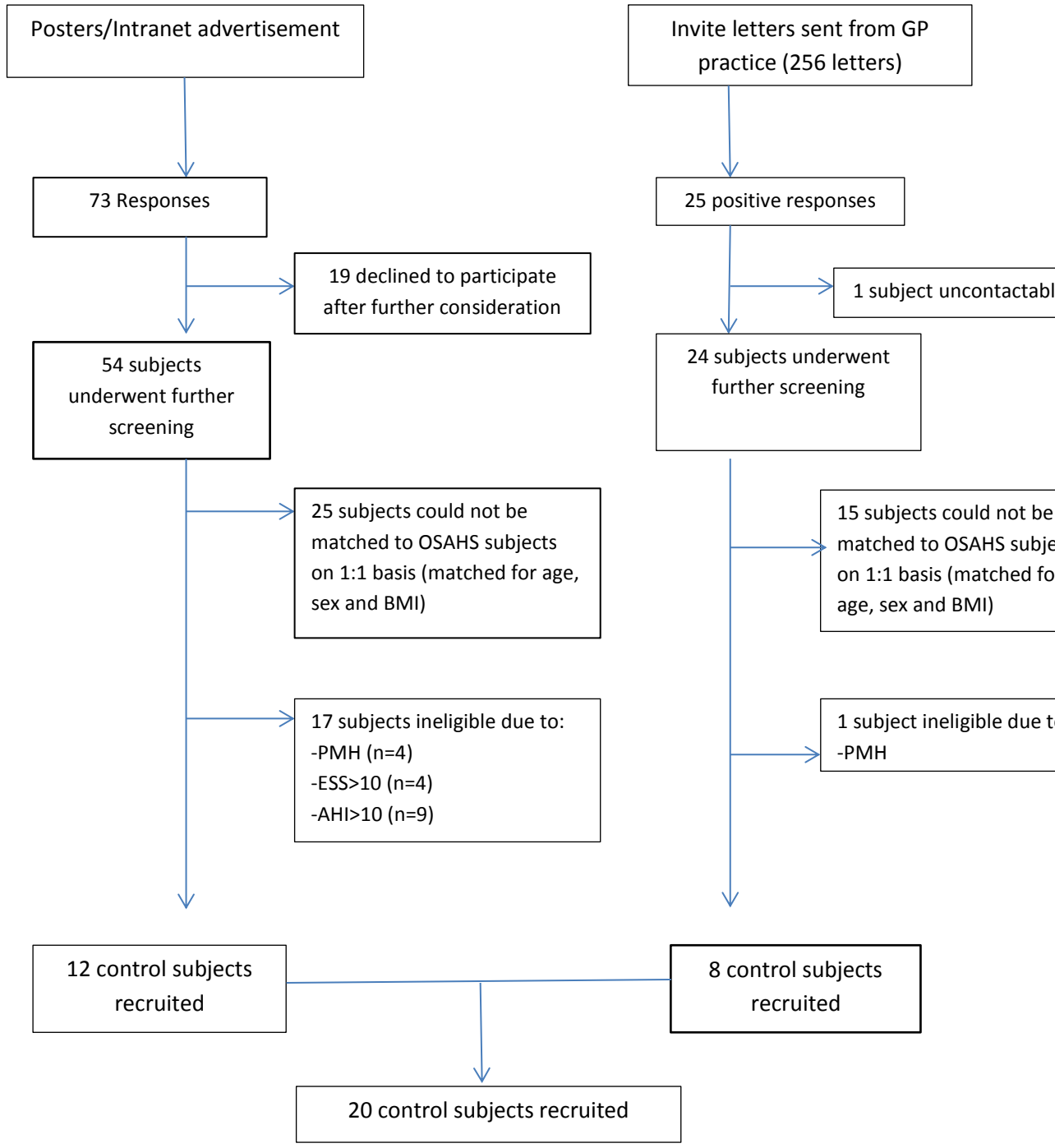

Table 5.1 Baseline demographics for the 20 patients with OSAHS matched to control subjects in the case-control study and the 43 patients completing the randomised controlled trial (RCT)

	Matched patients with OSAHS (n=20)	Patients completing the RCT (n=43)	p value
Age (years)	44 (7)	46 (9)	0.57
Sex	65% male	65% male	0.99
BMI (kg/m^2)*	29.7 (27.4-32.7)	29.9 (27.3-31.6)	0.84
Neck circumference (cm)*	40.5 (38.1-41.9)	40.0 (37.0-41.5)	0.39
Waist to hip ratio	0.94 (0.07)	0.95 (0.07)	0.48
Systolic blood pressure (mmHg)	127 (14)	128 (13)	0.87
Diastolic blood pressure (mmHg)	76 (10)	76 (9)	0.99
Mean Arterial Pressure (mmHg)	93 (11)	93 (10)	0.95
Fasting glucose (mmol/L)	4.9 (0.5)	4.9 (0.4)	0.67
Total cholesterol (mmol/L)	5.5 (1.0)	5.3 (1.0)	0.58
Current smokers	30%	23%	0.38
Ex-smokers	35%	23%	0.38
Apnoea/hypopnoea index (AHI)*	32 (22-41)	31 (20-41)	0.69
4% O_2 desaturation rate/hour	8.1 (11.7)	9.3 (12.3)	0.73
Minimum O_2 saturation (%)	87.4 (4.3)	86.1 (5.1)	0.33
TST90 (%)	1.2 (3.3)	4.4 (13.2)	0.32
Epworth sleepiness score (ESS)*	16 (14-18)	13 (6-15)	**0.002**

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: body mass index (BMI); obstructive sleep apnoea/hypopnoea syndrome (OSAHS); randomised controlled trial (RCT), percentage of sleep time spent with oxygen saturations of less than 90% (TST90)

Table 5.2 Vascular measurements for the 20 patients with OSAHS matched to control subjects in the case-control study and the 43 patients completing the randomised controlled trial (RCT)

	Matched patients with OSAHS (n=20)	Patients completing the RCT (n=43)	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	5.5 (1.6)	5.0 (1.9)	0.43
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)	4.9 (1.4)	4.8 (3.3-5.4)*	0.68
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	6.9 (2.1)	6.5 (1.9)	0.40
PWV (m/s)	6.8 (2.6)	7.6 (1.5)	0.11
AIx (%)	19.3 (10.9)	17.1 (11.6)	0.50
Change in AIx (%) following salbutamol	-4.3 (3.2)	-4.5 (-6- -3)*	0.58
Change in AIx (%) following GTN	-15.1 (5.5)	-14.0 (4.6)	0.38

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range) Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); glyceryl trinitrate (GTN); obstructive sleep apnoea/hypopnoea syndrome (OSAHS); randomised controlled trial (RCT)

The demographics of the subjects with OSAHS and controls subjects are shown below in Table 5.3. Baseline characteristics for the subjects with OSAHS and matched controls were similar with the exception of neck circumference. Given the association between neck circumference and OSA, this is unsurprising (Davies 1990, Davies 1992). However, BMI and waist-to-hip ratio were not statistically different between the groups. Blood pressure results given in Table 5.3 below reflect office blood pressure measurements. Ambulatory blood pressure measurements (ABPM) were similar in subjects with OSAHS and control subjects [systolic pressure 117 (8) vs. 119 (7) mmHg; p=0.54, diastolic pressure 72 (6) vs. 73 (6) mmHg; p=0.77, mean arterial pressure 88 (6) vs. 87 (10) mmHg; p=0.71] but complete data was only available for 14 of the 20 pairs.

There were more current smokers in the OSAHS group, but this was not statistically significant. The number of ex-smokers was similar in each group. Cigarette pack

year history (amongst current and ex-smokers) was similar in subjects with OSAHS and control subjects [14.6 (14.5) vs. 11.4 (4.71) years; p=0.47].

The 20 patients with OSAHS had a median AHI of 32 (IQR 22-41) which suggests at least moderate disease severity. The 4 % desaturation rate was however relatively low at 8.1 (11.7) per hour with a mean minimum oxygen saturation of 87.4 (4.3)%. The mean percentage of total sleep time with oxygen saturations below 90% was also low at 1.2 (3.3)%.

Table 5.3 Baseline characteristics of patients with OSAHS and controls

	Patients with OSAHS (n=20)	Control subjects (n=20)	p value
Age (years)	44 (7)	44 (7)	0.94
Sex	65% male	65% male	1.0
BMI (kg/m^2)*	29.7 (27.4-32.7)	29.4 (27.4-33.5)	0.20
Neck circumference (cm)*	40.5 (38.1-41.9)	39.0 (36.6-41.5)	**0.02**
Waist to hip ratio	0.94 (0.07)	0.93 (0.08)	0.54
Systolic blood pressure (mmHg)	127 (14)	124 (11)	0.33
Diastolic blood pressure (mmHg)	76 (10)	75 (9)	0.75
Mean Arterial Pressure (mmHg)	93 (11)	91 (9)	0.42
Fasting glucose (mmol/L)	4.9 (0.5)	4.9 (0.3)	0.68
Total cholesterol (mmol/L)	5.5 (1.0)	5.6 (0.9)	0.79
Current smokers	30%	5%	0.13
Ex-smokers	35%	40%	1.00
On no regular medication	60%	80%	0.22
Inhaled asthma medication	20%	5%	0.38
Antidepressant medication	10%	5%	1.00
Thyroxine medication	10%	5%	1.00
Proton pump inhibitor medication	0%	5%	1.00
Apnoea/hypopnoea index (AHI)*	32 (22-41)	4 (3-6)	Part of selection criteria
4% O_2 desaturation rate/hour	8.1 (11.7)	0.7 (0.9)	0.01
Minimum O_2 saturation (%)	87.4 (4.3)	89.3 (2.3)	0.05
TST90 (%)	1.2 (3.3)	0.6 (1.5)	0.44
Epworth sleepiness score (ESS)*	16 (14-18)	4 (2-8)	Part of selection criteria

Results presented as mean (standard deviation) unless otherwise stated. *Median (Interquartile range). Abbreviations: body mass index (BMI); obstructive sleep apnoea/hypopnoea syndrome (OSAHS),
percentage of sleep time spent with oxygen saturations of less than 90% (TST90)

5.2 Arterial Stiffness

The AIx was significantly higher in patients with OSAHS compared to control subjects, but no difference in PWV or aortic distensibility (at any of the three levels) was seen (Table 5.4).

Several of the MRI images obtained were of insufficient quality to determine aortic distensibility at one or more of the three levels. This, along with one case of claustrophobia meant it was not possible to compare aortic distensibility in two pairs in the ascending aorta and in five pairs in the descending aorta and at the level of the diaphragm. For reasons of body habitus, PWV could only be compared in 18 of the 20 matched pairs.

Table 5.4 Arterial stiffness in patients with OSAHS and controls

	Patients with OSAHS	Control subjects	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=18)	5.6 (1.6)	5.9 (2.4)	0.65
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=15)	4.9 (1.4)	4.9 (1.4)	0.87
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=15)	7.2 (2.2)	6.6 (2.0)	0.45
PWV (m/s) (n=18)	7.6 (1.2)	7.4 (1.2)	0.55
AIx (%) (n=20)	19.3 (10.9)	12.6 (10.2)	**0.017**

Table 5.4 The 'n' value represents number of paired samples available for analysis using the paired t-test. The reasons for missing data are given in Section 5.2 above. Results presented as mean (standard deviation. Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); obstructive sleep apnoea/hypopnoea syndrome (OSAHS)

As noted above in Section 5.1, a greater proportion of the subjects with OSAHS were current smokers, although this was not statistically significant. Across the group as a whole (n=40) no difference in AIx was seen between smokers, ex-smokers or non-smokers (Table 5.5)

Table 5.5 The influence of smoking history on the augmentation index (AIx)

	Smokers (n=7)	Ex-smokers (n=15)	Non-smokers (n=18)	p value
AIx (%)	18.3 (9.6)	16.8 (9.8)	14.3 (12.6)	0.67

Results presented as mean (standard deviation)

Abbreviation: augmentation index (AIx)

5.3 Endothelial Function

Endothelium-dependent change in AIx, a measure of vasomotor endothelial function, was impaired in patients with OSAHS compared to controls (p=0.02). No difference in endothelium-independent change in AIx was seen between the two groups (Table 5.6).

Table 5.6 Endothelial function in patients with OSAHS and controls

	Patients with OSAHS (n=20)	Control subjects (n=20)	p value
Change in AIx (%) following salbutamol (endothelium-dependent change)	-4.3 (3.2)	-8.0 (4.9)	**0.02**
Change in AIx (%) following GTN (endothelium-independent change)	-15.1 (5.5)	-14.1 (3.7)	0.45

Results presented as mean (standard deviation). Abbreviations: augmentation index (AIx); glyceryl trinitrate (GTN); obstructive sleep apnoea/hypopnoea syndrome (OSAHS)

Across the group as a whole (n=40) no difference in endothelium-dependent change in AIx was seen between smokers, ex-smokers or non-smokers (Table 5.7).

Table 5.7 The influence of smoking history on the endothelium-dependent change in augmentation index (AIx)

	Smokers (n=7)	Ex-smokers (n=15)	Non-smokers (n=18)	p value
Change in AIx (%) following salbutamol	-4.6 (3.3)	-7 6 (5.7)	-5.4 (3.6)	0.25

Results presented as mean (standard deviation. Abbreviation: augmentation index (AIx)

5.4 Association between OSAHS severity and vascular function

In the 20 patients with OSAHS in the case-control study, the AHI was negatively correlated with aortic distensibility at the level of the descending aorta (r = -0.56; p=0.015) and at the diaphragm (r = -0.45; p=0.049), but not at the level of the ascending aorta (r= -0.33; p=0.20) as shown in Figure 5.2. Fasting glucose was also negatively correlated with aortic distensibility in the descending aorta (r= -0.47: p=0.05). In a stepwise linear regression model with distensibility of the descending aorta as the dependent variable, and AHI and fasting glucose as independent variables, only AHI remained as an independent predictor (β-coefficient = -0.49; p=0.037). The addition of smoking status to the stepwise linear regression model did not alter this, with smoking status being excluded from the model (β-coefficient =0.12; p=0.61). There was no significant correlation between aortic distensibility at any of the three sites and nocturnal hypoxia; as measured by the 4% oxygen desaturation rate, minimum oxygen saturations or total sleep time with oxygen saturations below 90%. There was a non-significant correlation between aortic distensibility at the level of the diaphragm and the 4% oxygen desaturation rate and the total sleep time with oxygen saturations below 90% (see Table 5.8).

Figure 5.2 Relationship between AHI and aortic distensibility in patients with OSAHS

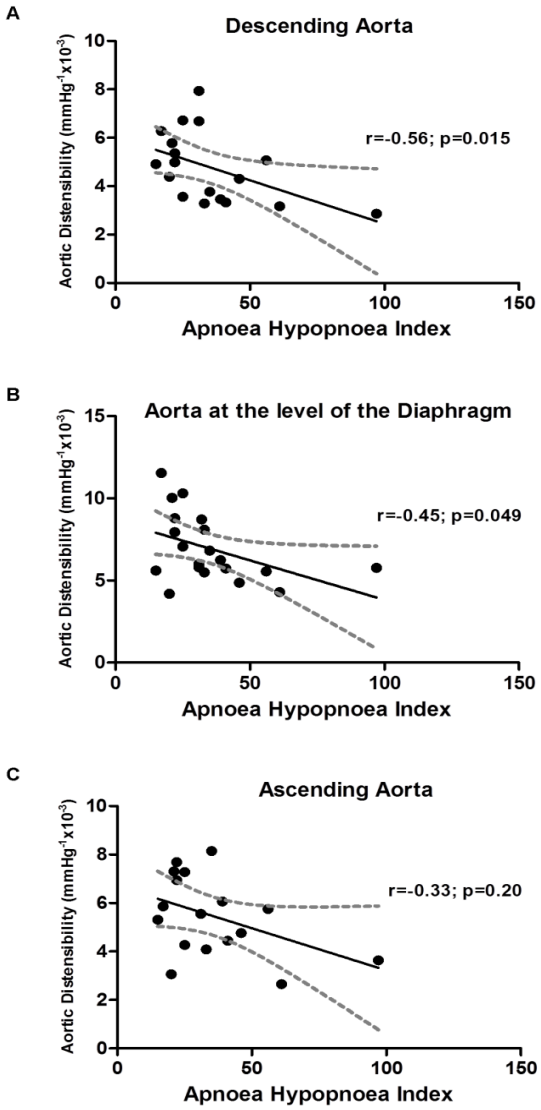

Figure 5.2 Apnoea hypopnoea index (AHI) plotted against aortic distensibility as measured at three levels within the thoracic aorta. The broken lines represent 95% confidence intervals and r values represent Spearman's rank correlation coefficients.

Table 5.8 Correlation coefficients for measures of nocturnal hypoxia and aortic distensibility (AoD) in patients with OSAHS

	4% oxygen desaturation rate	Minimum oxygen saturation	Total sleep time with O_2 saturations below 90%
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	- 0.41 (p=0.10)	- 0.21 (p=0.64)	- 0.33 (p=0.19)
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)	- 0.39 (p=0.14)	- 0.14 (p=0.62)	- 0.38 (p=0.15)
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	- 0.46 (p=0.08)	0.05 (p=0.86)	- 0.48 (p=0.07)

Table 5.8 Values represent correlation coefficients (r).
Abbreviations: aortic distensibility (AoD); oxygen (O_2)

AHI correlated non-significantly with PWV (r=0.46; p=0.055), but did not correlate with either AIx or endothelium-dependent change in AIx. There was no correlation between any measure of arterial stiffness or endothelial function and office blood pressure in this group of patients. Aortic distensibility at the level of the diaphragm (n=16) did however correlate with mean systolic blood pressure recorded during ABPM (r= -0.54; p=0.030).

5.5 Summary of results presented in Chapter 5

In this study of normotensive patients with OSAHS, AIx was increased and endothelium-dependent change in AIx was impaired when compared to well-matched control subjects and in the absence of overt CVD. Thus, even in the presence of the relatively modest nocturnal oxygen desaturation seen here, patients with OSAHS had evidence of increased arterial stiffness and impaired endothelial function. Furthermore, disease severity, as measured by the AHI, was shown to correlate with aortic distensibility, a measure of arterial stiffness in the descending

aorta and at the level of the diaphragm. These results will be discussed in detail along with the potential limitations of the study in Chapter 7.

Chapter 6: A comparison of the different methods of measuring arterial stiffness

6.1 Introduction

Aortic pulse wave velocity (PWV) measured by applanation tonometry is considered the gold standard measurement of arterial stiffness (Laurent 2006) and as such, the other measurements of stiffness, namely aortic distensibility and AIx, were compared to this. Baseline measurements of arterial stiffness were available for 73 subjects in total (20 control subjects and 53 patients with OSA) and these were used for the analyses presented in this chapter. All 73 were free of known CVD and their baseline demographics and measures of arterial stiffness are shown in Table 6.1 below.

Table 6.1 Demographics and baseline measures of arterial stiffness (n=73)

Age (years)	45 (8)
Sex	66% male
BMI (kg/m^2)*	29.8 (27.4-32.6)
Systolic Blood pressure (mmHg)	127 (13)
Diastolic Blood Pressure (mmHg)	76 (9)
Mean Arterial Pressure (mmHg)	93 (10)
PWV (m/s) (n=69)	7.6 (1.4)
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$) (n=60)	5.3 (2.1)
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)* (n=60)	4.8 (3.5-5.7)
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$) (n=65)	6.6 (2.1)
AIx (%) (n=73)	16.1 (10.9)

Results presented as mean (standard deviation) unless otherwise stated

*Median (Interquartile range)

Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); augmentation index (AIx); body mass index (BMI)

Baseline PWV measurements were available for 69 subjects (95 %). Missing measurements resulted from difficulties obtaining a satisfactory pressure wave at the femoral pulse site due to body habitus. As reported in Chapter 3 (section 3.11.2.1), the intraclass correlation coefficient (ICC) for repeated PWV measurements was 0.95 (95% CI 0.92-0.97); $p<0.001$.

6.2 PWV compared to aortic distensibility measured using cardiac MRI

Baseline aortic distensibility measurements were available for 60 subjects (82%) in the ascending and descending aorta and in 65 subjects (89%) at the level of the diaphragm. In two cases this was due to subjects experiencing unexpected claustrophobia on entering the MRI scanner. In the remainder of cases this was due to the quality of the MR images obtained being of insufficient quality to allow for distensibility calculations. As reported in Chapter 3 (Section 3.11.1.1) the ICC for aortic distensibility measurements across all three sites was 0.85 (95% CI 0.60-0.95); $p<0.001$.

PWV was significantly and negatively correlated with measurements of aortic distensibility at each of the three measurement sites, as shown in Table 6.2 below. This negative correlation persisted when control subjects and patients with OSA were examined separately.

Table 6.2 Correlation coefficients for comparison of PWV and aortic distensibility

	PWV (m/s)	p value
AoD Ascending aorta ($mmHg^{-1}$ $x10^{-3}$)	-0.59 (n=57)*	<0.001
AoD Descending aorta ($mmHg^{-1}$ $x10^{-3}$)	-0.59 (n=57)*	<0.001
AoD at level of diaphragm ($mmHg^{-1}$ $x10^{-3}$)	-0.61 (n=61)*	<0.001

Table 6.2 *denotes number of paired measurements available for comparison
Values represent correlation coefficients (r).
Abbreviations: aortic distensibility (AoD); aortic pulse wave velocity (PWV); metres per second (m/s)

6.3 PWV compared to the augmentation index (AIx)

Baseline AIx measurements were available in all 73 subjects at baseline. As reported in Chapter 3 (section 3.11.3.1), the ICC for AIx measurements was 0.96 (95%CI 0.94-0.98; p<0.001). PWV and AIx were significantly correlated with an r value of 0.43; p<0.001 based on 69 paired measurements (see Figure 6.1 below). The relationship persisted when control subjects and patients with OSA were examined separately.

Figure 6.1 The relationship between PWV and AIx

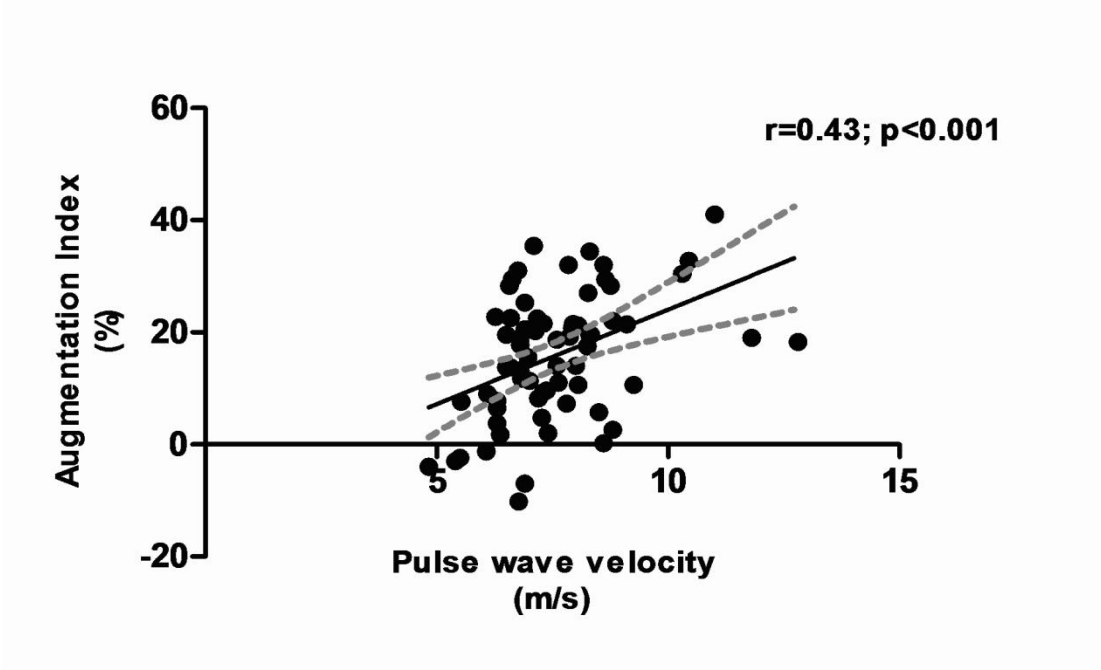

Figure 6.1 The relationship between pulse wave velocity (PWV) and the augmentation index (AIx). The broken lines represent 95% confidence intervals and r value represents the correlation coefficient.

6.4 A comparison of distensibility measurements at different sites along the aorta

Aortic distensibility was measured in the ascending and descending aorta (at the level of the right pulmonary artery) and at the level of the diaphragm. Measurements across all three sites showed good correlation. Distensibility in the ascending aorta correlated with that of the descending aorta ($r= 0.74$; $p<0.001$), and at the level of the diaphragm ($r=0.65$; $p<0.001$). Distensibility in the descending aorta correlated with that at the level of the diaphragm ($r=0.68$; $p<0.001$).

6.5 Discussion of results

In this study, three measure of arterial stiffness were made; namely aortic distensibility, measured directly during cardiac magnetic resonance imaging (MRI), pulse wave velocity (PWV) and augmentation index (AIx) both measured by applanation tonometry. These are measures of local aortic stiffness, regional stiffness

and wave reflection, respectively, providing a comprehensive assessment of arterial stiffness throughout the arterial tree. As this work was not the main aim of the study, the results are discussed here rather than in Chapter 7, the main discussion chapter.

PWV, the gold standard measurement of arterial stiffness, is non-invasive, reproducible (Wilkinson 1998) and widely used. However in obese patients (Laurent 2006) and particularly at the femoral pulse site it can be difficult to obtain pressure waves of sufficient quality for analysis. In determining PWV, the path length is usually measured externally using a tape measure and this too can be affected by obesity. Reproducibility in this study was high as reported above, but in four patients it was not possible to obtain a PWV measurement due to body habitus.

MRI scanning is also non-invasive and as aortic distensibility can be measured directly, should be less influenced by issues of body habitus and as such, is considered free from operator bias (Oliver 2003). Measured in this way, aortic distensibility has previously been shown to decrease with age (Oliver 2003) and is lower in patients with hypertension (Resnick 1997), type II diabetes mellitus (van der Meer 2007), end stage renal disease (Zimmerli 2007) and more recently in moderate to severe OSA (Kylintireas 2012).

Although MRI determination of aortic distensibility is a well-validated technique (Oliver 2003), prior to commencing this study we were not aware of any studies directly comparing aortic distensibility measured in this way, with the gold standard measurement of PWV. We found aortic distensibility at all three measurement sites to be significantly and negatively correlated with PWV measured by applanation tonometry. Since completing our study two groups have reported similar findings in obese subjects (Joly 2009) and a mixed group comprising patients with primary aldosteronism or hypertension and controls (Mark 2014). Additionally Redheuil *et al* concluded that compared to other measures of aortic function, aortic distensibility in the ascending aorta measured using MRI was the best marker of subclinical large artery stiffening in subjects under 50 years of age (Redheuil 2010).

As reported above, the reproducibility of aortic distensibility measurements in this study was acceptable, but on a number of occasions it was not possible to obtain aortic distensibility measurements, either due to the quality of the scans or due to unexpected claustrophobia (which may be more likely in obese patients due to the

internal diameter of the MRI scanner). Additionally a number of otherwise eligible subjects were unable to participate in the study at all due to claustrophobia or the presence of implanted/embedded metallic foreign bodies. Despite the above noted benefits, these issues, along with the financial costs associated with MRI scanning are likely to currently limit the measurement of aortic distensibility to research studies or exceptional circumstances.

It is not always possible, particularly in obese subjects, to obtain PWV measurements (Bakker 2011), with pressure waves at the femoral pulse presenting the greatest problem in this study. AIx is however determined, using a generalised transfer factor, from the radial pressure wave. The radial pulse is usually relatively superficial and easy to palpate, even in obese subjects and accordingly AIx measurements were available for all 73 subjects at baseline. Measurement of AIx is relatively straightforward and as it is obtained at the wrist, is easier for patients to undergo than a PWV measurement, making it ideal for use in large numbers of patients.

PWV and AIx are not interchangeable terms, although AIx is in part dependent on PWV. PWV is a direct measure of regional aortic stiffness along the aortic and aorto-iliac pathways, recording the speed at which the systolic pressure wave reaches the peripheries (Laurent 2006). AIx is a measure of arterial wave reflection and as such is an indirect measure of stiffness throughout the entire arterial tree (see Chapter 3, sections 3.11.2 and 3.11.3 for further details of PWV and AIx). A higher PWV results in the reflected wave arriving earlier, leading to increased AIx during early systole (Kelly 2001). AIx has been shown, in a large meta-analysis, to be predictive of cardiovascular events and all-cause mortality (Vlachopoulos 2010). In this study, PWV and AIx were significantly correlated overall and this remained the case when subjects with OSA and control subjects were examined separately. This is in keeping with a previous large study in healthy adults (McEniery 2005), as well as in smaller studies of patients with CVD and controls (Yasmin1999, Woodman 2005). This has not however been a universal finding, with several studies finding no association between PWV and AIx (Vyas 2007, Lemogoum 2004). As described above, AIx is a composite measure of wave reflection comprising not only arterial stiffness, but also the magnitude and timing of the reflected wave and additionally is influenced by heart rate, height and left ventricular function (Protogerou 2007). McEneiry *et al*

reported that age-related changes in AIx and PVW were non-linear with age, affecting the AIx more in subjects aged under 50 years with changes in PWV more prominent in older subjects (McEneiry 2005), suggesting the involvement of more than one physiological process. Subjects in this study were relatively young and when considered overall (n=73) or separately (i.e. subjects with OSA, subjects with OSAHS and control subjects), had PWV values falling within the recently published 'normal' range based upon their mean ages and blood pressures (Boutouyrie 2010). In conclusion, in this study all three measures of arterial stiffness were reproducible, but unlike AIx, measurements of PWV and aortic distensibility were not always technically possible. Both aortic distensibility and AIx were well correlated with PWV, the gold standard measurement of arterial stiffness.

Chapter 7: Discussion of findings

7.1 Summary of the key findings

This study shows that patients with OSAHS have evidence of increased arterial stiffness and impaired endothelial function compared to well-matched controls, even in the absence of cardiovascular disease (CVD) or hypertension. The median AHI in this study suggested at least moderate to severe disease, but patients did not experience significant nocturnal hypoxia. This is important as intermittent hypoxia (IH) has been postulated as the most important factor in any causal link between OSA and CVD (Lavie 2003, Foster 2007), and this will be discussed below.

Despite the findings of the case-control study (Chapter 5), in a larger group of patients with OSA (also without CVD or hypertension), 12-weeks of CPAP therapy in the context of this randomised placebo-controlled trial, did not lead to an improvement in any measure of arterial stiffness or endothelial function. Sub-group analysis similarly showed CPAP to have no significant effect upon arterial stiffness or endothelial function in subjects with excessive daytime sleepiness (EDS) or those with CPAP compliance of ≥4 hours per night. As was previously described in the early randomised controlled trials (Faccenda 2001, Pepperell 2002) and subsequently confirmed in large meta-analyses (Bazzano 2007, Haentjens 2007, Schein 2014), CPAP therapy did effect a small reduction in blood pressure.

The implications of these results will be discussed below in the context of the other published work in this area, along with the limitations of this study and suggestions for future work.

7.2 Findings of the case-control study

7.2.1 OSAHS is associated with increased arterial stiffness

Patients with OSAHS had increased arterial stiffness, as measured by the augmentation index (AIx), compared to well-matched control subjects. Additionally aortic distensibility was negatively correlated with the AHI.

Prior to starting this study, there had been several reports of increased arterial stiffness in patients with OSA, as measured by ankle-brachial pulse wave velocity

(Nagahama 2004, Shiina 2006), aortic distensibility (Kasikcioglu 2005) and PWV (Drager 2005). Since then, there has been an explosion of work in this area, with a recent review highlighting 23 subsequent studies examining the relationship between OSA and a variety of measures of arterial stiffness (Phillips 2013b). Nineteen of these studies reported results for either AIx, aortic distensibility or PWV**,** all three of which were measured in this study. Four of the five studies measuring AIx reported a higher AIx in patients with OSA compared to control subjects (Phillips C 2005, Noda 2008, Kohler 2008a**,** Buchner 2012). However all of these studies included at least some subjects with either CVD or hypertension. The severity of OSA in these studies varied and this is discussed below in the context of the disease severity reported in the study presented in this thesis.

Importantly, the study presented in this thesis is the first to examine the effect of OSAHS on AIx in subjects without known CVD or hypertension. It is difficult to adequately match patients with varying degrees of CVD and the exclusion of such patients is likely to reduce confounding. Additionally, control subjects were true controls recruited from the community (as opposed to being subjects who had been found not to have OSA after referral to a sleep clinic, as is often the case in the literature) without evidence of EDS and who subsequently underwent full polysomnography to exclude OSA.

One previous study did not find the AIx to be raised in subjects with OSA (Yim-Yeh 2010). In that study, obese but otherwise healthy subjects were recruited from the community and underwent polysomnography. Those with an AHI ≥10 were deemed to have OSA (irrespective of symptoms) with those with an AHI of less than 10 forming the control group, and in this respect the study was quite different from the work in this thesis. In the study by Yim Yeh *et al*, subjects with OSA were older, had higher blood pressure, a greater waist-to-hip ratio, a higher HbA1c and were more likely to be male than the control group. However despite this, they did not find AIx to be increased in subjects with OSA. However, it must be noted that in the study by Yim Yeh *et al*, although the mean AHI was 23, an AHI of ≥10 was considered to represent OSA and at this lower level would represent very mild disease. The authors suggest that their findings might reflect the inclusion of females (studies in this area often exclude or contain very few females), the raised BMI or the exclusion of

subjects with known CVD. Given that our study included a similar percentage of female subjects and that the mean AIx was similar (19.6% vs. 16.2% in their study) it is tempting to speculate whether the lack of EDS in the study by Yim Yeh *et al* may be an important factor. The importance of EDS in the association between OSA and CVD is unclear, with evidence of increased CVD risk associated with EDS of any cause in older people (Newman 2000) and a recent meta-analysis suggesting that CPAP is ineffective in reducing blood pressure in patients with OSA in the absence of EDS (Bratton 2014). This issue will be addressed in detail with reference to the results of the randomised controlled trial in Section 7.4.4 below.

An unintended consequence of excluding patients with CVD and hypertension was that subjects eligible for our study had milder disease in terms of nocturnal hypoxia, than often appears in publications in this area. Whilst the median AHI [32 IQR (22-41)] in patients with OSAHS indicated moderate to severe disease, nocturnal hypoxia was relatively mild, with a 4 % desaturation rate of 8.1 (11.7) per hour, mean minimum oxygen saturations of 87.4% (4.3%) and a mean of only 1.2% (3.3%) of sleep time with oxygen saturations less than 90%. This level of hypoxia is relatively modest compared to that reported in previous studies and probably reflects the relative youth of subjects in our study and the exclusion of patients with a history of respiratory failure or CVD. Indeed with a 4% desaturation rate of 8.1, irrespective of symptoms, a diagnosis of OSAHS may not have been made on the basis of oximetry alone in many of these cases. Kohler *et al* found the AIx to be increased in subjects with minimally symptomatic OSA (i.e. in the absence of EDS) (Kohler 2008a). OSA was diagnosed by Kohler *et al* on the basis of the ODI, with a mean ODI of 23.1, suggesting more severe hypoxia than seen in the study presented in this thesis. Similarly, a higher 4% desaturation rate than seen in our study was reported by Phillips *et al* (Phillips C 2005) and in a subsequent study, sleep time with oxygen saturations of less than 90% was 18.3% (Buchner 2012). In the other study reporting an increased AIx in patients with OSA (Noda 2008), the overall mean severity of OSA (either in terms of AHI or nocturnal hypoxia) was not reported.

Despite relatively modest nocturnal hypoxia, and in the absence of known CVD, patients with OSAHS had a higher AIx than well-matched controls in our study. The absolute difference in AIx between patients with OSAHS and controls was 6.7%

[19.3 (10.9) vs. 12.6 (10.2)] which is likely to be of clinical significance. AIx is a reproducible measurement of wave reflection, and reflects arterial stiffness throughout the entire arterial tree (Wilkinson 1998). In this study, measurement of AIx was possible in all patients, even in the presence of significant obesity. AIx has been shown to predict cardiovascular disease (Weber 2004) and a recent meta-analysis reported that an absolute increase in AIx of 10% leads to a 26% increase in cardiovascular events and a 38% increase in all-cause mortality (Vlachopoulos 2010).

In this study, aortic distensibility in the descending aorta and at the level of the diaphragm was negatively correlated with the AHI, with a non-significant trend towards a correlation between aortic distensibility at the level of the diaphragm and measures of nocturnal hypoxia. Prior to starting this study, it had been hypothesised that additional effects on the structure and function of the thoracic aorta may be seen due to intra-thoracic pressure swings during obstructive events leading to shear stress (Cistulli 1997, Sampol 2003). Furthermore, two studies (Kasikcioglu 2005**,** Tanriverdi 2006) the latter of which excluded patients with CVD and hypertension, had reported that aortic distensibility, determined at echocardiogram, was reduced in patients with OSA. Tanriverdi *et al* also showed, as in our study, that aortic distensibility was negatively correlated with the severity of sleep apnoea (Tanriverdi 2006). Serizawa *et al* reported an increase in the diameter of the ascending aorta in patients with OSA (Serizawa 2008) and subsequent work using MRI scanning in healthy volunteers demonstrated changes in proximal aortic diameter during simulated hypopnoeas (but not apnoeas) (Stöwhas 2011). In the study presented in this thesis, despite finding a negative correlation between aortic distensibility and the AHI, aortic distensibility was not lower in patients with OSA compared to well-matched controls. Four further studies have been published examining the aortic distensibility in patients with OSA, all of whom included some patients with CVD or hypertension. Three of these studies found distensibility was lower in patients with OSA (Tavil 2007, Keles 2009, Kylintireas 2012), with a fourth reporting no independent effect of OSA upon aortic distensibility (Lee 2010). In the one other study to use cardiovascular magnetic resonance imaging (MRI) to directly determine aortic distensibility, distensibility was lower in patients with OSA and correlated

with the ODI, although this did not retain significance following multivariate analysis (Kylintireas 2012). That study differed from our study in that subjects with CVD or hypertension were not excluded. As has been discussed above, patients recruited to the study presented in this thesis, had milder OSAHS than is often reported in the literature, particularly with regard to levels of nocturnal hypoxia, which may at least in part explain this finding. Additionally, with the exception of the study by Keles *et al*, the mean ages of subjects in the studies examining aortic distensibility in patients with OSA and controls were higher than the patients in the case-control study reported in this thesis [mean age 44(7) years]. This is important as aortic distensibility decreases with age (van der Heijden-Spek 2000) and certainly when all the participants entering the randomised controlled trial were considered together (n=53), baseline distensibility at each of the three measured sites was significantly inversely correlated with age (see Chapter 4, section 4.6.6). It is possible that any effect of OSAHS on the aorta might be more significant in the presence of an already stiffened aorta. The relatively young age of the subjects in our study also means that they are likely, on average, to have had undiagnosed OSAHS for less time than older subjects included in the studies above, and therefore it is possible that it may be too early to detect a deleterious effect on the aorta in these patients.

Unlike a number of previous studies we did not find PWV to be higher in patients with OSAHS (Phillips 2013b). However this has not been a universal finding, with two studies also reporting no difference (Korcarz 2010, Tonini 2010). With the exception of the study by Drager *et al* (Drager 2005), published before the start of this study, all of the subsequent studies have included at least some patients with CVD or hypertension. Indeed, although Drager *et al* showed PWV to be higher in subjects with severe OSA, defined as an AHI of ≥30 (but with a mean AHI of 55.7 and almost a third of sleep time spent with oxygen saturations less than 90%), compared to control subjects, they did not find PWV to be higher in subjects with mild-moderate disease, in keeping with the findings presented in this thesis. In our study there was a non-significant correlation between AHI and PWV ($r=0.46$; $p=0.055$). Drager *et al* did find PWV to be correlated with the AHI and this may reflect the inclusion of patients with more severe OSA. However it should also be

noted that despite the exclusion of patients with known cardiovascular disease, hypertension and diabetes mellitus (as in our study), values for PWV were much higher, with a mean value of >10m/s compared to a mean of 7.6m/s in this study. Recently published 'normal' values for PWV (Boutouyrie 2010) suggest that the mean PWV measured in the work presented here falls within the normal range based upon the mean age and blood pressure of the group and this may further explain the lack of difference seen between OSAHS patients and controls. The evidence for this normative data comes from a group of non-smoking subjects without overt CVD, hypertension, diabetes mellitus or dyslipidaemia (n=1455). In that cohort however, patients were not screened for the presence of OSA and given the prevalence figure of 20% for OSA in the general population (Young 2002a), many of the 'normal' subjects would be expected to have evidence of OSA at polysomnography. Chung *et al* also found increased PWV in severe OSA, but did not find it to be increased in patients with mild to moderate disease (Chung 2010). Thus it would appear that mild to moderate OSA, in the absence of significant hypoxia and pre-existing cardiovascular disease, is not associated with increased PWV.

AIx, PWV and aortic distensibility are all measures of arterial stiffness. Although AIx and PWV have been shown in a number of studies, as in this study (see Section 6.3), to be positively correlated (Yasmin 1999, Woodman 2005**,** McEniery 2005), they are not interchangeable. This is important with regard to the results of the study presented in this thesis and when comparing studies of differing measurements of arterial stiffness. PWV is considered to be the gold standard measurement of arterial stiffness, measuring regional vessel stiffness in the aorta which leads directly on from the left ventricle. As such, aortic stiffness is responsible for many of the pathophysiological effects of arterial stiffness (Laurent 2006). Arterial stiffness as measured by PWV has been well studied and has been shown, in a number of populations, to predict cardiovascular morbidity and mortality (Blacher 1999a, Blacher 1999b, Willum-Hansen 2006). AIx is a measure of wave reflection throughout the arterial tree and therefore is an indirect measure of global arterial stiffness, and has been shown to predict CVD and death (Vlachopoulos 2010). AIx therefore, unlike PWV or aortic distensibility, is a composite measure of stiffness in both the elastic and more peripheral arteries and as described in Chapter 2

(section 2.3), stiffness in the peripheries is primarily modulated by vasomotor tone, which is in part influenced by endothelial function. As discussed below in section 7.2.2, patients with OSAHS had evidence of impaired endothelial function and this may have contributed to the finding that arterial stiffness as measured by AIx, but not PWV or aortic distensibility, was increased in OSAHS patients.

Although AIx was found to be higher in subjects with OSAHS, the mean values for AIx and PWV in both patients with OSAHS and controls are similar to previously published 'normative' data (McEniery 2005, McEniery 2006, Boutouyrie 2010, Janner 2010). In a large study of over 4000 subjects, age-related changes in AIx and PVW were non-linear with age, affecting the AIx more in subjects aged under 50 years with changes in PWV more prominent in those over 50 years (McEneiry 2005). This non-linear relationship between age and measures of arterial stiffness has now been confirmed in a number of studies (Mitchell 2004, Wojciechowska 2006, Janner 2010) with a plateau in AIx noted at around 60 years of age (Janner 2010). This has led to the suggestion that AIx may be a more sensitive marker of arterial ageing in younger subjects (McEneiry 2005, Janner 2010). The mean age of subjects in the case-control study was 44 years and it may be that at this relatively young age, AIx is a better marker of increased arterial stiffness than PWV.

Less is known about the prognostic significance of aortic distensibility, although this is a direct measure of local aortic stiffness following on from the left ventricle. A recent large study of 2122 subjects without overt CVD (other than hypertension) showed aortic distensibility to predict cardiovascular morbidity and mortality over a mean follow-up period of 7.8 years. After adjustment for known cardiovascular risk factors, aortic distensibility remained a significant predictor of non-fatal cardiovascular events with hazard ratio of 1.45 (95% CI 1.18-1.78; $p<0.0005$) per standard deviation change in aortic distensibility (Maroules 2014).

7.2.2 OSAHS is associated with impaired endothelial function

In this study endothelium-dependent vasodilation was significantly impaired in patients with OSAHS, suggesting that even in patients with relatively mild disease and in the absence of known cardiovascular disease there is evidence of endothelial dysfunction. Endothelial dysfunction predicts future cardiovascular disease in a

variety of high risk patient groups and in healthy controls. It has been proposed as a key mechanism linking OSA and CVD (Kohler 2010, Hoyos 2015). Prior to starting this study, a number of small studies had reported endothelial dysfunction in patients with OSA (Kato 2000, Duchna 2000, Imadojemu 2002, Ip 2004, Oflaz 2006) and in a small study, endothelial function correlated with nocturnal hypoxia (Kraiczi 2001). Since then there has been a rapid expansion of work in this area, with a recent review identifying 35 studies (including this one) measuring endothelial function in adult patients with OSA compared to control subjects (Hoyos 2015). The overwhelming majority of these studies have shown, using a variety of means of assessing vasomotor endothelial function, that OSA is associated with impaired endothelial function. Only a minority of these studies have definitely excluded patients with CVD and hypertension and in doing so in the study presented here, the potential for confounding due to underlying CVD is reduced. The majority of studies to date have used flow mediated dilatation (FMD) to measure endothelial function. Although FMD is a non-invasive means of assessing endothelium-dependent vasodilation, it requires a highly skilled operator and expensive equipment (Stoner 2012). Changes in brachial artery diameter are determined using ultrasound following the reactive hyperaemia seen after a period of ischaemia (Lekakis 2011). Vasomotor endothelial function can also be assessed by measuring the effects of beta-2 agonists and GTN upon the AIx as used in this study (see Sections 2.4 and 3.12 for further details). Although a relatively novel technique prior to starting our study, this method of using beta-2 agonists to assess endothelium-dependent vasodilation had been shown to be reproducible (Wilkinson 2002B, Hayward 2002) and correlated with invasively measured endothelial function measured at venous occlusion plethysmography (Wilkinson 2002B). Blunting of the AIx response to beta-2 agonists has been shown in conditions known to be associated with endothelial dysfunction, including diabetes mellitus (Chowienczyk 1999), coronary artery disease (Hayward 2002), hypercholesterolaemia (Wilkinson 2002b) and peripheral vascular disease (Kals 2006). Using the same technique, Kohler *et al* showed that endothelial function is impaired in subjects with minimally symptomatic OSA (and in many cases concomitant CVD or hypertension). In that same study, FMD was also impaired in patients with OSA (Kohler 2008a). Changes in AIx following salbutamol

administration reflect global endothelium-dependent vasodilation (Hayward 2002) and the work in this thesis shows, for the first time, an impairment of endothelial function measured in this way, in patients with OSAHS, but without underlying CVD or hypertension.

In our study, endothelial function did not correlate with measures of OSAHS severity. A number of previous studies have reported a correlation between endothelium-dependent vasodilation and AHI and measures of nocturnal hypoxia (Hoyos 2015). This has not however been a universal finding with several other clinic-based studies finding no association (Kato 2000, Kohler 2008a). In a large population based study, FMD correlated with AHI and measures of nocturnal hypoxia, however when adjusted for BMI, the association was no longer significant overall, persisting only in the subset of patients with concomitant hypertension (Nieto 2004). Similarly, no association between FMD and severity of OSA was seen in a subsequent population-based study (Chami 2009). Given the population-based nature of these studies, the median AHI was low and it has been speculated that this, along with the associated lack of nocturnal hypoxia, may explain this finding (Hoyos 2015). However, the results presented here show impairment of endothelial function in relatively young patients with only very mild nocturnal hypoxia. It is nonetheless difficult to compare clinic- and population-based studies as it may be that for any given AHI, subjects who present to a sleep service may differ from those who do not. In particular it is speculated that those who present to a sleep service are more likely to be experiencing EDS (Young 2002), the importance of which in the association between OSA and CVD in unclear and is discussed further in Section 7.4.4**.** They may also differ in other, as yet unidentified ways.

That we found evidence of impaired endothelial function (and arterial stiffness) in a relatively young group of patients is perhaps not surprising, given that several studies have shown that the association between OSA and hypertension is greatest in younger patients (Bixler 2000, Grote 2000, Nieto 2000). More recently, two studies examining endothelial function in OSA have also concluded that the effect of OSA on endothelial function is greatest in younger patients (Chung 2009**,** Yim Yeh 2010**).** This would be in keeping with the idea that OSA may appear phenotypically different at different ages**.** As discussed in Section 1.7.2, the aetiology and clinical

features of OSA may be different in an older population (Launois 2007, Edwards 2014).

Endothelium-independent vasodilation (measured as the change in AIx following the administration of GTN) is primarily mediated by vascular smooth muscle and was not impaired in patients with OSAHS in our study. Despite an initial report to the contrary (Carlson 1996) and one subsequent study showing impaired endothelium-independent vasodilation in subjects with moderate to severe OSA (Butt 2011), the other studies in which it has been measured, have also reported endothelium-independent vasodilation to be unaffected in patients with OSA (Hoyos 2015).

7.3 Pathophysiological implications of the main findings of the case-control study

Arterial stiffness and endothelial dysfunction represent pre-clinical CVD and as discussed in Chapter 2 (Sections 2.3 and 2.4), have been shown to predict cardiovascular morbidity and mortality**.** Thus, the finding that there is evidence of increased arterial stiffness and endothelial dysfunction in patients with OSAHS, in the absence of known CVD or hypertension has important implications, not least with regard to the potential for early therapeutic intervention.

Much of the previous work in this area is potentially confounded by the inclusion of patients with underlying CVD. OSAHS is common, affecting 2-4 % of the population and OSA is commoner still with prevalence rates of 20% and as such any association with CVD has significant public health implications. As previously discussed in Chapter 2, any causal link between OSA and CVD is likely to be multifactorial and the pathophysiological mechanisms still remain incompletely understood. The acute effects of OSA include intermittent hypoxia, repeated arousals/sleep fragmentation and intra-thoracic pressure swings (McNicholas 2007). The subsequent biological consequences of which are likely to include activation of the sympathetic nervous system, systemic inflammation and oxidative stress. Based largely upon cell culture (Ryan 2005) and animal models (Fletcher 1992, Greenberg 1999, Prabhakar 2005) prior to starting this study it was generally accepted, albeit with much of the evidence showing an indirect association, that intermittent hypoxia

(IH) was the most important mechanism underlying any link between OSA and CVD (Lavie 2003, Foster 2007) and this remains the case (Levy 2013).

Animal models, although enabling IH to be studied separately from the arousals and intra-thoracic pressure changes seen in OSA, are not perfect models of OSA-related IH (Foster 2007) and in cell culture models the duration and severity of IH may be greater than that seen in OSA (Ryan 2005). More recent work in healthy humans exposed to more physiologically relevant IH over an eight hour period at night (i.e. IH more closely resembling that seen in OSA) demonstrated an increase in diurnal blood pressure and sympathetic activity after one night, with a further increase after two weeks of nocturnal exposure (Tamisier 2011). Additionally, healthy males exposed to six hours of IH (whilst awake) for four consecutive days had increased plasma levels of markers of oxidative stress, which returned to normal after the period of exposure to IH had ceased (Pialoux 2009).Using the same model Foster *et al* showed increased blood pressure and decreased nitric oxide bioavailability after 4 days, which improved following removal of IH (Foster 2009). However, despite the undoubted importance of IH, patients in the study presented in this thesis had demonstrably stiffer arteries and impaired endothelial function in the absence of significant nocturnal hypoxia.

Recent work by Jafari *et al* confirmed the presence of endothelial dyfunction in patients with OSA, but this was independent of the degree of nocturnal hypoxia (Jafari 2013). If the only important driver of any association between OSA and CVD was IH, then it might reasonably be expected that nocturnal supplemental oxygen might be effective in reducing CVD risk. Gottlieb *et al* randomised 318 patients with moderate to severe OSA to receive healthy lifestyle and sleep education alone or in combination with either CPAP therapy or supplemental oxygen therapy, with indices of nocturnal hypoxia similarly improved in both the CPAP and supplemental oxygen arms of the study (Gottlieb 2014). Despite this, and the higher reported adherence in subjects receiving supplemental oxygen, only the subjects receiving CPAP therapy experienced a (small) reduction in blood pressure after 12 weeks, confirming the results of a previous, smaller study (Norman 2006). The mean ESS at baseline of those receiving CPAP and supplemental oxygen were 8 and 9.6 respectively, suggesting that patients were not particularly symptomatic. The authors do not report

whether any of the interventions led to a symptomatic improvement. Limited sleep studies were performed at baseline and after 12 weeks, so the authors were not able to draw further conclusions regarding the importance or otherwise of intra-thoracic pressure swings or arousal/sleep fragmentation in their study.

The evidence above suggests that the other acute effects of OSA, namely arousals/sleep fragmentation and intra-thoracic pressure swings may also play an important role. As discussed above (Section 7.2.1) there has been recent interest in the possibility of direct shear stress to the aorta caused by intra-thoracic pressure swings and further work is required in this area. Additionally little is known about the effect of intra-thoracic pressure swings on the sympathetic nervous system (see Section 2.13.4).

Although previous studies had linked arousal/sleep fragmentation to increased sympathetic nervous system activity (Somers 1993a, Horner 1995), evidence for a possible link with CVD came largely from studies of sleep deprivation (Irwin 2006, Chaput 2007, Knutson 2007). More recently, evidence largely from observational studies, suggest that sleep fragmentation due to non-respiratory sleep disorders may be associated with hypertension (Walters 2009, Batool-Anwar 2009). In a recent large (n=780) population study of older individuals (mean age 68.7 years) without known coronary artery disease, sleep fragmentation was associated with increased diurnal blood pressure (Chouchou 2013). This association was independent of other confounders, including indices of sleep disordered breathing. Additionally the measured arousal index was associated with an increase in sympathetic nervous system activity as measured by pulse transit time and heart rate variability (Chouchou 2103). Similarly in a large population sample (n=1021) drawn from the Wisconsin Sleep Cohort, the sleep fragmentation index (defined as the number of awakenings and transition to stage I sleep from deeper NREM or REM sleep per hour of sleep) was associated with awake systolic blood pressure in subjects without evidence of sleep disordered breathing (AHI <1), suggesting an independent role for arousals in the aetiology of hypertension (Morrell 2000). There is limited evidence to suggest that the sympathetic nervous system may be implicated in vascular remodelling (Schiffrin 2002), and in a small study of healthy subjects, stimulation of

the sympathetic nervous system led to an acute impairment of endothelial function (Hijmering 2002).

The results of the case-control study presented in this thesis are important and raise a number of issues which are summarised below:

1. In the absence of known CVD or hypertension, subjects with OSAHS are at increased risk of CVD. This adds to the evidence base in this area, but of course this study cannot exclude the possibility of CVD as an epiphenomenon of OSA, for example due to obesity or underlying genetic factors. Subjects and controls were well matched for BMI and waist-to-hip ratio, but neck circumference in patients with OSAHS was higher raising the possibility of increased central obesity (see Section 7.5 for further discussion of this).
2. Subjects with OSAHS have evidence of increased arterial stiffness and impaired endothelial function even in the absence of significant nocturnal hypoxia. On the basis of what is often indirect evidence, the dominant paradigm is that IH is the most important pathophysiological link between OSA and CVD. This study suggests that other mechanism such as intra-thoracic pressure swings and sleep arousal/fragmentation may play an important role and should be given greater consideration.
3. Given the lack of nocturnal hypoxia in these patients and despite being symptomatic (all had EDS), many of them would not have been diagnosed with OSAHS on the basis of oximetry alone. This is important as even this relatively young group, free from known CVD, have evidence of subclinical CVD, as evidenced by increased arterial stiffness and impaired endothelial function compared to well-matched controls. Such patients may benefit symptomatically from CPAP treatment. These findings underline the importance, particularly in young, otherwise healthy patients of being able to measure obstructive events and arousals that are not associated with significant hypoxia.
4. The subjects in the case-control study may not traditionally have been considered to be at significant CV risk given their relatively young age, absence of cardiovascular comorbidity and lack of significant nocturnal hypoxia. However despite this they have been shown to have evidence of

arterial stiffening and endothelial function. This underlines the importance of looking for and addressing other modifiable cardiovascular risk factors in all patients with OSAHS.

7.4 Results of the randomised controlled trial

In the context of the placebo-controlled randomised trial, 12 weeks of CPAP therapy did not lead to a significant improvement in any measure of arterial stiffness or endothelial function in subjects with OSA (in the absence of known CVD or hypertension). A trend towards a lower AIx with CPAP therapy was seen, but this was non-significant. This, despite having shown in the case-control study in subjects with similarly mild nocturnal hypoxia (see Table 4.4) that arterial stiffness was increased and endothelial function was impaired in patients with OSAHS. As has been previously shown in a number of studies (Bazzano 2007, Haentjens 2007, Schein 2014), CPAP therapy did lead to a small reduction in blood pressure. No significant correlation was seen amongst patients who entered the randomised controlled trial between any measure of arterial stiffness or endothelial function and severity of OSA. A small, but statistically significant rise in BMI was noted between the first and third visits. In an attempt to reduce confounding, a cross-over design was employed and patients with known CVD, hypertension and diabetes mellitus (DM) were excluded from the study.

7.4.1 CPAP therapy is not associated with a reduction in arterial stiffness in patients with OSA

Prior to commencing this study, one non-randomised study had reported a reduction in brachial-ankle pulse wave velocity with CPAP therapy (Kitihara 2006). However, a recent review identified 12 subsequent intervention studies investigating the effect of CPAP therapy on arterial stiffness (Phillips 2013b), with further studies published since then (Chung 2011, Litvin 2013, Kohler 2013, Kartali 2014). Only two of these studies reported no effect of CPAP on arterial stiffness (Bakker 2011, Kohler 2013), although in a further three studies, a reduction in arterial stiffness was only seen in patients deemed ‘compliant’ (detail of what constituted acceptable compliance were only stated in one of the studies) with CPAP therapy (Phillips 2013b).

Importantly, there have only been three randomised controlled studies comparing CPAP therapy with control (Drager 2007, Kohler 2008b, Kohler 2013) and only one of these excluded patients with CVD and hypertension (Drager 2007). Drager *et al* randomised 24 patients with severe OSA to receive either CPAP therapy or no treatment for four months. PWV fell in all twelve CPAP treated patients, with a mean reduction of 1.1m/s with no change in PWV seen in the control group. This is a clinically significant reduction, with a recent meta-analysis suggesting that a 1 m/s reduction in PWV leads to a 14% decrease in cardiovascular events (Vlachopoulos 2010). Similarly Kohler *et al* randomised 102 patients with moderate to severe OSAHS to receive either CPAP therapy or sham CPAP (as used in our study) and showed a significant reduction in the AIx from 14.5% to 9.1% (Kohler 2008b). Approximately a quarter of patients in that study had pre-existing treated hypertension and significantly more of those receiving CPAP therapy had known coronary artery disease than those receiving sham CPAP (7.4% vs. 0%; p=0.04). Evidence of EDS was not an inclusion criterion in the first of these studies (Drager 2007), but the mean ESS in subjects receiving CPAP therapy was raised at 14/24. EDS was a requirement for entry into the study by Kohler *et al* (Kohler 2008b). More recently however, the same author found no change in the AIx in patients *without* evidence of EDS randomised to CPAP therapy (Kohler 2013), despite the previous finding by the same group that AIx was higher in subjects with minimally symptomatic OSA (Kohler 2008a). In the above intervention studies Kohler *et al* have shown that CPAP improved arterial stiffness in one group of patients, but not in the other. The two study populations differed in a number of ways, not least in the presence (Kohler 2008b) or absence (Kohler 2013) of EDS and in terms of reported CPAP compliance (4.7 hours determined as CPAP use on the last night of the study vs. 2.8 hours). Those treated with CPAP in the former study (Kohler 2008b) also had a higher baseline AHI (41.9 vs. 9.5), were younger (48.7 vs. 58.4 years) and had a lower baseline AIx (14.5 vs. 27.9 %) than those in the subsequent study (Kohler 2013). Additionally there were almost twice as many subjects receiving treatment for hypertension in the later study than in the former. Whilst the presence or absence of EDS may be important in the efficacy of CPAP in effecting an improvement in arterial stiffness, as it appears to be for improving blood pressure, the higher CPAP

compliance in the former study (Kohler 2008b) may well be important. Additionally the subjects with EDS were younger and healthier and had more severe OSA and thus it could be argued that OSA (and hence its treatment with CPAP) may have played a more significant role in measured arterial stiffness than in the older, more hypertensive subjects without EDS.

Evidence from two reviews (Doonan 2011, Phillips 2013b) and one meta-analysis (Vlachantoni 2012) suggests that in the majority of studies to date, CPAP therapy has a beneficial effect upon arterial stiffness. The fact remains however that until now there had only been one randomised controlled study in patients without underlying CVD or hypertension (Drager 2007). Indeed only one other study definitely excluded patients with CVD and hypertension and was a small non-randomised study (n=27) showing a reduction in arterial stiffness measured by the cardio-ankle vascular index (Kasai 2011). The possible reasons for the discrepancy between the results of the study resented in this thesis and previously published work will be considered below in section 7.4.3.

7.4.2 CPAP therapy is not associated with an improvement in endothelial function in patients with OSA

Before commencing this study there had been one randomised controlled trial (Ip 2004) and several non-randomised studies showing that CPAP had a beneficial effect upon endothelial function. As with arterial stiffness, there has been an explosion of work in this area with a recent review identifying 30 (including the research presented in this thesis) studies reporting on the effect of CPAP therapy on endothelial function (Hoyos 2015). These studies have employed a number of different techniques for assessing endothelial function and the authors of the review sub-divide studies into those examining endothelial function in the micro-vasculature or macro-vasculature. The two are not interchangeable (see Section 2.4), but have both been shown to correlate to coronary artery endothelial function (Anderson 1995, Bonetti 2004) and will be considered together here.

The review identified 21 non-randomised studies, 11 of which definitely excluded patients with CVD and hypertension. The majority of these assessed endothelial function by FMD and the vast majority of studies reported an improvement in

endothelial function following CPAP therapy, with duration of therapy between 1 night and 26 weeks. Two studies however, only reported an improvement in endothelial function in patients with CPAP compliance of ≥4 hours per night (Jelic 2008, Jelic 2010). Most of these studies comprise small numbers of patients with an 'n' of between 6 and 50 patients. Interestingly the largest non-randomised study (n=50) is one of only two studies to find that CPAP therapy was not associated with an improvement in endothelial function. Shiina *et al* did not find any improvement in venous occlusion plethysmography following three months of what is described as 'optimal' CPAP therapy (average compliance not stated) despite demonstrating a reduction in brachial-ankle pulse wave velocity over the same time period (Shiina 2010). Similarly, in a much smaller study of 8 CPAP-compliant subjects without CVD, Jelic *et al* found no improvement in FMD after 4 weeks of CPAP therapy (Jelic 2009).

As would be expected, there have been fewer randomised control studies in this area and because of the cross-over design, the study presented in this thesis reports findings for the largest number of subjects to receive CPAP therapy and is only the second randomised study to definitely exclude subjects with CVD or hypertension. The first by Ip *et al* showed an improvement in FMD in 14 male patients with moderate to severe OSA, randomised to receive CPAP therapy (average compliance 4.3 hours per night) for four weeks (Ip 2004). In a subset of eight patients (AHI not stated) using CPAP for more than three months, FMD measurements were made one week after CPAP withdrawal, at which time endothelial function was impaired and similar to baseline readings. Similarly, in a subset of patients from the MOSAIC study, Kohler *et al* showed an improvement in FMD in 31 patients with minimally symptomatic (and rather mild) OSA randomised to receive CPAP (Kohler 2013). The overall median CPAP compliance in the MOSAIC study was 2.84 hours per night. Compliance in the subjects in whom FMD was measured is not specifically reported, but the authors do note that the effect upon endothelial function was greater in those using CPAP for four or more hours per night (Kohler 2013). Kohler *et al* have previously shown that a two-week period of CPAP withdrawal (using sham CPAP) leads to a deterioration in endothelial function, not seen in those randomised to continue with therapeutic CPAP (Kohler 2011). Three other studies, in relatively

small numbers of patients, have shown improvements in endothelial function with CPAP therapy (Cross 2008, Trzepizur 2009, Nguyen 2010). This has not been a universal finding however, with two studies finding no impact of CPAP therapy on endothelial function (Comodore 2009, Simpson 2013). These are both relatively small studies and in the larger of the two, patients with moderate to severe OSA were randomised to receive either twelve weeks of CPAP therapy or sham CPAP. Mean CPAP compliance was 3.5 hours per night and endothelial function was assessed by peripheral artery tonometry (Simpson 2013)

Overall, the majority of previous studies suggest an improvement in endothelial function with CPAP therapy; however most of these have not been randomised and contain relatively small numbers of patients. This current study is the largest randomised placebo-controlled trial of the effect of CPAP on endothelial function and additionally patients were free of CVD and hypertension which are known confounders. Potential reasons for the discrepancy between these results and previously published work are discussed below in section 7.4.3

7.4.3 Differences between the results of this study and the published literature

The findings of this study could be considered to be at odds with the majority of published literature in this area, in that CPAP therapy did not lead to an improvement in arterial stiffness or endothelial function in this group of patients with OSA. There are a number of possible explanations for this:

1. Patients in this study had milder OSA, particularly in terms of nocturnal hypoxia, than often appears in publications in this area. As such, it could be argued that if IH is the main driver of cardiovascular disease in OSA, then subjects without significant hypoxia would have less to gain in terms of cardiovascular benefit from CPAP therapy. However the results of the case-control study showing increased arterial stiffness and impaired endothelial function in subjects with similarly low levels of nocturnal hypoxia suggest that IH is not the only important mechanism linking OSA and CVD. Work by Marin *et al* examining the mortality of untreated patients with OSAHS found no increase in cardiovascular morbidity and mortality in those with

mild to moderate disease compared to healthy controls (Marin 2005). Whilst the mean AHI placed patients in the moderate to severe category in this study, they were not particularly hypoxic, perhaps due to their relatively young age and the exclusion of patients with respiratory failure and CVD. There was no intention to specifically recruit patients without significant hypoxia, but after excluding patients with CVD, hypertension and diabetes mellitus (DM), those patients who remained eligible had, on the whole, milder disease in term of hypoxia. Given the clear association between CVD and OSA this is an unsurprising consequence of excluding those with CVD or hypertension.

BMI is known to be a predictor of oxygen desaturation in OSA (Peppard 2009), but little is known about the differences between patients with OSA who desaturate and those who do not. A recent study compared patients with severe OSA (defined as AHI >30) with and without nocturnal hypoxia (lack of hypoxia was arbitrarily defined as minimum oxygen saturation >88% and >80% of sleep time with oxygen saturations of >96%) (Palma 2014). They found subjects without hypoxia to have a lower BMI and waist circumference than those who did, consistent with the findings of Peppard *et al*. Apnoea duration was shorter in those without hypoxia and heart rate variability analysis suggested a lower sympathetic tone in those without hypoxia, compared to patients with hypoxia (Palma 2014). There are likely to be pathophysiological differences between patients with and without nocturnal hypoxia and further work in this area is required.

2. In an attempt to reduce confounding, subjects with CVD, hypertension and DM were excluded and a crossover design was employed. Confounding has been a significant issue in previous studies examining the effect of CPAP on arterial stiffness with only two studies definitely excluding patients with overt CVD and hypertension (Drager 2007, Kasai 2011) and no previous studies using a cross over design (Ryan 2013). Only one other randomised controlled study of the effect of CPAP on endothelial function excluded patients with CVD and hypertension (Ip 2004). Arterial stiffness and endothelial dysfunction are early or sub-clinical markers of CVD (Celermajer 1992, Reddy 1994, Laurent 2006, Lane 2006) and as such, the inclusion of subjects with known CVD is difficult to adequately control for.

3. Compliance with CPAP therapy was relatively low at three hours per night in this study and this has to be an important consideration. CPAP (and sham CPAP) compliance was very accurately measured with time clock data downloaded at the end of each limb giving figures for the amount of time spent 'at pressure' (i.e. not just with the CPAP machine switched on) every night giving an average nightly usage over each 12 week period. This CPAP usage was associated with a reduction in ESS overall [7(4-11) vs. 10(5-13); $p<0.001$] and so clearly had a symptomatic benefit but may have been insufficient to lead to a change in arterial stiffness and endothelial function. A figure of four hours per night on 70% of nights is often quoted as representing adequate compliance (Sawyer 2011), but it is clear that a significant proportion of subjects in research studies are non-compliant by this standard (Weaver 2008) and compliance out-with research studies is likely to be lower. Thus, the very accurately measured compliance reported here is likely to represent 'real-life' compliance and is similar to compliance in previous studies in which CPAP therapy led to a reduction in blood pressure (Faccenda 2001) and improvements in cognitive function at this centre (Engleman 1994). Mortality in a large series of patients with OSA was reduced in subjects who used CPAP for between one and six hours per night (mean use 3.9 hours) compared to those with lower usage (Campos-Rodriguez 2005). Several previous non-randomised studies have however shown that arterial stiffness and endothelial function did not change in subjects deemed to have insufficient compliance with CPAP (Jelic 2008, Jelic 2010, Phillips 2013b). The two randomised controlled studies showing a beneficial impact of CPAP therapy on arterial stiffness report average CPAP compliance as 6.6 and 4.7 hours per night respectively (Drager 2007, Kohler 2008b). The third randomised controlled study reported lower compliance at 2.8 hours per night and showed no effect of CPAP therapy on arterial stiffness, but did show an improvement in endothelial function at that level of compliance (Kohler 2013). In the other randomised studies showing an improvement in endothelial function with CPAP therapy, all reported CPAP compliance of greater than four hours per night. The two randomised studies reporting no effect of CPAP on endothelial function reported CPAP compliance of 3.5 and 5.5 hours per night. In our study, sub-group analysis of

those with nightly CPAP compliance of ≥4 hours did not show any improvement in either arterial stiffness or endothelial function, but obviously this was in a smaller number of patients and the study was not powered for this.
Overall compliance was lower than was ideal and whilst this may well represent real life CPAP usage in patients with this degree of OSA, may limit the generalizability of these findings amongst patients with greater compliance. Equally, it could be argued that studies reporting on any benefit derived from especially good CPAP compliance are of limited generalizability to the average patient with OSA who may never achieve such high levels of CPAP compliance (Simpson 2013).

4. In the only previous randomised control trial examining the effect of CPAP therapy on arterial stiffness to exclude subjects with CVD or hypertension (Drager 2007), baseline PWV was much higher than in this study, suggesting that patients were certainly at higher risk of CVD. Indeed recently published 'normal' values suggest that the mean PWV measured in our study falls within the normal range based on the mean age and blood pressure of the group (Boutouyrie 2010), which could have resulted in a ceiling effect. Also it is important to note that the results of the study by Drager *et al* are based upon only 12 patients who were randomised to receive CPAP therapy.

7.4.4 Is EDS important in the development of CVD in OSA?

Despite finding that subjects with OSAHS had increased arterial stiffness and impaired endothelial function compared to control subjects, there was no improvement in either of these measures following CPAP therapy in patients with OSA. A non-significant trend towards a lower AIx with CPAP was however seen in subjects with EDS [16.5 (11.4)% vs. 18.2 (11.2)%; p=0.058]. The only difference between the subjects with OSAHS in the case-control study and the patients with OSA in the randomised controlled study (see Table 5.1) was that the presence of EDS was mandatory for the former and not for the latter. Those in the case-control study all reported EDS and had a median ESS of 16 and whilst the median ESS in the randomised controlled study was 13, nineteen of the subjects had an ESS of <11.

Although a number of studies have demonstrated a relationship between the severity of EDS and severity of OSA (Gottlieb 1999, Koutsourelakis 2008, Mediano 2007), there is wide inter-individual variation in the susceptibility to EDS (Young 2002a) and the reasons for this are not clear. In population based studies however, most subjects with an elevated AHI will not report EDS (Kapur 2005). These findings may in part reflect the subjective nature of the ESS, commonly used to define EDS and the often multifactorial nature of EDS. In a large study of patients with OSA (defined as an RDI >5) and controls, Koutsourelakis *et al* reported that although the RDI accounted for 17% of the variability in the ESS, a history of depression and diabetes accounted for a further 11% and 7% respectively of the variability in ESS (Koutsourelakis 2008).

The initial observational studies showing increased cardiovascular risk in patients with OSA not treated with CPAP seem to consist predominantly (but certainly not exclusively) of patients with EDS (Marti 2002, Marin 2005, Doherty 2005), although only one reports ESS results (Doherty 2005). In a more recent randomised controlled trial examining the effect of CPAP in patients without EDS, CPAP treatment did not reduce the incidence of hypertension or cardiovascular events after a median of four years of follow up (Barbe 2012). The authors do however report that this study may have been underpowered and in *post hoc* analysis of patients using CPAP for ≥4 hours per night, the risk of hypertension or a cardiovascular event was lower ($p=0.04$). Recently published work suggests that calculated cardiovascular risk in OSA patients without EDS is not reduced by six months of CPAP therapy (Craig 2012). As will be outlined in section 7.4.5 below, there are a number of studies showing that CPAP is effective in reducing blood pressure in OSA, however several studies and a recent meta-analysis show no effect overall in patients without EDS. In another meta-analysis, Montesi *et al* have shown that the expected blood pressure reduction with CPAP therapy increases with every five point increase in the baseline ESS (Montesi 2012), suggesting that EDS is important.

The question as to whether the presence of EDS matters is important, not least because 20% of the population have evidence of OSA in the absence of EDS, compared to a prevalence of 2-4% for OSAHS. Kohler *et al* have previously shown increased arterial stiffness and impaired endothelial function (using the same

measures as in this study) in subjects without EDS, although a proportion of patients also had CVD (Kohler 2008a). As in our study, the inclusion criteria for patients entering randomised controlled trials examining the effect of CPAP on arterial stiffness and endothelial function are often based solely on the AHI. With regard to arterial stiffness, there has been one randomised controlled trial recruiting only patients with EDS (Kohler 2008b) and one specifically recruiting subjects without EDS (Kohler 2013); the former reported an improvement in arterial stiffness with CPAP therapy whereas the latter did not. In the eight previously published randomised controlled studies of the effect of CPAP on endothelial function, the presence of EDS was mandated in three (Cross 2008, Nguyen 2010, Kohler 2011) and the absence of EDS mandated in another (Kohler 2013). In the two studies reporting no improvement in endothelial function, EDS was not an inclusion criterion (Comondore 2009, Simpson 2013).

An underlying mechanism for any association between EDS and CVD in OSA has not been elucidated and certainly is beyond the scope of this study. EDS is generally felt to be a marker for arousals and subsequent sleep fragmentation in OSA (Young 2002a, Robinson 2006) and has been shown in a small study to be associated with increased sympathetic nervous system activity (SNA) (Donadio 2007). EDS is not exclusive to OSA and has been reported in a number of cardiovascular diseases (Choi 2006), although it is possible that this relates to the known prevalence of undiagnosed OSA in the general population. In an older population, EDS has also been shown to be an independent predictor of cardiovascular morbidity and mortality, but again undiagnosed OSA was not excluded in these subjects (Newman 2000). In a study of 86 patients with severe OSA, daytime sleepiness (as measured by the ESS) was related to daytime measures of cardiac function (namely the stroke index and cardiac index) after controlling for the RDI and degree of nocturnal hypoxia (Choi 2006). The authors suggest that the presence of EDS may not just be a marker of OSA severity but may reflect underlying cardiovascular dysfunction.

In our study, sub-group analysis of patients with and without EDS did not show CPAP to have an effect on arterial stiffness or endothelial function in either group, although a non-significant trend towards a reduction in AIx with CPAP in those with EDS was seen. These findings may of course be due to the smaller numbers of

patients with EDS, as the study was not powered for this analysis. This study therefore does not help to answer the question as to the importance of EDS when considering the association between OSA and CVD and further work is required in this area.

7.4.5 CPAP therapy is associated with a reduction in blood pressure in patients with OSA

CPAP therapy led to a small reduction in office systolic blood pressure [126 (12) vs. 129 (14) mmHg; p=0.03]. There was no difference in ambulatory blood pressures between the two limbs of the study and the reason for this is unclear but may reflect the lack of a complete ambulatory blood pressure data set. Complete ambulatory blood pressure measurements were available for only 33 of the 43 subjects who completed the study (see section 4.6.5), increasing the likelihood of type II statistical error. The effect of CPAP therapy on blood pressure *per se* was not the main aim of this thesis, and the results will only be discussed briefly here. OSA is an independent risk factor for hypertension (Stradling 2001, McNicholas 2007) and CPAP has been shown in numerous studies to reduce blood pressure in patients with OSA (Faccenda 2001, Pepperell 2002). In a meta-analysis, the overall reduction in blood pressure with CPAP therapy is around 2-3mmHg (Bazzano 2007, Haentjens 2007, Schein 2014), with greater reductions in patients with more severe OSA, higher BMI and hypertension at baseline (Bazzano 2007). In this study only a small reduction in systolic blood pressure was seen and this may reflect the fact that, as discussed above (section 7.4.3), patients had relatively mild OSA and all were normotensive. However, even at this level of systolic blood pressure reduction, the findings of a large meta-analysis suggest a 10% reduction in stroke mortality and a 7% reduction in other vascular disease could be expected (Lewington 2002). An area of uncertainty in this area has been whether CPAP therapy has a blood pressure lowering effect in patients without EDS. Several randomised controlled trials have now shown no beneficial effect of CPAP therapy on blood pressure in patients without EDS (Barbe 2001, Robinson 2006, Barbe 2012**,** Craig 2012). A recently published meta-analysis of over 1000 patients without EDS reported the same conclusion, with the caveat that exploratory analyses suggested there may be a small reduction in diastolic blood

pressure in patients with CPAP compliance of ≥4 hours per night (Bratton 2014). Patients in our study had a mean ESS of 13; however 44% of subjects had an ESS ≤10 and hence did not have EDS. Sub-group analysis of patients with and without EDS demonstrated no effect of CPAP therapy on any measure of blood pressure (office or ambulatory) after 12 weeks. This may of course be due to the smaller numbers in each group and the study was not powered for this.

7.4.6 Increase in BMI over the study period

Interestingly, there was a small, but significant increase in BMI between the first and third visits in this study [29.9 (27.3-31.6) kg/m2 vs. 30.1 (27.4-33.3) kg/m2; $p<0.001$]. Theoretically one might imagine that if CPAP treatment led to a reduction in ESS (as in this study), then patients would have more energy and hence if anything should lose weight. Initial small non-randomised studies suggested a reduction in weight (Loube 1997) and visceral fat (Chin 1999) following CPAP therapy. However as part of the recent APPLE (Apnea Positive Pressure Long-term Efficacy) study, 812 patients randomised to receive CPAP therapy or sham CPAP had body weight measurements at baseline and after 6 months (Quan 2013). This also showed that those in the CPAP arm of the study had a small, but statistically significant increase in weight, with a small reduction in weight seen in the sham arm of the study. Additionally, weight gain was greater in those with better CPAP compliance. A very recent meta-analysis attempts to address the issue of the effect of CPAP upon body weight and identified 25 randomised controlled studies involving CPAP therapy that reported BMI results before and after intervention (Drager 2014). In keeping with the results presented here, this confirmed a small but significant increase in BMI following a minimum of four weeks CPAP therapy.

Of course, weight gain is not always due to an increase in adiposity and may reflect an increase in muscle mass associated with increased activity. In the study presented in this thesis and in the APPLE study there is no quantification of body composition. However a previous study, employing imaging techniques to determine body composition, showed that eight months of CPAP was associated with an increase in lean body mass (Münzer 2010). There are a number of possible mechanisms for this weight gain, further elucidation of which are certainly beyond the scope of this study.

The potential mechanisms may include reductions in the work of breathing and sympathetic nervous system activity at night with effective CPAP therapy, leading to a reduction in energy expenditure and subsequent weight gain (Quan 2013, Drager 2014). Additionally, inflammatory cytokines due to untreated OSA may promote anorexia which is reversed by CPAP (Quan 2013). Under normal conditions during slow wave sleep, growth hormone is released and cortisol release is inhibited (Van Cauter 2008). When sleep is fragmented by OSA the relative amounts of growth hormone and cortisol release may be affected, potentially leading to a catabolic state, which if reversed by CPAP may lead to weight gain. Additionally the role of CPAP in reversing the effects of OSA on leptin is unclear (Drager 2014). From a practical point of view, the increase in weight after commencing CPAP therapy should prompt physicians treating patients with OSA to emphasise the need for active attempts at weight reduction.

7.5 Limitations of the study

This study has a number of limitations, two of which have already been discussed above, namely the relatively mild degree of nocturnal hypoxia in patients with OSA and compliance with CPAP therapy (see section 7.4.3).

Subjects with OSAHS and controls were well-matched in all respects except for neck circumference, which given the known association between neck circumference and OSA (Davies 1990, Davies 1992) is perhaps unsurprising. There have been a number of recent studies suggesting an association between neck circumference and increased cardiovascular risk (Preis 2010, Zhou 2013). It is unclear whether this is an independent association or relates to the relationships of neck circumference to central obesity (Onat 2009) and OSA (Davies 1990, Davies 1992). Body mass index (BMI) measurements do not provide information about the distribution of fat deposition in the body. Thus, it could be argued that a higher neck circumference in the OSAHS patients is indicative of increased central or upper body obesity, known to be more associated with CVD than other patterns of fat distribution (Jensen 2008). Against this would be the fact that another measure of central obesity, the waist-to hip ratio, was not significantly different between the two groups in our study. Although not statistically significant, a greater number of subjects with OSAHS were

current smokers than in the control group. Proportions of ex-smokers were similar within each group. Smoking has long been established as a risk factor for CVD and has been shown to be associated with increased arterial stiffness (Mahmud 2003, Li 2006) and impaired endothelial function (Celermajer 1993). Acute changes in arterial stiffness (Mahmud 2003) and endothelial function (Lekakis 1997, Karatzi 2007) are also reported after cigarette smoking. To avoid confounding from the acute effects of cigarette smoking, subjects were asked not to smoke for at least 10 hours prior to attending for vascular assessment. Across the group as a whole (20 subjects with OSAHS and 20 control subjects) no difference between any measure of arterial stiffness or endothelial function was noted between smokers, ex-smokers and never smokers. Subjects were not however matched for smoking history and thus cigarette smoking remains a potential confounder in the case-control study.

In contrast to the arterial stiffness and endothelial function data obtained using pulse wave analysis (AIx data), it was not possible to obtain full data sets for PWV and aortic distensibility. With regards to PWV this was related to body habitus and the consequent difficulties in obtaining a reliable trace at the femoral or carotid pulse site in two of the 20 subjects with OSAHS. The difficulties of obtaining adequate traces at these sites have been recognised by others (Bakker 2011). Due to movement or other artefact, it was not always possible to determine aortic distensibility from the MRI scans and this, along with one case of unexpected claustrophobia, meant a direct comparison was only available for 15 of the 20 pairs. A consequence of missing data is the increased likelihood of a type II statistical error**.** As described in section 3.2.2, the case-control study was not formally powered and as such may have been underpowered, raising the possibility of type I statistical error.

A potential criticism of our study is the methodology used to assess endothelial function. Vasomotor endothelial function was assessed using the *relatively* novel technique of measuring the effects of salbutamol and GTN on the AIx. As discussed above in section 7.2.2, this has been shown to be a reproducible technique that correlates with invasively obtained measurements of endothelial function (Wilkinson 2002B, Hayward 2002). Additionally impairment of the AIx response to inhaled salbutamol has been described in numerous conditions associated with endothelial dysfunction (Lekakis 2011). There have been concerns regarding the use of this

technique to assess endothelial function in this situation (Ryan 2013, Hoyos 2015). These are based upon two studies published around the time that this study was being conducted (Donald 2006, Westhoff 2007). The first reported that the reproducibility of this technique was lower than that of FMD (coefficient of variation 11.5 vs 7.1 %) in 16 adults who were assessed on consecutive days and had AIx measurements before and after sub-lingual GTN and inhaled salbutamol (Donald 2007). A potential criticism of that study is the timing of the administration of salbutamol only 23 minutes after the administration of GTN. It has previously been shown that the effects of GTN on the AIx persist for 25 minutes (Greig 2005), hence the 30 minute interval used in our study. The second study examines the effect of inhaled salbutamol on the *reflection index* (not augmentation index) measured by a photoplethysmographic device attached to the index finger in a subset of subjects and the authors concluded that this automated pulse wave analysis procedure cannot be used to assess endothelial function (Westhoff 2007). Although the technique referred to utilises the same principle as used in this study, it cannot be viewed as the same technique. Indeed, Donald *et al* also measured the change in reflection index following salbutamol in their study and found it to be much less reproducible than either FMD or changes in AIx following salbutamol (Donald 2007). FMD has been the most widely studied means of assessing endothelial function to date and at present represents the gold standard measurement. However it is an expensive technique, requiring a great deal of operator expertise. The use of arterial tonometry to determine the effects of salbutamol on AIx, whist undoubtedly requiring a degree of operator skill, is suitable in even the most obese subjects and is well tolerated by subjects. This makes it a useful technique in cross-over studies such as this one, in which subjects undergo numerous assessments and is recognised as a valid method of assessing endothelial function in the most recent position paper from the European Society of Cardiology Working Group on Peripheral Circulation (Lekakis 2011). Notwithstanding the above however, the use of this means of assessing endothelial function has not been as widespread as might have been predicted when this study was designed and conducted. Indeed the above noted position paper (Lekakais 2011) comments on its relative novelty as a technique. Kohler *et al* used the same method (alongside the more commonly used FMD technique) of assessing endothelial

function in patients with minimally symptomatic OSA patients, published whilst this study was underway (Kohler 2008a). However no subsequently published study in the field of OSA has employed this technique, with the majority favouring FMD for the assessment of endothelial function (Hoyos 2015). Therefore despite the advantages described above, I could not recommend its use as the sole means of assessing endothelial function in future studies in this area and given the prominence of the use of FMD, this would seem a logical alternative option.

The power calculation for this study was based upon pilot data comparing aortic distensibility in patients with OSA and healthy controls. On this basis and for a crossover design it was calculated that a sample size of 40 was required (see Chapter 3, section 3.14) and indeed 43 patients completed the study. This may however mean that the study is underpowered with regard to the other measures of arterial stiffness and endothelial function and along with the incomplete data set may have increased the likelihood of type II statistical error.

As discussed in Chapter 3, there has been much debate around the ideal placebo for trials involving CPAP (Karlawish 2001, Brown 2011). For the reasons outlined in section 3.7.2, sham CPAP (i.e. CPAP set to deliver sub-therapeutic pressure) was chosen as the placebo. Sham CPAP replicates some of the experience surrounding the provision of a CPAP machine and the wearing of a CPAP mask at night but has previously been shown to have little (Rodway 2010) or no (Jenkinson 1999, Farre 1999) effect on respiratory events during sleep. In CPAP naïve patients, such as those in our study, the use of sham CPAP should assist with the blinding of patients and researchers, along with reducing the impact of any placebo effect associated with the use of therapeutic CPAP. Several studies have shown that CPAP use does not differ from sham CPAP use (Brown 2011) and this approach has been used successfully in previous trials at this centre (Smith 2007, Cross 2008). In this study however, CPAP compliance was significantly higher than compliance with sham CPAP, which could suggest that subjects were not completely blinded. If this were the case, then perhaps one might imagine that sham CPAP use would be lower in those patients who entered the CPAP limb first and thus had experience of the benefits of therapeutic CPAP. This proved not to be the case however, with sham CPAP use unaffected by the order in which patients received CPAP and sham CPAP.

The ESS fell more with CPAP than sham CPAP and a lack of perceived symptomatic benefit may have led to lower sham CPAP use. Whilst acknowledging that there is no perfect placebo for CPAP, sham CPAP is probably the most rigorous placebo and is currently felt to represent the placebo of choice (Rodway 2010, Brown 2011). Although the study did not include a formal washout period, the vascular assessments were made 12 weeks after crossover making the persistence of carry over effects unlikely. Indeed, recent work by Kohler *et al* demonstrated a rapid recurrence of OSA (within two days) of switching from therapeutic to sham CPAP. Two weeks after switching from CPAP to sham CPAP they also showed an increase in blood pressure and an impairment of endothelial function compared to subjects who continued on therapeutic CPAP (Kohler 2011).

It could be argued that in order to effect a change in vascular function patients would need to be treated with CPAP therapy for longer than 12 weeks. Whilst this may be the case, many of the previous studies showing improvements in blood pressure (Faccenda 2001, Pepperell 2002) and vascular function (Ip 2004, Cross 2008, Kohler 2008b) with CPAP therapy were conducted over a four to 12 week period. Similarly, anti-hypertensive therapy has previously been shown to have a beneficial effect on aortic distensibility after 12 weeks of treatment (Honda 1999) and it was on this basis that the 12-week duration was chosen. Indeed, there have only been two randomised controlled trials of the effect of CPAP therapy on arterial stiffness or endothelial function that have been conducted over longer time periods with treatment durations of four (Drager 2007) and six months (Kohler 2013). Notwithstanding the above, OSA is a chronic disease and for that reason a longer treatment duration, particularly in relatively young patients without evidence of CVD, may be desirable. In a placebo controlled study such as this one this would however also prolong the period that patients received the placebo intervention (in this case sham CPAP). This presents an important ethical challenge particularly given the known efficacy of CPAP in the treatment of OSA (McDaid 2009) and the longer term use of sham CPAP may be difficult to justify. Additionally with 12 weeks in each arm of the study, subjects are already being asked to maintain their interest and willingness to participate for six months. It is not clear whether extending the study duration would lead to an increase in the drop-out rate.

7.6 Conclusions

This study extends previous observations by showing that even relatively young, normotensive patients with OSAHS, free of overt CVD have evidence of increased arterial stiffness and impaired endothelial function. OSAHS is common and prevalence rates are likely to increase with increasing obesity rates and as such, any association between OSAHS and CVD has significant public health implications. Early detection of those at increased cardiovascular risk may enable timely therapeutic intervention and risk reduction.

Importantly in this study, patients had evidence of increased arterial stiffness and impaired endothelial function, despite a lack of significant nocturnal hypoxia. Intermittent hypoxia (IH) has generally been considered to represent the most important mechanism underlying any link between OSA and CVD; however these findings would suggest that other mechanisms may also play an important role. Less is known about the other acute effects of OSA, namely arousals/sleep fragmentation and intra-thoracic pressure changes with regard to an association between OSA and CVD, and further work is needed in this area. A better understanding of the underlying pathophysiological mechanisms may help to identify patients at risk of CVD and potentially lead to new treatment strategies for OSA.

Despite the finding that patients with OSAHS had increased arterial stiffness and impaired endothelial function in the case-control study, CPAP therapy did not lead to a significant improvement in either of these measures in a larger group of patients with OSA in the randomised controlled trial. There are a number of possible reasons for this. Firstly, in subjects with relatively mild OSA and in the absence of underlying CVD, CPAP therapy may be ineffective in improving vascular structure and function. Patients in this study were assessed after 12 weeks and it is possible, particularly in those with milder disease, that any improvement would require a longer period of treatment.

It is possible that the lack of effect seen relates to the relatively low CPAP compliance in this study. As described above, a number of studies have only shown improvements in vascular structure and function in subjects with (variably defined) good compliance with CPAP therapy. CPAP compliance in this study was very accurately measured and at three hours per night was sufficient to lower the ESS and

effect a small reduction in diurnal systolic blood pressure. In patients such as those studied here, this probably reflects 'real life' CPAP compliance.

This study again raises the question about the importance of EDS in the link between OSA and CVD. Patients in the case-control all had EDS as compared to only a proportion of those in the randomised controlled trial. This may provide an explanation for the lack of improvement in vascular function in patients with OSA, despite finding that patients with OSAHS had increased arterial stiffness and impaired endothelial function at baseline. Although CPAP has been shown in many studies to reduce blood pressure in OSA, this does not seem to occur in patients without EDS and the reasons for this are as yet unclear.

In considering the findings of this study from a clinical perspective, it is important to remember that the cohort of patients studied here (in both the case-control study and the RCT) represent a very specific sub-population of patients with OSA overall. OSA is known to be an independent risk factor for hypertension, co-existing in up to 40% of patients (Phillips B 2005, Parati 2013) and although not established as an independent risk factor for CVD, there is a clear association between OSA and CVD. In an attempt to reduce confounding in this study, patients with known CVD, hypertension or DM were deliberately excluded. As such, the finding that CPAP did not improve arterial stiffness or endothelial function in this sub-population does not exclude a beneficial effect of CPAP therapy upon vascular function in patients with pre-existing CVD or indeed in those with more significant nocturnal hypoxia. Subjects in this study did not have evidence of significant nocturnal hypoxia, indeed the 4% desaturation rate was low at 9.3 (12.3) per hour and the percentage of sleep time with oxygen saturations of less than 90% was also low at 4.4 (13.2)%.

The findings of this study, coupled with the finding from the case-control study that patients with OSAHS have evidence of increased arterial stiffness and impaired endothelial function, clearly emphasises the need to carefully consider and address other modifiable cardiovascular risk factors in all patients with OSA. This should be in addition to any consideration of CPAP provision.

CPAP is clearly an effective treatment for the symptoms of OSA, but on the basis of previous evidence and the work contained within this thesis, the case has not yet been made for the use of CPAP therapy in OSA solely to reduce cardiovascular risk.

In order to answer the question of whether CPAP treatment is effective in improving vascular structure and function and subsequent cardiovascular morbidity and mortality, large prospective randomised studies are ideally required with long term follow up. Given the already proven benefits of CPAP in terms of symptom control however, this would be ethically challenging and this approach may only be possible in subjects without significant symptoms.

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Appendix I

EPWORTH SLEEPINESS SCALE

0	=	would **never** doze
1	=	**slight** chance of dozing
2	=	**moderate** chance of dozing
3	=	**high** chance of dozing

Situation	Chance of Dozing
Sitting and reading	.....
Watching TV	.....
Sitting, inactive in a public place (eg a theatre 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 traffic	.....
TOTAL	==========

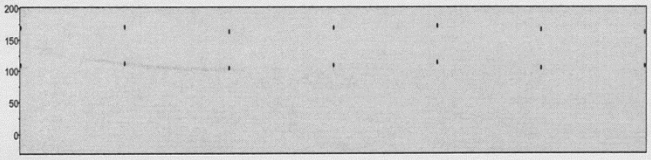
SphygmoCor®
Pulse Wave Velocity Report

PATIENT DATA

Patient Name
Patient ID
Patient Code
Age, Sex

STUDY DATA

09 Dec 2008, 09:36:13 Operator ID: **anne jones**
Algorithm **Intersecting tangent** Distance: **425 mm** Blood Pressure: **117/74 (-)**
Medication
Notes control



QUALITY CONTROL

	aPH	PHv	PLv	BLv
Site A				
Pressure	**168**	**3**	**2**	**10**
ECG	**418**	**4**	**1**	**3**
Site B				
Pressure	**124**	**6**	**2**	**16**
ECG	**406**	**4**	**2**	**3**

PULSE WAVE VELOCITY CALCULATION

ECG-FEM	166.7	3.6	7	51
ECG-CAR	109.1	3.3	7	48
FEM-CAR	57.6	4.9		

Pulse Wave Velocity = 7.4 ± 0.6 (m/s)

AtCor Medical SCOR-2000 7.0 (00445) 120 PWCPAP 17 Dec 2008

Example of a PWA Report

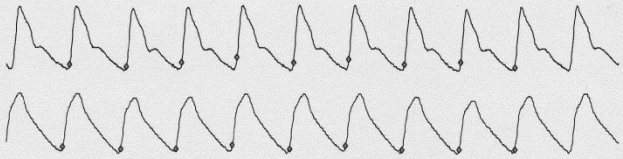
SphygmoCor®
Evaluation Report

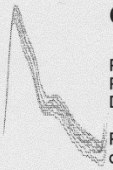
PATIENT DATA

Patient Name
Patient ID
Patient Code
Age, Sex

STUDY DATA

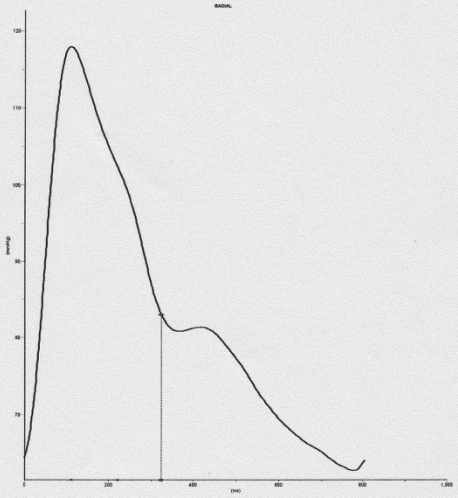
19 Nov 2008, 10:07:44 Operator ID: **annejones**
Medication:
Notes: t0



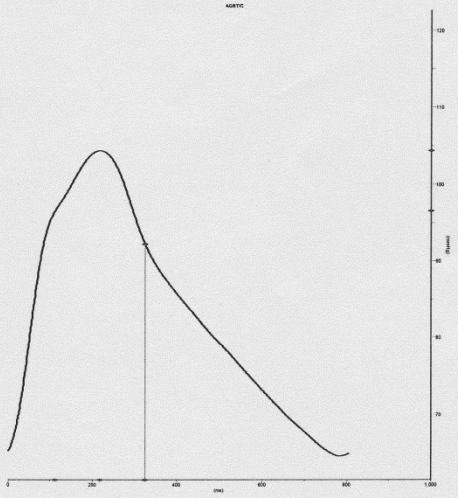


QUALITY CONTROL

Pulse Height	56
Pulse Height Variation	2%
Diastolic Variation	3%
Pulse Length Variation	1%
dP/dt Max	775



Sp	Sp
118	104
Dp	Dp
64	65
PP	PP
54	39
MP	MP
83	83



Peripheral T1, T2, AIx 110ms, 221ms, 71%

Aortic AIx (AG/PP) @HR75 20%

CENTRAL HAEMODYNAMIC PARAMETERS

Heart Rate, Period	**75 bpm, 805 ms**	P1 Height(P1 - Dp)	**31 mmHg**	Buckberg SEVR (Ad/As)	**120% (2721/2273)**
Ejection Duration (ED)	**324 ms, 40%**	Augmentation (AG)	**8 mmHg**	MP. (Systole, Diastole)	**94, 76 mmHg**
Aortic T1, T2, Tr	**110, 218, 144 ms**	Aug. Index (AG/PP, P2/P1)	**20%, 125%**	End Systolic Pressure	**92 mmHg**

AtCor Medical SCOR-2000 7.0 (00445) 120 PWCPAP 09 Dec 2008

Effect of Continuous Positive Airway Pressure (CPAP) on Aortic Distensibility in Patients with Obstructive Sleep Apnoea/Hypopnoea Syndrome (OSAHS)

INFORMATION SHEET FOR POTENTIAL SUBJECTS

You are invited to participate in a study being conducted within the Department of Sleep Medicine at the Royal Infirmary of Edinburgh. Before you decide whether to take part in the study it is important that you understand the objectives of this research and what it will involve. Please take time to read the information below carefully and discuss it with friends, relatives or your GP if you wish. Please do not hesitate to ask us if there is anything you do not understand or if you would like any further information.

It is entirely up to you as to whether you wish to participate in this study. If you decide to do so you will be given a copy of this information sheet to keep and will be asked to sign a written consent form. If you decide not to participate or if you wish to withdraw at any time during the study you are entirely free to do so and this will not in any way affect the standard of care that you receive.

The aim of this study is to look at the effect that treatment of Obstructive Sleep Apnoea/Hypopnoea Syndrome (OSAHS) with Continuous Positive Airway Pressure (CPAP) has on the stiffness of the body's main blood vessel, the aorta.

OSAHS can cause high blood pressure and this is associated with an increase in cardiovascular risk (i.e. heart attacks and strokes). The degree of stiffness of the aorta may be important in this increased risk. We aim to look at whether this degree of stiffness is altered by treatment with CPAP. It is not known exactly what leads to this aortic stiffening in OSAHS, it may be related to dips in the oxygen levels overnight due to breathing pauses (apnoeas), or it may be related to factors associated with daytime sleepiness. By looking at different groups of patients within the Department of Sleep this may become clearer.

In the study you will receive two different types of CPAP treatment. CPAP machines can be set to provide air at different pressures. You will receive one such pressure for three months, and a different pressure for the second three month period. This will better enable us to detect any changes attributable to CPAP treatment. You will be randomised to determine which of these two forms of CPAP you receive first. During the study neither you nor the principal researcher will know in which order you receive the two forms of treatment. Prior to being randomised you will have an overnight sleep study to ascertain your suitability for inclusion.

We would also check some routine blood tests, including your cholesterol and glucose level, along with a 24-hour blood pressure monitoring (this is applied in the hospital but then can be worn at home or at work whilst going about your normal daily activities).

We will look at the stiffness of the aorta using Magnetic Resonance Imaging (MRI). This is a non-invasive imaging technique that does not involve ionising radiation. Some people are not able to have such a scan if they have certain implants or foreign bodies inside them. You will be asked specifically about this prior to entry into this study, but if you think you might fall into this category then please let us know.

We will also perform what is known as pulse wave analysis at the same visit. This again is a non-invasive technique and looks at blood flow at the wrist, neck and groin. In order to look at the response of the blood vessels, measurements will be repeated following administration of Glyceryl Trinitrate (better known as GTN, a commonly used medicine for angina) by a spray under the tongue, and Salbutamol, (a commonly used medicine for asthma) taken via an inhaler.

These measurements (MRI and pulse wave analysis) will be taken before treatment is commenced, and then repeated after each three month period of treatment (i.e. three times in total)

Any information collected about you during the course of the study will be kept strictly confidential. Any information that leaves the hospital will have all identifying details removed such that you could not be recognised from it. We will inform your General Practitioner of your participation in this study. If during the course of your participation in the study we find any unexpected results, for example on the blood tests or MRI scan, we will of course discuss these with you fully and inform your GP. It may be something simple that can be dealt with by your GP, if not we will refer you on to the relevant hospital department.

Any travel expenses incurred by you during your participation in the study will be reimbursed.

Though it is not expected, if taking part in this research harms you, there are no special compensation arrangements. If you are harmed due to negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way in which you have been approached or treated during the course of this study then the normal National Health Service complaints mechanism is available to you.
A group called Consumers for Ethics in Research (CERES) publish a leaflet entitled ‘Medical Research and You’. This leaflet gives information about medical research and addresses some of the questions you may have. A copy can be obtained from their website, www.ceres.org.uk or by writing to CERES, PO Box 1365, London, N16 0BW.

This research is being undertaken by the following investigators:

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Professor Neil Douglas
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Dr Graham McKillop
Sister Marjorie Vennelle

Thank you for taking the time to read this information sheet, please do not hesitate to contact us if you require further information.

Appendix II

Transcripts of publications arising from this thesis

Sleep Medicine 14 (2013) 428–432

Contents lists available at SciVerse ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Arterial stiffness and endothelial function in obstructive sleep apnoea/hypopnoea syndrome

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ARTICLE INFO

Article history:
Received 21 July 2012
Received in revised form 4 January 2013
Accepted 10 January 2013
Available online 23 February 2013

Keywords:
Obstructive sleep apnoea/hypopnoea syndrome (OSAHS)
Cardiovascular disease
Arterial stiffness
Endothelial function
Pulse wave velocity
Augmentation index
Aortic distensibility

ABSTRACT

Background: Obstructive sleep apnoea–hypopnoea syndrome (OSAHS) is associated with increased cardiovascular morbidity and mortality. Our study examined arterial stiffness and endothelial function in subjects with OSAHS with no known cardiovascular disease compared to well-matched controls.
Methods: Twenty subjects with OSAHS (defined as apnoea–hypopnoea index [AHI] $\geqslant$15 and Epworth Sleepiness Scale score $\geqslant$11) without cardiovascular disease and 20 well-matched controls underwent a comprehensive evaluation of arterial stiffness and endothelial function. Arterial stiffness was measured by applanation tonometry and cardiovascular magnetic resonance imaging (MRI) and endothelial function assessed by measuring vascular reactivity after administration of glyceryl trinitrate and salbutamol.
Results: Subjects with OSAHS had increased arterial stiffness (augmentation index 19.3 [10.9] vs. 12.6 (10.2)%; $p = 0.017$) and impaired endothelial function (change in augmentation index following salbutamol −4.3 (3.2) vs. −8.0 (4.9)%; $p = 0.02$) compared to controls. Aortic distensibility, a measure of arterial stiffness, was negatively correlated with the AHI.
Conclusions: Our findings suggest that even in the absence of known cardiovascular disease, subjects with OSAHS have increased arterial stiffness and impaired endothelial function and are at increased risk for cardiovascular disease.

1. Introduction

Obstructive sleep apnoea is caused by repetitive obstruction of the upper airway during sleep and when it leads to excessive daytime somnolence is called obstructive sleep apnoea–hypopnoea syndrome (OSAHS). OSAHS affects 2% to 4% of the middle-aged population [1] with a further 20% having frequent apnoeas and hypopnoeas in the absence of excessive daytime somnolence [2].

Obstructive sleep apnoea is an independent risk factor for hypertension [3] and also has been associated with increased cardiovascular morbidity and mortality [4–8].

The mechanisms for these associations are not completely understood, but each obstructive event is associated with transient increases in blood pressure [9], arterial stiffness [10], and sympathetic activity [11] that may contribute to endothelial dysfunction. Prior to beginning our study, previous studies in small numbers of patients suggested that subjects with obstructive sleep apnoea had increased arterial stiffness [12] and evidence of endothelial dysfunction [13,14].

The aim of our study was to comprehensively examine both arterial stiffness and endothelial function in subjects with OSAHS, without overt cardiovascular disease (CVD) or diabetes mellitus (DM) compared to well-matched controls. It was our hypothesis that OSAHS leads to increased arterial stiffness and endothelial dysfunction independent of pre-existing CVD.

2. Methods

2.1. Subjects

Twenty subjects with OSAHS (defined as apnoea-hypopnoea index [AHI] $\geqslant$15 on overnight polysomnography (PSG) and an Epworth Sleepiness Scale score $\geqslant$11) with no history of CVD or DM were recruited through the Department of Sleep Medicine. Exclusion criteria were: previous continuous positive airway pressure, respiratory failure, medications affecting blood pressure,

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http://dx.doi.org/10.1016/j.sleep.2013.01.001

sleepiness when driving, professional driving, contraindications to magnetic resonance imaging (MRI), and intercurrent illness. Twenty control subjects with no history of OSAHS, CVD, or DM were recruited via advertisement (within the hospital and on the hospital and university intranet) and a local general practitioner practice. Control subjects were required to have an AHI $\leqslant$10 on PSG and an Excessive Sleepiness Scale score <11 and also were subject to the exclusion criteria. We were able to match 20 subjects with OSAHS for age (within 5 years), sex, and body mass index (within 10%) on a one-to-one basis with the control subjects (Table 1).

To exclude a history of CVD, a detailed medical history was taken from all subjects and their medical records examined. Subjects with a raised clinic blood pressure also were excluded and all subjects underwent a fasting blood glucose measurement to exclude DM.

A 16-channel in-patient PSG (Compumedics Ltd., Abbotsford, Victoria, Australia) was performed in all subjects. Sleep was scored using standard Rechtschaffen and Kales criteria [15]. Apnoea was defined as a reduction in airflow of $\geqslant$90% from baseline for at least 10 s and hypopnoea as a reduction in airflow of $\geqslant$30% for at least 10 s with an oxygen desaturation of $\geqslant$4% from baseline or a $\geqslant$50% reduction in airflow for at least 10 s with a $\geqslant$3% oxygen desaturation or associated arousal [16].

Subjects were recruited between March 2007 and February 2009. Our study was approved by Lothian Local Research Ethics Committee (ref. 06/S1102/54) and written informed consent obtained from all subjects.

2.2. Study design

All vascular studies were conducted at the same time of day and subjects and controls had fasted overnight and abstained from smoking, alcohol, and caffeine for at least 10 h prior to the vascular studies.

2.3. Assessment of arterial stiffness and endothelial function by applanation tonometry

All studies were carried out by a single operator (AJ) in accordance with the Expert Consensus Document on Arterial Stiffness [17]. Measurements were recorded supine in a temperature-controlled room after at least 30 min of rest. Resting blood pressure and heart rate were recorded in duplicate using an automated sphygmomanometer (Omron 705IT, Milton Keynes, UK) and the mean used for analysis.

2.3.1. Pulse wave analysis

Radial artery pressure waveforms were continuously measured by tonometer (Colin Corp., Komaki City, Japan) and the SphygmoCor® system (version 7, AtCor Medical, Sydney, Australia). A validated mathematical transfer function was applied to the mean of approximately 10 waveforms to derive an aortic pressure waveform [18]. From this, the augmentation index (AIx), a measure of central-pressure augmentation, was determined. AIx is a measure of stiffness throughout the arterial tree and is calculated as the difference between the first and second systolic peaks, expressed as a percentage of the pulse pressure. AIx is affected by heart rate and was corrected to a heart rate of 75 beats per minute, as previously described [19]. Readings with >10% variability in pulse height or in the diastolic portion of the waveform were excluded. Repeated measurements of AIx were taken at baseline, with the mean value used for analysis.

Endothelial function was assessed by measuring endothelium-dependent change in AIx following inhaled salbutamol and endothelium-independent change in AIx following sublingual glyceryl trinitrate (GTN) [20,21].

After baseline recordings, 500 μg of GTN tablet was given sublingually for 3 min and then removed. AIx was then recorded every minute for 10 min and then every 5 min for a further 15 min. Previous studies demonstrated that the haemodynamic effects of GTN can persist for up to 25 min [22] and therefore, 400 μg of inhaled salbutamol via a spacer device was given 30 min after the GTN. AIx was then recorded every minute for 10 min and then every 5 min for a further 10 min. The greatest change in AIx following the administration of each drug was used for analysis [20].

2.3.2. Pulse wave velocity

Pulse wave velocity (PWV) increases with arterial stiffness and records the time taken by the systolic pressure wave to reach the peripheries. Carotid–femoral (aortic) PWV was measured by a micromanometer (Millar Instruments, Houston, TX, USA) and the SphygmoCor® system by sequential acquisition of carotid and femoral pressure waveforms gated to the R wave of a simultaneously recorded electrocardiogram. The SphygmoCor® system applied the intersecting tangent algorithm to determine the onset of the forward wave and the difference in transit time between the carotid and the femoral locations was used to calculate the PWV. Readings that met quality control standards were accepted for analysis [23]. We aimed to repeat each recording three times and the mean PWV was used for analysis.

Table 1
Baseline characteristics.

	Subjects with OSAHS (n = 20)	Control subjects (n = 20)	p Value
Age (y)	44 (7)	44 (7)	0.94
Sex	13 men	13 men	
BMI (kg/m^2)*	29.7 (27.4–32.7)	29.4 (27.4–33.5)	0.20
Neck circumference (cm)*	40.5 (38.1–41.9)	39.0 (36.6–41.5)	0.02
Waist to hip ratio	0.94 (0.07)	0.93 (0.08)	0.54
Systolic blood pressure (mmHg)	127 (14)	124 (11)	0.33
Diastolic blood pressure (mmHg)	76 (10)	75 (9)	0.75
MAP (mmHg)	93 (11)	91 (9)	0.42
Fasting glucose (mmol/L)	4.9 (0.5)	4.9 (0.3)	0.68
Total cholesterol (mmol/L)	5.5 (1.0)	5.6 (0.9)	0.79
Current smokers	30%	5%	0.13
Ex-smokers	35%	40%	1.00
Apnoea/hypopnoea index *	32 (22–41)	4 (3–6)	Part of selection criteria
Epworth Sleepiness Scale score*	16 (14–18)	4 (2–8)	Part of selection criteria

Mean (standard deviation) unless indicated.
BMI, body mass index; MAP, mean arterial blood pressure; OSAHS, obstructive sleep apnoea–hypopnoea syndrome.
* Median (interquartile range).

2.4. Assessment of aortic distensibility

Aortic distensibility was measured using cardiovascular MRI. Studies were performed on a 1.5-Tesla MRI scanner (Philips Medical Systems, Best, The Netherlands). Sagittal oblique images of the aorta in the supine position were used to define planes orthogonal to the aortic axis for electrocardiogram-gated, steady-state, free precession cine imaging with full R–R coverage in the ascending and descending aorta at the level of the right pulmonary artery and at the level of the diaphragm. Noninvasive blood pressure recordings were taken in the scanner immediately before and after each image was acquired (S/5™ monitor, Datex-Ohmeda, Helsinki, Finland), with the mean used to calculate the pulse pressure. Image analysis was performed by a single operator (MC) using an automated algorithm (EasyVision, Philips Medical Systems). Aortic distensibility was calculated as $(A_{max} - A_{min})/(A_{min} \times \text{Pulse pressure})$ where A_{max} and A_{min} were the maximum and minimum aortic areas during the cardiac cycle.

2.5. Data analysis

Data are expressed as mean (standard deviation) unless stated. Statistical analyses were performed using SPSS version 17 (SPSS, Chicago, IL, USA). Differences between groups were analysed using paired *t* tests or Wilcoxon signed rank tests. Differences in categorical data were analysed using McNemar test. Differences in vascular parameters between different smoking categories were examined using a one-way analysis of variance. Spearman rank correlation coefficient was used to determine correlations between vascular parameters and AHI. A p value of <0.05 was considered statistically significant. A stepwise linear regression analysis was performed with variables entered into the model if the initial significance of their correlation with the dependent variable was $\leqslant 0.05$.

3. Results

Baseline characteristics for the subjects with OSAHS and matched controls were similar with the exception of neck circumference (Table 1). There were more current smokers in the subject group, but this was not statistically significant; the numbers of ex-smokers were similar in each group. Cigarette pack-year history (among current and ex-smokers) was similar in subjects with OSAHS and controls (14.6 [14.5] vs. 11.4 [4.71] years; $p = 0.47$). The median AHI among subjects with OSAHS was 32, suggesting at least moderate disease; however, the mean minimum oxygen saturation was relatively high at 87.4 (4.3)% and the mean percentage of sleep time with oxygen saturations below 90% was low at 1.2 (3.3)%.

AIx was higher in subjects with OSAHS than in controls (19.3 [10.9] vs. 12.6 [10.2]%; $p = 0.017$).

We were able to measure PWV in 18 of the matched pairs (missing measurements due to patient body habitus) and this was similar in subjects with OSAHS and controls (7.6 [1.2] vs. 7.4 [1.2] m s^{-1}; $p = 0.55$). There was no difference in aortic distensibility between the subjects with OSAHS and controls in the ascending aorta (5.6 [1.6] vs. 5.9 [2.4] $mmHg^{-1} \times 10^{-3}$; $p = 0.65$), or descending aorta (4.9 [1.4] vs. 4.9 [1.4] $mmHg^{-1} \times 10^{-3}$; $p = 0.87$) or at the level of the diaphragm (7.2 [2.2] vs. 6.6 [2.0] $mmHg^{-1} \times 10^{-3}$; $p = 0.45$). For technical reasons and one case of claustrophobia, we were unable to compare aortic distensibility in two pairs in the ascending aorta and in five pairs in the descending aorta and at the level of the diaphragm.

Endothelium-dependent changes in AIx, a measure of endothelial function, was impaired in subjects with OSAHS compared to controls ($p = 0.02$). There was no difference in endothelium-independent change in AIx between the two groups (Table 2).

Table 2
Endothelium-dependent and endothelium-independent changes in augmentation index in subjects with OSAHS and controls.

	Subjects with OSAHS ($n = 20$)	Control subjects ($n = 20$)	p Value
Change in AIx (%) following salbutamol	−4.3 (3.2)	−8.0 (4.9)	0.02
Change in AIx (%) following GTN	−15.1 (5.5)	−14.1 (3.7)	0.45

Mean (standard deviation).
AIx, augmentation index; GTN, glyceryl trinitrate; OSAHS, obstructive sleep apnoea–hypopnoea syndrome.

A greater proportion of the subjects with OSAHS were smokers; however, this was not statistically significant. Across the group ($n = 40$) no difference in AIx or endothelium-dependent change in AIx was seen between current smokers, ex-smokers, or nonsmokers.

In subjects with OSAHS, AHI was negatively correlated with aortic distensibility in the descending aorta and at the level of the diaphragm ($r = -0.56$; $p = 0.015$ and $r = -0.45$; $p = 0.049$, respectively) (Fig. 1). Fasting glucose also was negatively correlated with aortic distensibility in the descending aorta ($r = -0.47$; $p = 0.05$). In a linear regression model with aortic distensibility in the descending aorta as the dependent variable and AHI and fasting glucose as independent variables, only AHI remained as an independent predictor.

A nonsignificant correlation between AHI and PWV was seen ($r = 0.46$; $p = 0.055$). AHI did not correlate with AIx or endothelial function in this group of subjects.

4. Discussion

In our study of subjects without overt CVD, we have shown that subjects with OSAHS have increased AIx and impaired endothelial function compared to well-matched controls.

We also have shown that disease severity, as measured by AHI correlates with aortic distensibility, a measure of arterial stiffness. Obstructive sleep apnoea is associated with increased cardiovascular morbidity and mortality [4–8], and numerous mechanisms are proposed including alterations in arterial stiffness and endothelial function.

AIx has previously been shown to be higher in patients with obstructive sleep apnoea than in control subjects [24–26], but these studies have all included patients with CVD. Our study extends previous observations by demonstrating that even young normotensive subjects with relatively mild OSAHS who are free of CVD have a higher AIx than well-matched controls (19.3 [10.9] vs. 12.6 [10.2]%; $p = 0.017$). AIx is a composite measure of central arterial stiffness and peripheral wave reflection and predicts cardiovascular events, with a recent meta-analysis suggesting that an absolute increase in AIx of 10% leads to a 26% increase in cardiovascular events and a 38% increase in all-cause mortality [27].

We did not find PWV to be significantly higher in subjects with OSAHS in our study. Several other studies have reported a higher PWV in patients with obstructive sleep apnoea when compared to control subjects [28]. However, in keeping with our findings Drager et al. [12]. did not demonstrate a difference in PWV in the absence of CVD between control subjects and those with mild-to-moderate disease. They found the AHI to be highly correlated with PWV ($r = 0.61$, $p < 0.0001$) [12]; in our study a trend towards a correlation between AHI and PWV was noted in subjects with

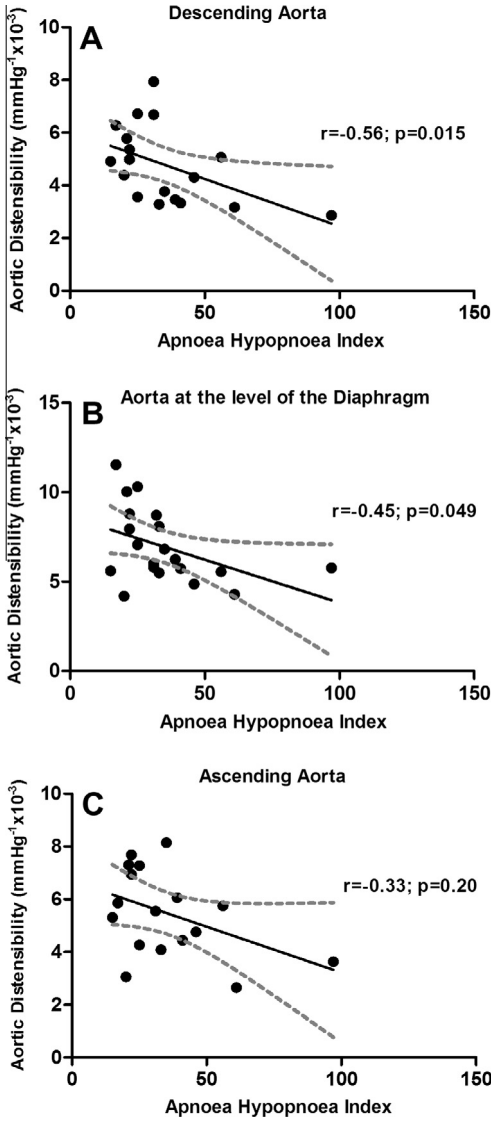

Fig. 1. Relationship between apnoea–hypopnoea index (AHI) and aortic distensibility. AHI plotted against aortic distensibility as measured at three levels within the thoracic aorta. The broken lines represent 95% confidence intervals.

OSAHS. It should be noted that in the aforementioned study, PWV was significantly higher than our data and recently published normal values suggest that the PWV measured in our study fall within the normal range based on the mean age and blood pressure of the group [29]. This finding may explain why, despite demonstrating a difference in AIx between patients with OSAHS and control subjects, we did not detect a difference in PWV between the two groups.

It has been hypothesized that additional effects on the structure and the function of the thoracic aorta may be seen due to intrathoracic pressure swings during obstructive events leading to shear stress, and recent work in healthy volunteers demonstrated changes in proximal aortic diameter during simulated hypopnoeic (but not apnoeic) events [30]. Similarly, aortic diameter has been shown to be greater in patients with sleep apnoea in a study that included patients with CVD [31]. Like Keles et al. [32] we found the AHI to be correlated with aortic distensibility despite the milder degree of obstructive sleep apnoea in our patients. However, we did not find a difference in aortic distensibility between subjects with OSAHS and well-matched controls. The few studies that have previously examined this hypothesis have produced conflicting results [32–34] and further work is required in this area.

Endothelial function was impaired in OSAHS subjects compared with control subjects. The magnitude of the difference that we have reported is similar to the findings of Kohler et al. [25] who used the same method of assessing endothelial function in subjects with minimally symptomatic obstructive sleep apnoea, a proportion of whom also had CVD. Several studies have previously reported impaired endothelial function as measured by flow-mediated dilatation in patients with obstructive sleep apnoea [13,14,35–38]. However, many of these studies involved small numbers of patients or included patients with CVD. In the largest of these studies [13,37], subjects had more severe obstructive sleep apnoea than in our study. The results of our study add to current knowledge, suggesting that endothelial function is impaired even in patients with less severe OSAHS in the absence of known CVD.

The mechanisms through which obstructive sleep apnoea leads to increased arterial stiffness and endothelial dysfunction are likely to be multifactorial. Obstructive sleep apnoea is associated with oxygen desaturation, sleep fragmentation and intrathoracic pressure swings with individual events associated with transient increases in blood pressure [9], arterial stiffness [10], and sympathetic activity [11]. Intermittent hypoxia has been proposed as a key mechanism and has been shown to lead to oxidative stress and systemic inflammation [39]. Obstructive sleep apnoea is an independent risk factor for hypertension [3], which may in turn lead to vessel damage.

In our study of normotensive patients with relatively modest nocturnal oxygen desaturation, arterial stiffness was increased and endothelial function was impaired when compared to well-matched control subjects. The AHI correlated with aortic distensibility, but in our patient group no correlation was seen between minimum oxygen saturation, percentage of sleep time with oxygen saturations below 90%, or blood pressure and aortic distensibility. This is only a small study but these results suggest that other aspects of obstructive sleep apnoea, such as sleep fragmentation, intrathoracic pressure swings and activation of the systemic nervous system also may be important in the development of increased arterial stiffness and endothelial dysfunction.

Identifying subjects with OSAHS without CVD or DM was difficult and meant that overall our subjects had milder disease than often appears in publications in this area, which may have contributed to the fact that we showed AIx but not PWV or aortic distensibility to be higher in patients with OSAHS than in the control group.

Our OSAHS and control groups were well matched in all respects except for the greater neck circumference seen in OSAHS subjects. Recent work suggests that neck circumference is associated with cardiovascular risk [40]. It remains unclear if this is an independent association or if it is due to the already known association of neck circumference with OSAHS.

Finally, it should be emphasised that this is a relatively small study and we were not able to obtain complete data sets for PWV and aortic distensibility, which may have increased the possibility of type II statistical error.

In conclusion, these findings suggest that even in our cohort of normotensive subjects with relatively mild OSAHS and no history of CVD, there is evidence of increased arterial stiffness and impairment of endothelial function.

Conflict of Interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2013.01.001.

Acknowledgments

This study was funded by a British Heart Foundation project grant (ref.: PG/06/092/21267).

We thank Dr. Tom Mackay and all of the staff within the Department of Sleep Medicine, the Department of Radiology, and the Clinical Research Facility at the Royal Infirmary of Edinburgh. We gratefully acknowledge the assistance of the Scottish Primary Care Research Network in recruiting control subjects.

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Sleep Medicine 14 (2013) 1260–1265

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

The effect of continuous positive airway pressure therapy on arterial stiffness and endothelial function in obstructive sleep apnea: a randomized controlled trial in patients without cardiovascular disease

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ARTICLE INFO

Article history:
Received 26 May 2013
Received in revised form 13 August 2013
Accepted 15 August 2013
Available online 14 October 2013

Keywords:
Obstructive sleep apnea (OSA)
Cardiovascular disease
Continuous positive airway pressure (CPAP) therapy
Arterial stiffness
Endothelial function
Augmentation index
Pulse wave velocity
Aortic distensibility

ABSTRACT

Background: Obstructive sleep apnea (OSA) is associated with increased cardiovascular morbidity and mortality which may be mediated by increased arterial stiffness and endothelial dysfunction. Continuous positive airway pressure (CPAP) therapy improves excessive daytime somnolence (EDS), but its effect on vascular function in patients without preexisting cardiovascular disease (CVD) is unclear.
Methods: Fifty-three patients with OSA defined as an apnea–hypopnea index (AHI) of $\geqslant$15 and without CVD were recruited into a double-blind, randomized, placebo-controlled, crossover trial of 12 weeks of CPAP therapy, of whom 43 participants completed the study protocol. Arterial stiffness was assessed by measuring the augmentation index (AIx) and pulse wave velocity (PWV) by applanation tonometry and cardiovascular magnetic resonance imaging to determine aortic distensibility. Endothelial function was assessed by measuring vascular reactivity after administration of salbutamol and glyceryl trinitrate.
Results: CPAP therapy lowered systolic blood pressure (SBP) (126 mmHg [standard deviation {SD}, 12] vs 129 mmHg [SD, 14]; $P = .03$), with a trend towards reduced AIx (15.5 [SD, 11.9] vs 16.6 [SD, 11.7]%; $P = .08$) but did not modify endothelial function. When subjects with ($n = 24$) and without ($n = 19$) EDS were separately examined, no effect of CPAP therapy on vascular function was seen.
Conclusions: In patients without overt CVD, CPAP therapy had a nonsignificant effect on AIx and did not modify endothelial function.

1. Introduction

Obstructive sleep apnea (OSA) is common and is caused by repetitive obstruction of the upper airway during sleep. When OSA leads to excessive daytime somnolence (EDS) it is termed *obstructive sleep apnea–hypopnea syndrome (OSAHS)*, affecting 2–4% of the middle-aged population with an even greater proportion having evidence of OSA without EDS [1].

OSA is associated with increased cardiovascular morbidity and mortality [2,3] and is an independent risk factor for hypertension [4]. Many mechanisms linking OSA and cardiovascular disease (CVD) have been proposed, with individual obstructive events associated with transient increases in blood pressure (BP) [5], arterial stiffness [6], and sympathetic activity [7]; all of which, along with systemic inflammation [8] and intrathoracic pressure swings, may contribute to endothelial dysfunction. We previously showed [9] that subjects with OSAHS in the absence of known CVD had evidence of increased arterial stiffness and impaired endothelial function when compared to well-matched control subjects.

Observational studies suggest that cardiovascular morbidity and mortality is lower in patients treated with continuous positive airway pressure (CPAP) therapy [2,10,11]. Randomized controlled trials (RCTs) have shown that CPAP therapy lowers BP [12,13], and it also has been shown to improve endothelial function in small studies [14,15]; however, many of these studies did not exclude patients with CVD [12,13,15]. To our knowledge, the effects of CPAP therapy on arterial stiffness had not yet been investigated prior to beginning our trial.

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http://dx.doi.org/10.1016/j.sleep.2013.08.786

The aim of our double-blind, randomized, placebo-controlled, crossover trial was to examine the effects of CPAP therapy on arterial stiffness and endothelial function in subjects with OSA in the absence of overt CVD or diabetes mellitus (DM).

2. Methods

2.1. Subjects

Fifty-three subjects with OSA (apnea–hypopnea index [AHI] of $\geqslant$15 on polysomnography), with no history of CVD or DM, were recruited through the Department of Sleep Medicine, Royal Infirmary of Edinburgh (Fig. 1). Exclusion criteria were previous CPAP therapy, respiratory failure, medications affecting BP, sleepiness when driving, professional driving, contraindications to magnetic resonance imaging, and intercurrent illness. EDS was defined as a score of $\geqslant$11 on the Epworth Sleepiness Scale (ESS). A detailed medical history was taken and medical records were examined to exclude a history of CVD or hypertension. A fasting venous glucose sample was taken to exclude DM.

Sixteen channel, inpatient, overnight polysomnography (Compumedics Ltd., Abbotsford, Australia) was performed in all subjects with sleep scored using standard Rechtschaffen and Kales criteria [16], and apnea and hypopneas were defined per the 2007 American Academy of Sleep Medicine standard criteria [17].

Subjects were recruited between March 2007 and August 2008. Sample size was determined using pilot data to provide a 90% chance of detecting a 10% difference in mean aortic distensibility (AoD) at a significance level of 5% ($n = 40$). Therefore, we aimed to recruit 60 subjects to allow for the occurrence of dropouts. The study was approved by Lothian Local Research Ethics Committee (ref. 06/S1102/54) and written informed consent was obtained from all subjects.

2.2. Study design

Our double-blind, randomized, placebo-controlled, crossover trial examined the effects of CPAP therapy on arterial stiffness and endothelial function in subjects with OSA. The study is registered with the International Standard Randomized Controlled Trial Number Register (ISRCTN48783995).

Subjects underwent vascular assessments at baseline and were randomized to receive either CPAP therapy (S8 Autoset™, ResMed, Abingdon, UK) or sham CPAP for 12 weeks before crossing into the second arm of the study for a further 12 weeks. Sham CPAP was achieved by setting the CPAP flow to the lowest possible setting and adding a flow-restricting connector and extra holes to the circuit, allowing air to escape [13,15]. Randomization was performed by a single researcher (MV) who was not involved in the measurement of vascular outcomes, using a computer generated balanced block. Both the subjects and the researchers who assessed vascular outcomes (AJ and MC) were blinded to treatment allocation. Optimum CPAP pressures were determined at overnight inpatient CPAP titration (Spirit™, ResMed, Abingdon, UK). CPAP usage was determined using time-clock data, which measured time spent at the prescribed pressure throughout the entire study period.

Vascular assessments were repeated after each arm of the study and were conducted at the same time of day, with subjects having fasted overnight and abstained from cigarettes, alcohol, and caffeine use for at least 10 h prior to beginning the study. Resting BP and heart rate were recorded in duplicate using an automated sphygmomanometer (Omron 705IT, Milton Keynes, UK) with the mean used for analysis.

2.3. Assessment of arterial stiffness and endothelial function by applanation tonometry

All studies were performed in the supine position in a temperature-controlled room after at least 30 min of rest and in accordance with the Expert Consensus Document on Arterial Stiffness [18] by a single operator (AJ).

2.3.1. Pulse wave analysis

Radial artery pressure waveforms were continuously measured by tonometer (CBM 7000, Colin Corp., Komaki City, Japan) and the SphygmoCor® system (version7, AtCor Medical, Sydney, Australia), which applied a validated transfer function to the mean of approximately 10 waveforms, giving an aortic pressure waveform. From this mean, the augmentation index (AIx) corrected to a heart rate of 75 beats per minute as previously described [9], was determined. AIx is a measure of stiffness throughout the arterial tree and represents the difference between the first and second systolic peaks, expressed as a percentage of the pulse pressure. Repeated measurements of AIx were taken at baseline and the mean value was used for analysis. Readings with >10% variability in pulse height or in the diastolic portion of the waveform were excluded.

Noninvasive assessment of endothelial function was performed by measuring endothelium-dependent change in AIx following the administration of salbutamol and endothelium-independent change in AIx following glyceryl trinitrate [19]. As previously described [9], after baseline recordings 500 μg of glyceryl trinitrate tablet was given sublingually for 3 min and then was removed. AIx was then recorded every minute for 10 min and then every 5 min for a further 15 min. A dose of 400 μg of inhaled salbutamol via a spacer device was subsequently administered, with recordings of AIx every minute for 10 min and then every 5 min for a further 10 min. The greatest change in AIx following the administration of each drug was used for analysis [19].

2.3.2. Pulse wave velocity

Pulse wave velocity (PWV) increases with arterial stiffness and measures the speed at which the systolic pressure wave reaches the peripheries. Carotid-femoral (aortic) PWV was measured as previously described [9] using a micromanometer (Millar Instruments, Houston, TX) and the SphygmoCor® system by sequential acquisition of carotid and femoral pressure waveforms gated to the *R*-wave of a simultaneously recorded electrocardiogram. Only those readings that met quality-control standards [20] were accepted for analysis. We aimed to repeat each recording three times, with the mean PWV used for analysis.

2.4. Assessment of aortic distensibility (AoD)

Cardiovascular magnetic resonance imaging (1.5 Tesla, Philips Medical Systems, Best, Netherlands) was used to determine AoD. Sagittal oblique images of the aorta in the supine position were used to define planes orthogonal to the aortic axis for electrocardiogram-gated steady-state free precession cine imaging with full *R–R* coverage in the ascending and descending aorta at the level of the right pulmonary artery and at the level of the diaphragm. BP measurements (S/5™ monitor, Datex-Ohmeda, Helsinki, Finland) were taken in the scanner immediately before and after each image was acquired, and the mean was used to determine the pulse pressure. Image analysis was performed using an automated algorithm (EasyVision, Philips Medical Systems) by a single operator (MC) who was blinded to treatment allocation, with AoD calculated as $(A_{max} - A_{min})/(A_{min} \times \text{pulse pressure})$ in which A_{max} and A_{min} were the maximum and minimum aortic areas during the cardiac cycle.

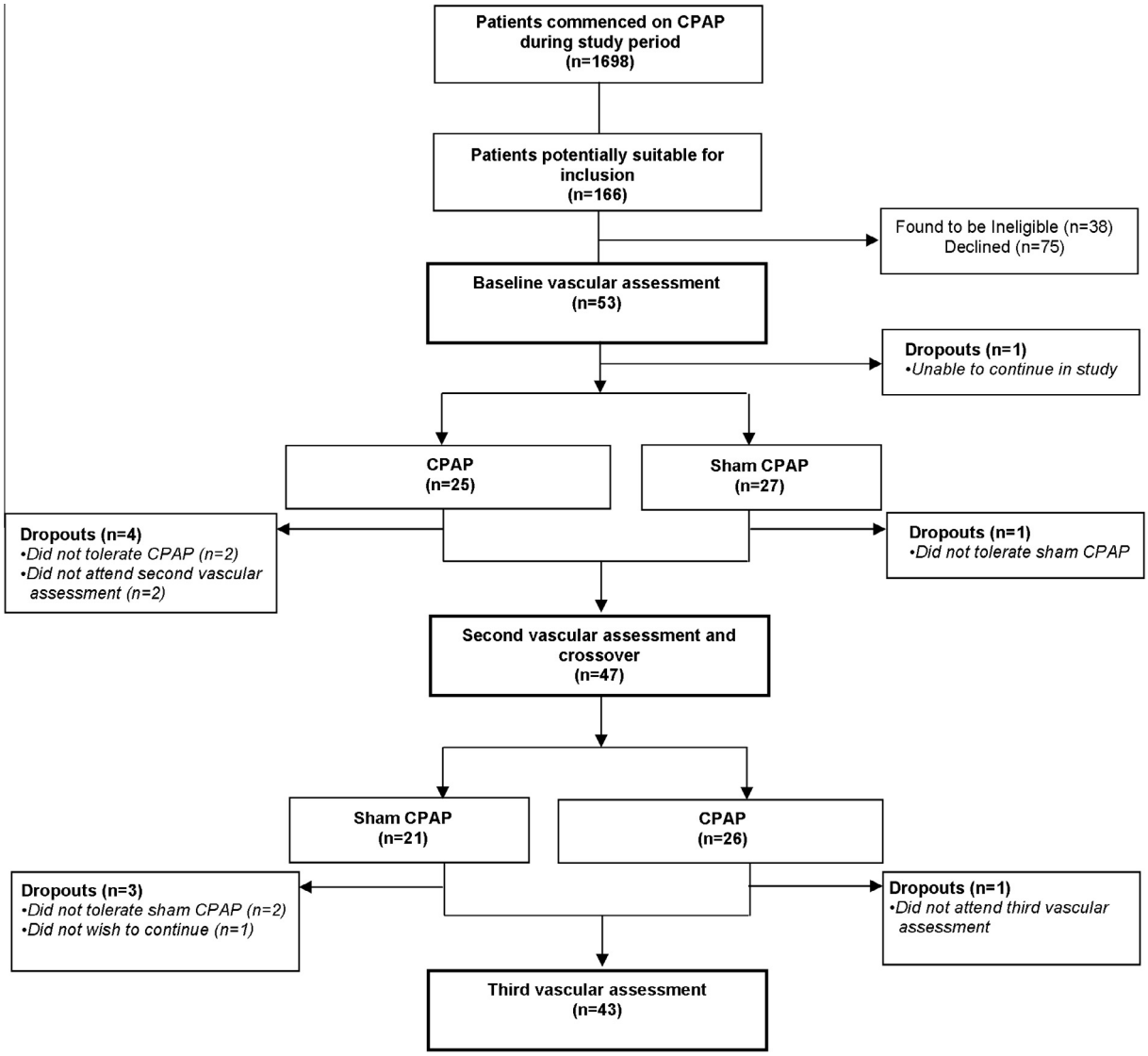

Fig. 1. Study profile. *Abbreviation:* CPAP, continuous positive airway pressure.

2.5. Data analysis

Statistical analyses were performed using SPSS version 17 (SPSS, Chicago, IL) and all data are expressed as mean (standard deviation [SD]) unless otherwise stated. Differences between treatment arms were analyzed using paired *t* tests or Wilcoxon signed rank tests. Repeated measures analysis of variance was used to look for evidence of an order effect. The unpaired *t* test, Mann–Whitney *U* test, and χ^2 test were used to analyze baseline differences between those who dropped out of the study and those who completed the follow-up, as well as between subjects with and without EDS. A *P* value of <.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics and vascular measurements

Fifty-three subjects with OSA underwent baseline vascular assessments, of whom 10 did not complete the RCT (Fig. 1); baseline data for these subjects did not significantly differ from the 43 patients who completed the study. Baseline characteristics for those completing the study are shown in Table 1. The median AHI was 31, suggesting moderate disease severity; however, the mean minimum oxygen saturations were relatively high and the mean percentage of sleep time spent with oxygen saturations below 90% was low (Table 1), which suggests that these patients did not experience significant nocturnal intermittent hypoxia. Baseline arterial stiffness and endothelial function are shown in Table 2.

3.2. CPAP usage

There was a difference in CPAP use ($P = \cdot001$) between the two limbs of the study, with subjects using CPAP for 3.0 h per night (range, 0–8.1 h) and sham CPAP for 2.0 h per night (range, 0–5.8 h). ESS scores were lower on CPAP therapy than on sham (median, 7 [interquartile range {IQR}, 4–11] vs median, 10 [IQR, 5–13]; $P < .001$).

3.3. Vascular outcomes

Systolic blood pressure (SBP) was lower on CPAP therapy than on sham (126 [SD, 12] vs 129 [SD, 14] mmHg; $P = .03$) (Table 3).

Table 1
Baseline characteristics of subjects who completed the study ($N = 43$).

Parameter	
Age (y)	46 (9)
Sex	28 men
BMI (kg/m^2)*	29·9 (27.3–31.6)
Neck circumference (cm)*	40 (37–41.5)
Waist to hip ratio	0·95 (0.07)
Systolic blood pressure (mmHg)	128 (13)
Diastolic blood pressure (mmHg)	76 (9)
MAP (mmHg)	93 (10)
Fasting glucose (mmol/L)	4·9 (0.4)
Total cholesterol (mmol/L)	5·3 (1.0)
Current smokers	23%
Ex-smokers	23%
Cigarette pack/y history (among smokers and ex-smokers)	11.4 (8.0)
Apnea–hypopnea index*	31 (20–41)
4% O_2 desaturation rate/h	9.3 (12.3)
Minimum O_2 saturation (%)	86.1 (5.1)
TST90 (%)	4.4 (13.2)
Epworth Sleepiness Scale score*	13 (6–15)

Abbreviations: y, year; BMI, body mass index; MAP, mean arterial pressure; O_2, oxygen; h, hour; TST90, percentage of sleep time spent with oxygen saturations of less than 90%.
Mean (standard deviation) unless otherwise indicated.
* Median (interquartile range).

Table 2
Baseline vascular function in subjects who completed the study ($N = 43$).

Vascular parameter	
Augmentation index (%) ($N = 43$)	17·1 (11.6)
Femoral PWV (meters/s) ($n = 40$)	7.6 (1.5)
AoD-ascending aorta (mmHg^{-1} × 10^{-3}) ($n = 35$)	5.0 (1.9)
AoD-descending aorta (mmHg^{-1} × 10^{-3}) ($n = 35$)*	4.8 (3.3–5.4)
AoD at level of diaphragm (mmHg^{-1} × 10^{-3}) ($n = 42$)	6.5 (1.9)
Change in AIx (%) following salbutamol ($N = 43$)* (endothelium-dependent)	−4.5 (−6 to −3)
Change in AIx (%) following GTN ($N = 43$) (endothelium-independent)	−14.0 (4.6)

Abbreviations: PWV, pulse wave velocity; s, second; AoD, aortic distensibility; AIx, augmentation index; GTN, glyceryl trinitrate.
Mean (standard deviation) unless otherwise indicated.
* Median (interquartile range).

No significant treatment order effects were seen for any of the measured vascular variables.

3.3.1. Arterial stiffness

There was a nonsignificant trend ($P = .08$) toward a lower AIx on CPAP therapy than on sham CPAP. No differences in PWV or AoD were seen (Table 3). For reasons of body habitus, we were unable to measure AIx in one subject and PWV in three subjects. For technical reasons and one case of claustrophobia, we were unable to measure AoD in the ascending aorta in six subjects; in the descending aorta in five subjects; and at the level of the diaphragm in four subjects.

3.3.2. Endothelial function

CPAP therapy did not affect endothelial function, and no difference in endothelium-dependent or endothelium-independent change in AIx observed (Table 3).

3.4. Subgroup analysis

Of the 43 subjects who completed our study, 24 had EDS and 19 did not. Nonsleepy subjects were older than those with EDS (50 years [SD, 9 years] vs 43 years [SD, 7 years]; $P = .005$), but baseline BP and body mass index were not statistically different. Subgroup analysis of subjects with and without EDS showed no difference in any of the measured vascular variables following CPAP therapy in either group.

A post hoc analysis of subjects who used CPAP therapy for at least 4 h per night ($n = 17$) did not reveal any significant differences in any of the measured vascular variables between the CPAP therapy and sham limbs.

4. Discussion

In a rigorous, double-blind, randomized, placebo-controlled, crossover trial, CPAP therapy lowered SBP with a trend towards a reduction in AIx after 12 weeks. However, CPAP therapy had no effect on PWV, AoD, or endothelial function in this group of subjects without CVD after 12 weeks.

OSA is associated with increased cardiovascular morbidity and mortality [2,3], and numerous mechanisms are proposed. Using the same measures, we previously showed [9] that subjects with OSA and EDS had increased AIx and impaired endothelial function compared to well-matched control subjects in the absence of CVD. In keeping with previous findings, CPAP therapy led to a reduction in BP [21]. A trend towards a reduction in AIx was seen, but it was not statistically significant. AIx is a measure of central arterial stiffness and peripheral wave reflection, with increased AIx associated with increased cardiovascular events and all-cause mortality [22]. Two studies have previously reported a reduction in AIx following CPAP therapy [23,24]; however, one of those studies was nonrandomized [24], and both studies included patients with CVD.

Carotid-femoral (aortic) PWV is the gold standard measurement of arterial stiffness [18] and is an independent predictor of cardiovascular outcome [25]. CPAP therapy did not lead to a reduction in PWV in our study, in contrast to the work by Drager et al. [26] who

Table 3
Vascular function following CPAP and sham CPAP treatment periods ($N = 43$).

	CPAP	Sham CPAP	*P* value
Systolic blood pressure (mmHg)	126 (12)	129 (14)	.03
Diastolic blood pressure (mmHg)	77 (8)	77 (8)	.90
MAP (mmHg)	94 (8)	94 (9)	.29
Augmentation index (%) ($n = 42$)	15.5 (11.9)	16.6 (11.7)	.08
Femoral PWV (meters/s) ($n = 40$)	7.5 (1.2)	7.6 (1.4)	.29
AoD-ascending aorta (mmHg^{-1} × 10^{-3}) ($n = 37$)	4.9 (1.7)	5.1 (1.9)	.44
AoD-descending aorta (mmHg^{-1} × 10^{-3}) ($n = 38$)*	4.6 (3.9–5.7)	4.5 (3.7–5.1)	.22
AoD at level of diaphragm (mmHg^{-1} × 10^{-3}) ($n = 39$)	6.8 (1.9)	6.9 (2.3)	.80
Change in AIx (%) following salbutamol ($n = 42$)* (endothelium-dependent)	−5 (−8 to −3)	−4 (−6.8 to −2.5)	.50
Change in AIx (%) following GTN ($n = 42$) (endothelium-independent)	−14.1 (5.1)	−15.3 (4.6)	.13

Abbreviations: CPAP, continuous positive airway pressure; MAP, mean arterial pressure; PWV, pulse wave velocity; s, second; AoD, aortic distensibility; Aix, augmentation Index, GTN, glyceryl trinitrate.
Mean (standard deviation) unless otherwise indicated.
* Median (interquartile range).

showed a significant reduction in PWV following CPAP therapy in a smaller, randomized, parallel trial of patients with severe OSA in the absence of CVD. However, it should be noted that baseline PWV in CPAP-treated patients in that study was higher at 10.4 (SD, 1.0) meters per second, compared to 7.6 (SD, 1.5) meters per second in our study. A reduction in PWV has been reported following CPAP therapy in several nonrandomized studies, some of which included subjects with CVD [24,27].

OSA may exert additional direct effects on the thoracic aorta, due to swings in intrathoracic pressure and subsequent shear stress during obstructive events. We and others have previously shown the AHI to be correlated with AoD [9,28], and recently published work showed that AoD was lower in subjects with OSA compared to matched control subjects [29]. Despite this, we did not find CPAP therapy to have any effect on AoD after 12 weeks of treatment, and to our knowledge our study is only the second study to examine this association. In a small nonrandomized study on OSAHS, subjects who were compliant with CPAP therapy (mean use, 6.3 [SD, 2.1] h/night) had an improvement in AoD at 6-month follow-up [28].

In our study CPAP therapy did not lead to an improvement in endothelial function after 12 weeks. Several smaller studies demonstrated an improvement in endothelial function following CPAP therapy, but those studies were in subjects with more severe OSA [14,15,30–32]. Some of the studies also included patients with CVD [15,30] and many were nonrandomized [30–32].

We previously showed that a subgroup of 20 subjects with OSA and EDS had increased arterial stiffness and impaired endothelial function compared to well-matched control subjects [9]. Aside from a higher ESS score (16 [IQR, 14–18] vs 13 [IQR, 6–15]), those patients did not significantly differ in baseline demographics, OSA severity, or vascular function compared to the group who completed the RCT. However, despite this, no treatment effect on vascular function was seen with CPAP therapy, and this may have been related to the inclusion of patients without EDS in this trial. Studies have shown that, although CPAP therapy lowers BP in patients with OSA [21], there may be little effect in those without symptoms of EDS [33,34]. While overall we have shown that CPAP therapy lowers SBP with a trend towards a reduction in arterial stiffness, a subgroup analysis of sleepy and nonsleepy subjects did not show CPAP therapy to affect BP, arterial stiffness, or endothelial function in either group. This finding may be due to the small numbers in each group, and therefore the effectiveness of CPAP therapy in modifying vascular function in nonsleepy subjects remains unclear. Thus further work is required in this area.

Identifying patients with OSA but without CVD or DM was difficult, and this led to an overall milder spectrum of disease than often is reported in the literature. Given the increasing evidence for the role of intermittent hypoxia in the pathogenesis of CVD in OSA [35], it is tempting to attribute the lack of any treatment effect to the absence of significant hypoxia in these patients. However, as discussed above, we previously showed a subset of patients with a similarly mild degree of nocturnal hypoxia to have increased arterial stiffness and impaired endothelial function, suggesting that although important, intermittent hypoxia is not the only factor affecting the cardiovascular system in OSA. Other factors including EDS and recurrent arousals also may play a role.

A limitation of our study is that mean CPAP use was 3 h at pressure per night. This compliance is disappointing, but it is similar to the compliance of 3.3 h reported in a previous study [12] of 68 patients that demonstrated a reduction in BP with CPAP therapy and in other consecutive series of new CPAP therapy users in this center [36]. Thus we believe that this CPAP use reflects clinical practice in patients with this degree of OSA, but this may of course limit the generalizability of these results among patients with greater compliance. Post hoc analysis of those using CPAP therapy for more than 4 h did not demonstrate any change in vascular function; however, the smaller number of subjects involved made it impossible to form a definitive conclusion regarding this, and indeed the study was not powered to do so. There is no ideal placebo for CPAP therapy but the most rigorous arguably is sham CPAP, which was used in our study [37]. It could be argued that an improvement in vascular function requires longer than 12 weeks of therapy; however, many of the previous studies that showed an improvement in BP [12,13] or vascular function [14,15,23,30,31] with CPAP therapy were conducted over a 4- to 12-week period. Equally, antihypertensive therapy has been shown to improve AoD after 12 weeks of treatment [38].

5. Conclusions

In this group of subjects with relatively mild OSA in the absence of CVD or DM and in the context of a rigorous, double-blind, randomized, placebo-controlled crossover trial, we found that CPAP therapy lowered BP but had a nonsignificant effect on arterial stiffness, as measured by AIx, and did not modify endothelial function. Although it is clear that CPAP therapy is an effective treatment for EDS, more evidence is required, especially in patients with less severe OSA who do not have evidence of CVD, to determine its cardiovascular benefits.

Funding sources

This study was funded by a British Heart Foundation project grant (PG/06/092/21267). We thank Dr. Tom Mackay and all of the staff within the Department of Sleep Medicine, the Department of Radiology and the Clinical Research Facility at the Royal Infirmary of Edinburgh.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2013.08.786.

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