CPAP withdrawal as a model of the cardiovascular effects of obstructive sleep apnoea

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Christopher D. Turnbull

St. John's College

Nuffield Department of Medicine

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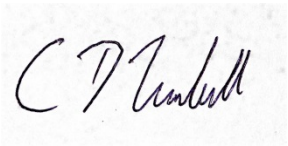
Declaration of originality

The work in Chapter Six includes work already published (see Appendix 8.7). I confirm that this is my own work and I have permission to include it my thesis.



Publications

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Outside the scope of this thesis:

4. Relationships between MRI fat distributions and sleep apnea and obesity hypoventilation syndrome in very obese patients. C. Turnbull, S. Wang, A. Manuel, B. Keenan, A. McIntyre, R. Schwab, J. Stradling. *Sleep and Breathing* E-published
5. Can postural OSA be usefully identified from its severity alone? A. Johar, C. Turnbull, J. Stradling *BMJ Open Respiratory Research* E-published
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8. To screen or not to screen for obstructive sleep apnea, that is the question. C. Turnbull & J. Stradling. *Sleep Med Reviews* 2017 36:125-127.
9. Night-to-night variability of obstructive sleep apnoea. A. Stoberl, E. Schwarz, S. Haile, C. Turnbull, V. Rossi, J. Stradling, M. Kohler. *J Sleep Res* 2017 26:782-788.
10. Overnight urinary isoprostanes as a marker of oxidative stress in OSA C. Turnbull, I. Akoumianakis, C. Antoniades, J. Stradling. *ERJ.* 2017 49(2).
11. Eye disorders associated with obstructive sleep apnoea. S. West & C. Turnbull. Curr Opin Pulm Med 2016 Nov; 22(6): 595-601 (Review)
12. In patients with minimally symptomatic OSA can baseline characteristics and early patterns of CPAP usage predict those who are likely to be longer-term users of CPAP. C. Turnbull, D. Bratton, S. Craig, M. Kohler, J. Stradling. *J Thorac Dis.* 2016 8:276-81.
13. Efficacy of EPAP. C. Turnbull, M. Kohler, J. Stradling. *Sleep Breath. 2016* Mar; 20(1): 259-60.
14. Quality of life, diet and exercise measurements in obese individuals with and without ventilatory failure. C. Turnbull, A. Manuel, J. Stradling. *Obes Res Clin Pract* 2015 9:639-40.
15. Does either obesity of OSA severity influence the response of autotitrating CPAP machines in very obese subjects? C.D. Turnbull, A.R. Manuel, J.R. Stradling. *Sleep Breath* 2016 May; 20(2): 647-52.
16. Withdrawal of continuous positive airway pressure therapy for 2 weeks in obstructive sleep apnoea patients results in increased circulating platelet and leucocyte-derived microvesicles. L Ayers, C. Turnbull, N. Petousi *et al. Respiration 2016 91(5):412-413*

CPAP withdrawal as a model of the cardiovascular effects of obstructive sleep apnoea

Christopher Turnbull, St. John's College, Doctor of Philosophy Submission, Hilary 2018

Abstract

Obstructive sleep apnoea (OSA) is associated with cardiovascular disease and hypertension. The underlying causes of cardiovascular disease and hypertension in OSA are not fully understood. Potential causes for cardiovascular disease and hypertension in OSA include; intermittent hypoxia, arousal mediated sympathetic activation, oxidative stress, endothelial dysfunction, and systemic inflammation. Continuous positive airway pressure (CPAP) is the standard treatment for OSA and CPAP withdrawal is used to model the consequences of OSA. In the CPAP withdrawal model patients with known OSA are withdrawn from their usual CPAP treatment and randomised to intervention or control for two weeks. Utilising a CPAP withdrawal model, I examined the effects of supplemental oxygen versus air (sham) during CPAP withdrawal on several outcomes, including; morning blood pressure, intermittent hypoxia, markers of arousal, and daytime sleepiness. In addition, I examined the effects of the return of OSA following CPAP withdrawal on several biomarkers. Supplemental oxygen virtually abolished the rise in morning blood pressure seen following CPAP withdrawal. Supplemental oxygen markedly attenuated intermittent hypoxia, whilst having little effect on markers of arousal, and no effect on daytime sleepiness. This suggests that intermittent hypoxia is the dominant cause of morning increases in blood pressure in OSA. Following CPAP withdrawal there was a small unexpected reduction in adrenomedullin, which is a potent vasodilator and may contribute to the blood pressure rise with CPAP withdrawal.

Contents

1. Chapter One: An introduction to obstructive sleep apnoea and its cardiovascular effects

1.1. Abstract (Chapter One)

Obstructive sleep apnoea (OSA) is a common disorder affecting approximately 2-12% of middle-aged adults. The dominant risk factors for OSA are obesity, older age, and male gender. OSA is associated with daytime sleepiness, road traffic accidents, and cardiovascular disease. Continuous positive airway pressure (CPAP) is the standard treatment for OSA. Whilst CPAP improves daytime sleepiness and reduces blood pressure, current evidence — from the SAVE trial — does not support its use solely for reducing cardiovascular events. New treatments are needed for individuals at risk of cardiovascular disease in OSA and understanding the underlying mechanisms is important to help develop new treatments. Potential causative mechanisms for cardiovascular disease in OSA include arousal induced sympathetic activation, large intrathoracic pressure swings leading to shear stress on the heart and great vessels, and intermittent hypoxia. In this chapter I will discuss OSA in more detail including, but not limited to; the aetiology, epidemiology, physiology, and cardiovascular effects of OSA.

1.2. Obstructive sleep apnoea (OSA)

Obstructive sleep apnoea (OSA) is a condition that is characterised by episodic upper airway narrowing during sleep. During sleep the activity of the upper airway dilator muscles decreases in a sleep stage dependent manner (1). This leads to narrowing of the upper airway, which can cause either partial reduction in airflow (a hypopnoea), or complete cessation of airflow (an apnoea). The exact definition of apnoeas and hypopnoeas depend on scoring criteria (2)(3). Apnoeas and hypopnoeas have several acute physiological effects. Cessation of airflow typically leads to intermittent hypoxia, increased respiratory effort with large swings in intrathoracic pressure, cortical arousals leading to sleep fragmentation, and sympathetic activation leading to blood pressure and heart rate surges. The most recognised daytime consequence of OSA is daytime sleepiness (4). OSA also causes impaired quality of life (5), increased road traffic accidents (6)(7), and is associated with significant health care and economic burden (8). OSA is also associated with numerous comorbidities including cardiovascular disease (9), which will be discussed in more detail later.

1.3. OSA definition

OSA is diagnosed by a combination of both typical symptoms and sleep study findings. The presence of obstructive sleep apnoea on a sleep study is usually termed OSA, and the combination of OSA with symptoms is often called obstructive sleep apnoea syndrome (OSAS). Thresholds are commonly used when describing the presence or absence of OSA on a sleep study, either using a threshold for the apnoea hypopnoea index (AHI) or the oxygen desaturation index (ODI). Sleep disordered breathing (SDB) is better described as a spectrum ranging from no snoring, snoring in some positions, habitual snoring, OSA in some postures

only, and all night severe OSA. Apnoeas are events where there is reduction in airflow to <10% of baseline (usually the preceding 60-120 seconds) for >10 seconds. Hypopnoeas are variably defined but describe a significant reduction in airflow, typically at least 30-50% reduction from baseline, lasting for >10 seconds. Hypopnoeas are usually also required to be associated with either; a reduction in oxygen saturations above a threshold of either 3 or 4%, or an arousal from sleep identified on electro-encephalogram (EEG) (2). The AHI is the total number of apnoeas and hypopnoeas divided by the total sleep time. The ODI describes the number of events where there is a sudden fall in oxygen saturations from baseline greater than either a 3 or 4% threshold per hour of sleep. Throughout this thesis, the oxygen desaturation index ≥4% will commonly be used ($ODI_{\geq 4\%}$). A 4% threshold for ODI will be used oxygen desaturations below this this threshold are not associated with cardiovascular disease (10). The ODI is closely correlated to the AHI (11), as typically apnoeas and hypopnoeas will cause an oxygen desaturation. There are notable exceptions to this relationship; in healthy individuals with high normal lung volumes, apnoeas and hypopnoeas may not cause an oxygen desaturation. In this case the ODI may underscore the amount of OSA (12). In contrast in individuals with underlying lung disease with low resting oxygen saturations and less “oxygen reserve” the ODI may overscore OSA (13).

The AHI and the ODI do not distinguish obstructive from central events; this can be done on the basis of respiratory effort during the events. Obstructive events are characterized by ongoing or increasing respiratory effort against a narrowed or collapsed upper airway. Bands sensing movement, applied around the thorax and/or abdomen, will detect this ongoing effort, although the magnitude of efforts/movements is reduced and the efforts/movements

are frequently paradoxical. Central apnoeas and hypopnoeas are characterized by an absence of respiratory effort and therefore the absence of movements detected by abdominal and thorax effort bands.

A threshold of an AHI or ODI of >5 events/hour is commonly used to determine the presence of OSA. This threshold is arbitrary as the AHI is not necessarily related to the degree of symptoms as will be discussed in more detail later. OSA is typically defined as mild when the AHI or ODI is between 5-15 events/hour, moderate OSA when the AHI is 15-30 events/hour, and severe when the AHI is >30 events/hour.

1.4. Epidemiology of OSA

OSA is common, although exact estimates of its prevalence depend on its definition and the population being studied. Community based study estimates of the prevalence of OSA widely vary between 2-80% of the population (14)(15). This variation is largely explained by the exact definition of apnoeas/hypopnoeas, and the presence or absence of symptoms (the difference between OSA and OSAS). Early work published in 1993 from the Wisconsin cohort study performed polysomnography (PSG) on a community based middle-aged population enriched for the presence of habitual snoring (14). This study recruited a random sample of community dwelling individuals employed by four state organisations in Wisconsin (USA). They used a two-stage sampling technique. Firstly, over 3500 individuals were sent questionnaires and secondly a cohort of 602 individuals — enriched for those who reported habitual snoring — had overnight sleep studies. In this study they firstly looked at the

prevalence of SDB defined by the AHI and individuals were divided into those with an AHI <5 (individuals without SDB), those with an AHI ≥5, ≥10 and ≥15 (individuals with different thresholds of SDB). Hypopnoeas were defined as a significant reduction in airflow in association with an oxygen desaturation ≥4%. Second, they estimated the prevalence of OSAS by looking at the presence of both a raised AHI and hypersomnolence, assessed using a questionnaire.

In total 625 individuals underwent polysomnography (250 women and 352 men). They estimated that 9% of women and 24% of men between the ages of 30-60 years had an AHI ≥5, and that 4% of women and 9% of men had an AHI of ≥15. They estimated the prevalence of OSAS as 2% amongst women and 4% amongst men (AHI ≥5 and hypersomnolence). A criticism is that the proportion of individuals with SDB who reported hypersomnolence, which was 23-24%, is similar to the proportion of the general population who have raised sleepiness scores, with between 14 and 21% of the general public estimated to daytime hypersomnolence (16)(17). It is not clear that the proportion of individuals with SDB and sleepiness is any higher than those without SDB in community based populations.

The original Wisconsin study is now over twenty years old and, with increasing levels of obesity, the prevalence of OSA is likely to be higher. The Wisconsin study was extended, both with new individuals included, and with original participants undergoing repeated sleep studies on a four-yearly basis between 1988-2011. In total 1520 individuals had at least one sleep study over this period and over two thirds of individuals had undergone between two

and six sleep studies. Sleep studies were scored with the same hypopnoea definition but after 1994 the Epworth Sleepiness Score (ESS, see section 1.6) was added and a score of >10 was used to define to hypersomnolence. Moderate or severe SDB prevalence was estimated to have increased from 8.8% of the population between 1988-1994 to 13.0% between 2007-2010 in men, and from 3.9% to 5.6% in women. Moderate or severe OSAS prevalence was estimated to have increased 3.9% to 5.6% in men and from 1.3% to 1.9% in women.

The Wisconsin studies defined hypopnoeas as significant reduction in airflow or respiratory effort in association with an oxygen desaturation >4%. In 2012, the American Academy of Sleep Medicine (AASM) produced its latest manual for scoring sleep-studies. Importantly the AASM 2012 manual defined hypopnoeas as a 30-90% reduction in airflow in association with either a >3% oxygen desaturation or an EEG scored arousal from sleep (2). The changes in the AASM 2012 were brought about for three reasons. First, these changes standardise AHI reporting as previously two hypopnoea definitions had existed. Second, hypopnoeas leading to arousal but without desaturation are now included as they are still associated with symptoms that improve with CPAP (18). Finally, hypopnoeas are scored with a 3% definition, as derivation of the AHI with both 3 and 4% definitions are associated with cardiovascular disease (19) — although this it has been eloquently demonstrated that this association is driven by desaturations ≥4% (10). AASM 2012 AHI definition changes have a significant impact on the estimated prevalence of OSA. For example, in patients investigated for potential OSA with PSG, the AHI increased from 15 /hour using the recommended AASM 2007 criteria to 38 /hour using the 2012 criteria (3). Similarly, a separate study, observed a 90% increase in the AHI using the 2012 criteria when compared to the 2007 criteria (20).

Whilst these studies of individuals referred for investigation of potential OSA suggest that using the 2012 criteria increase the AHI, they do not show how this impacts upon estimates of prevalence of OSA in the community.

Using the AASM 2012 criteria, the HypnoLAUS study conducted over 2000 sleep studies on a community based population in Lausanne, Switzerland. Subjects were recruited from a previous study and were invited consecutively. Hypersomnolence was defined as an ESS >10. SDB was estimated to be present in 84% of men and in 61% of women studied using an AHI >5, and in 50% of men and in 23% of women using an AHI >15. The estimated prevalence of OSAS was much lower, for example in men under 60 years old approximately 40% of individuals had an AHI of 5-15 and only approximately 6% had an AHI of 5-15 with an ESS >10.

The high prevalence rates observed in the HypnoLAUS study occurred in a cohort with relatively low levels of obesity. The HypnoLAUS study included patients with a mean BMI of 25.6 kg/m^2, likely to be much lower than the updated Wisconsin study where 80.5% of those studied were overweight or obese. It is therefore likely that in the Wisconsin cohort OSA prevalence estimates would be even higher. The definition of a hypopnoea clearly impacts upon the estimates of the prevalence of SDB. An important limitation when considering this increase in estimated OSA prevalence are the older age groups sampled in the HypnoLAUS and follow-up Wisconsin studies compared to the original Wisconsin sleep cohort study, although statistical adjustments were made for age. SDB can be identified in middle aged

adults in 9-60% of women and 24-80% of men, with the variance in estimates depending mainly on the hypopnoea definition. The prevalence of OSAS based on the finding of SDB and hypersomnolence in middle aged adults is estimated to be lower, at 2-10% of women and 4-12% of men.

1.5. Aetiology of OSA

OSA is caused by intermittent collapse of the oropharynx at the retroglossal or retropalatal level during sleep. Oropharyngeal patency is maintained during the daytime by strong upper airway dilator muscles. During sleep, there are decreases in both tonic and phasic activity in the pharyngeal dilator muscles. The primary pharyngeal dilator muscle is the genioglossus and it acts to move the tongue anteriorly, thereby increasing pharyngeal space. During the second stage of non-rapid eye movement (non-REM) sleep there are decreases in both tonic and phasic genioglossus muscle activity. During rapid eye movement (REM) sleep there are further reductions in muscle activity (1). This decrease in upper airway dilator muscle activity leads to increased upper airway resistance in both healthy individuals and in patients with OSA (21), leading to propensity of upper airway collapse at the retroglossal or retropalatal level. This loss in muscle activity is not the only determinant of OSA. Risk factors such as obesity, pharyngeal crowding, older age and male gender, all increase the likelihood of OSA.

1.5.1. Obesity and OSA

The major risk factor for OSA is obesity and with increases in the levels of obesity in Western populations the prevalence of OSA is likely to increase. There are both local and distant effects of obesity that predispose to the development of OSA.

Locally, increased external neck circumference and increases in size of upper airway structures with fatty infiltration predispose to the development of OSA. Epidemiological studies show a clear relationship between the external neck circumference and the severity of OSA, as measured by the AHI (22). This is probably due to increasing adipose tissue around the neck causing external loading pressures on the pharynx and causing extra-luminal compression of the upper airway. In addition to associations with severity of OSA, neck circumference has been used to predict the CPAP setting required to treat OSA (23). The pressure required to treat sleep apnoea, or CPAP setting, is a measure of upper airway collapsibility (24), and so therefore increased external neck circumference is associated with increased loading pressures on the oropharynx. One limitation is that this relationship has not been found in very obese populations (13).

In addition to increasing neck circumference, the size of upper airway structures has been shown to be associated with the AHI. Using magnetic resonance imaging (MRI) patients with OSA have been shown to have larger fatty infiltrated tongues, parapharyngeal fat pads and other upper airway structures when compared to control subjects (25)(26). In addition, three dimensional MRI reconstructions show patients with OSA to have smaller airway volumes at

both the retroglossal and retropalatal levels. These reductions in airway volume are likely to be secondary both to intra-luminal factors such as increased size of the tongue and extra-luminal factors like increased neck circumference.

Acoustic pharyngometry is a technique used to measure the cross-sectional area of the upper airway. Sound is emitted from a mouthpiece device and reflected waveforms are received by the device. Patients with OSA have a smaller mean pharyngeal minimal cross-sectional area (27). Similarly to MRI measurements of the retropalatal and retroglossal airway volumes the minimal cross-sectional airway area is dependent on both intra-luminal and extra-luminal factors.

There are also distant effects of obesity on the upper airway. Thoracic and abdominal obesity lead to restriction of lung volume and reduced end-expiratory lung volume. Experimental reductions in end-expiratory lung volume have been shown to increase pharyngeal collapsibility (28). Reductions in end-expiratory lung volume decreases caudal traction on the pharynx thereby increasing pharyngeal collapsibility.

1.5.2. OSA and age

OSA is increasingly common with increasing age. In the Wisconsin study the prevalence of sleep disordered breathing (AHI ≥5) increased from 17% in men aged 30-39, to 31% in men aged 50-60, and from 6.5% to 16% in women aged 30-39 and 50-60 respectively (14). In the HypnoLAUS study, increased risk of OSA was observed with increasing age (15). The reasons

for increasing prevalence of OSA with increasing age are not fully understood but probably include increasing upper airway fatty infiltration of soft tissue structures, reduced pharyngeal dilator muscle responsiveness, decreased end-expiratory lung volume, and changes in control of ventilation (29). Alternatively, changes in testosterone levels (30), or hyoid bone position (31), may contribute to age related increases in OSA prevalence.

1.5.3. OSA and gender

OSA is more common in men than women (14)(15). There are several potential reasons why this might be the case. First, there are anatomical differences in the upper airway between men and women. On average men tend to have longer upper airways and this may explain much of the male tendency to increased upper airway collapsibility (32). In addition, men tend to have larger tongues and other upper airway structures (33).

There may also be hormonal influences on the propensity towards developing OSA. There is a stronger relationship between age and risk of OSA in women than in men which is perhaps explained by hormonal changes around the time of the menopause (15). Indeed, post-menopausal women who are not treated with hormone replacement therapy have an increased prevalence of OSA (2.6%), when compared to post-menopausal women who have hormonal replacement therapy and premenopausal women (0.6%) (34). The median AHI was 2.8 /hour in premenopausal women, and 8.7 /hour in postmenopausal women in the HypnoLAUS study (15). Theories as to why hormonal changes may influence OSA severity include stimulatory effects of oestrogen and progesterone on ventilatory drive (35), effects

of oestrogen on genioglossus fatiguability (36), and alterations in rostro-caudal fluid shifts (37). It is worth noting that these analyses were not adjusted for differences in obesity or comorbidities so it possible that increases in the AHI following the menopause could be due to confounding factors such as increased obesity.

In addition to obesity, age and male gender, risk factors for OSA include retrognathia and tonsillar enlargement, both of which increase pharyngeal crowding, predisposing to pharyngeal collapse (38).

1.6. OSA and sleepiness

Daytime sleepiness is the most recognised consequence of OSA. Patients with OSA have been shown to have increased subjective and objective sleepiness. The Epworth Sleepiness Score (ESS) was developed as a subjective measure of daytime sleepiness by Johns. He showed that patients with sleep disorders including OSA scored more highly on the ESS than control subjects (39). Furthermore, in patients referred for investigation of potential SDB increasing severity of OSA was associated with increasing ESS values (40). It is worth noting that there was significant overlap particularly between those with simple snoring and those with mild to moderate OSA.

The Multiple sleep latency test (MSLT) involves electro-encephalogram (EEG) recordings whilst an individual lies in a darkened room multiple times during the day and attempts to sleep. In patients referred for investigation of SDB, increasing severity of OSA is associated

with shorter sleep latency on the MSLT, indicative of daytime sleepiness (41). Arousals from sleep due to respiratory events, the apnoea index, and the degree of hypoxaemia are all modestly correlated with the MSLT. With correlation coefficients of -0.36, -0.32 and -0.34 respectively, they each only explain approximately 10-13% of the variation in objective sleepiness. Arousals, apnoeas and hypoxaemia are all related to each other and it is not clear which of these factors, or if other factors, determines risk of daytime sleepiness.

The association between the severity of SDB and daytime sleepiness in community based populations is not as clear. As previously mentioned, the Wisconsin sleep cohort estimated that 23-24% of those with SDB had daytime sleepiness, which is comparable to levels of an elevated ESS in the general population. Results from other large epidemiological studies are contrasting. In the Sleep Heart Health Study investigators found increasing levels of daytime sleepiness as measured by the ESS with increasing severity of sleep disordered breathing (42). There was a high degree of variability, many individuals without measurable SDB had an ESS ≥11 (21%), and many individuals with severe SDB had an ESS <11 (65%). The HypnoLAUS study found no correlation between the severity of SDB and the ESS (15). Therefore, whilst patients being investigated and treated for OSA have greater levels of daytime sleepiness, the association between daytime sleepiness and degree of sleep disordered breathing is less clear in community-based populations.

The effects of treatment on daytime sleepiness are discussed in the next section. Other potential complications of OSA are less clearly improved by treatment and are discussed later.

1.7. Treatments for OSA

1.7.1. Treatments for OSA: Continuous Positive Airway Pressure (CPAP)

Continuous positive airway pressure (CPAP) is the standard treatment for OSA. CPAP generally is delivered via a portable bedside machine connected to a tight-fitting face mask. CPAP works as a pneumatic splint, holding the upper airway open during sleep. CPAP has been shown to be an effective therapy in improving patient symptoms in OSAS (4)(5)(43). An early randomised controlled trial (RCT) comparing CPAP to sham CPAP in patients with a raised ESS and significant OSA (ODI>10) showed a marked improvement in sleepiness with CPAP (5). There was an 8.5 point reduction in ESS with CPAP compared to a 2 point reduction with sham CPAP. Marked improvements in objective sleepiness were also observed over four weeks. Meta-analyses have found the effect of CPAP on subjective sleepiness to be less marked (4)(43). Trials included in these meta-analyses have differing methodologies and are heterogeneous. All severities of OSA were considered as were patients with minimal symptoms from OSA. CPAP appeared to have a more marked effect on the ESS in those with severe OSA, where CPAP was estimated to reduce ESS by 2.6 points, than with mild OSA, where CPAP was estimated to reduce the ESS by 1.2 points (4). CPAP also significantly improves quality of life indices (5)(44)(45). Studies have shown improvements in sleepiness and quality of life even in those with minimally symptomatic or

milder OSA (46)(47)(48)(49). This has not been a universal finding in minimally symptomatic patients (50).

1.7.2. Other treatments for OSA

Other treatment options in OSA include weight loss, reducing alcohol intake, mandibular advancement devices (MAD), an implantable hypoglossal nerve stimulator, and positional therapies. Weight loss has been shown to be an effective treatment for OSA, reducing pharyngeal collapsibility and severity of sleep disordered breathing (51). Both dietary weight loss (52), and bariatric surgery (53), are effective in reducing the AHI, although bariatric surgery tends to lead to larger reductions in both weight and AHI. Although CPAP is more effective in reducing the AHI than dietary weight loss, there is little data on patient related outcome measures following weight loss.

MAD have been shown to reduce both the AHI and to improve the ESS in patients with mild to moderate OSA (54). Meta-analyses have shown small improvements favouring CPAP over MAD in the ESS (4), and small improvements favouring MAD over CPAP for quality of life (45), but neither results were significant. There is a lack of direct data comparing CPAP to MAD to make firm conclusions as to the non-inferiority or superiority of CPAP or MAD.

Newer therapies that are being developed for OSA include implantable hypoglossal nerve stimulators and positional therapies. Implantable hypoglossal nerve stimulators were shown to reduce the severity of OSA, improve subjective sleepiness, and improved quality of life

scores in a non-randomised trial in patients who had not tolerated CPAP therapy (55). Patients were only included if their BMI was <32 kg/m^2. A subset of patients underwent randomised therapy withdrawal compared to continued therapy for one week after 12 months of effective therapy. This showed a marked increase in the AHI in the therapy withdrawal group. Given that only 46 patients were included and that therapy withdrawal lasted for only one week no patient related outcome measures were assessed.

Recently new devices have been developed to treat positional obstructive sleep apnoea. Supine positioning worsens pharyngeal collapsibility and propensity to OSA (56). Over 40% of patients with OSA have positional OSA, where changing their sleeping positional would be likely to treat or significantly improve their OSA (57). New positional therapy devices are attached to bands, either around the abdomen or neck, and sense sleeping position. When supine posture is detected these devices then vibrate to stimulate the patient into moving to an alternate sleeping position. A non-randomised trial of such a positional therapy device reduced the severity of OSA compared to baseline but did not significantly improve daytime sleepiness or quality of life. Patients included had positional OSA and a BMI <35 kg/m^2 (58). In a trial comparing MAD to positional therapies, positional therapies showed a reduction in AHI at both three and twelve months, but this was not superior to MAD therapy at either time point (59)(60). Patients included had mild to moderate positional OSA. Data collected on daytime sleepiness and quality of life was of poor quality and therefore firm conclusions cannot be drawn. With both implantable hypoglossal nerve stimulators and positional therapy devices high quality RCT data looking at patient related outcome measures are lacking, and are needed before recommending these therapies more widely.

1.8. OSA and road traffic collisions

Road traffic collisions are a potential complication of OSA that are likely to relate to sleepiness. Early work from Canada demonstrated an approximately threefold increased rate of road traffic collisions in patients with OSA prior to treatment, compared to post treatment collisions in the same patients and in control subjects (61). This study did not adjust for driving exposure although average driving distances were comparable before and after CPAP treatment. In addition, control subjects were randomly selected and had not been questioned about sleepiness or undergone sleep studies. Following on from this work many trials have assessed driving performance in a simulated setting. Simulated driving performance is worse in patients with OSA than in control subjects (6), and CPAP improves performance in patients with OSA when compared to sham (62).

A meta-analysis of studies assessing driving risk and OSA found a 2.4 fold increased risk of road traffic collisions in patients with OSA compared to control subjects (7). Studies included had varying methodology and assessed disease severity and sleepiness in different ways. A diagnosis of OSA was a significant predictor of road traffic collision, but trends between both increasing AHI and ESS were not significant. Only three studies were included in the meta-analysis that assessed the relationships between the AHI or the ESS and road traffic collisions, so this non-significant finding could be an issue of statistical power. A further meta-analysis in patients with OSA from Tregear *et al.* (63), showed that CPAP treatment decreases the risk of road traffic collisions. In fact either full or partial CPAP adherence

probably mitigates the risk of road traffic collisions in lorry drivers, whilst the risk remains high in those who are entirely non-adherent with CPAP therapy (64).

1.9. OSA and hypertension

There is compelling evidence of a causal link between OSA and hypertension, both from epidemiological studies and RCTs. In 1993, in a small number of patients with OSA, increasing severity of OSA, as measured by the AHI, was associated with both increasing systolic and diastolic blood pressure (65). In 2000 three important studies were published supporting a link between OSA, elevated blood pressure, and hypertension (66)(67)(68). Davies *et al.* published a small case-control series with 45 patients with OSA and 45 age and BMI matched control subjects without OSA (68). Patients with OSA had elevations in both daytime and night-time systolic and diastolic blood pressure, along with the loss of the normal nocturnal dip in blood pressure. The Sleep Heart Health Study was a cross-sectional cohort study with over 6,000 individuals with robust data collection for blood pressure and PSG assessments. Individuals underwent prospective assessment of blood pressure during the daytime and subsequently underwent unattended home PSG. Participants were only assessed if they had data from blood pressure and PSG assessments and relatively few patients were not included on this basis (~5% for each). There was a clear relationship between increasing AHI and both systolic and diastolic blood pressure, and between increasing AHI and current diagnosis of hypertension (67). Prospective data after four years of follow-up in the Wisconsin sleep study showed that an elevated AHI increased the odds of developing hypertension (66). In the more recent HypnoLAUS study the highest quartile of AHI had an odds ratio of 1.6 of having a diagnosis of hypertension when compared to the

first quartile of AHI (15). The association between sleep-disordered breathing, elevated blood pressure and hypertension is only partially explained by BMI.

Randomised controlled trials have shown that the effect of OSA on blood pressure is partially treatable with CPAP (69)(70). Pepperell *et al.* randomised 118 patients with OSA to one month of either CPAP or sham CPAP therapy (69). There was a reduction of 3.3mmHg in mean 24-hour ambulatory blood pressure with CPAP compared to sham. This was partially made up of a small 0.8mmHg increase in mean ambulatory blood pressure in the sham CPAP group. This study looked only at patients with significant OSA (an ODI >10) and daytime sleepiness (ESS >9). A meta-analysis published more recently estimated the treatment effect of CPAP to be slightly less than this, 2.3mmHg on systolic blood pressure and 2.0mmHg on diastolic blood pressure (70). The seven RCTs included in this meta-analysis had significant differences in their methodology. The control group was sham CPAP in three studies whereas the other four studies had no active control. In addition, some studies included non-sleepy patients. The lack of sham control treatment may have introduced bias into studies but it is also worth noting that sham CPAP may further disrupt sleep and actually cause elevations in blood pressure. The inclusion of non-sleepy patients may have affected the results if the effect of OSA on blood pressure is isolated to sleepy patients.

The meta-analysis by Hu *et al.* (70), estimated the effect of CPAP on reducing blood pressure to be greater in both patients with resistant hypertension and patients who are already on at least one anti-hypertensive medication. The combination of losartan and CPAP has been

explored in patients with OSA (71). Whilst losartan lowered blood pressure in patients with OSA similarly to patients without OSA, the addition of CPAP to losartan in patients with OSA did not lower mean 24-hour ambulatory blood pressure. Reductions in night time systolic blood pressure were seen with the addition of CPAP to losartan. Robinson *et al.* looked at patients with a diagnosis of resistant hypertension, OSA (defined as an ODI >10), and who were not sleepy (defined as an ESS <10) (72). This study probably lacked statistical power to find a small effect on blood pressure, including 35 patients in a cross-over design. It was powered to find a difference in mean 24-hour ambulatory blood pressure of 5mmHg. There were small non-significant reductions in awake (2.1mmHg) and sleeping (2.9mmHg) mean 24-hour ambulatory blood pressure. A similar small RCT looked at the effect of CPAP versus sham on 24 hour ambulatory blood pressure and found a 9.3mmHg reduction in mean systolic blood pressure (73). The confidence intervals for this estimate were wide (0.4 to 17.9mmHg) due to the small sample size (n=45).

The HIPARCO study is the largest RCT to date looking at the effect of CPAP in resistant hypertension, including 194 patients (74). Patients with moderate or severe OSA and resistant hypertension, despite usage of three or more anti-hypertensive medications, were included. Patients were randomised 1:1 to either CPAP or no intervention. Compliance with anti-hypertensive medications was assessed by questionnaire and pill count prior to randomisation. CPAP led to a 3.9mmHg reduction in mean 24-hour blood pressure. One limitation of this study is the lack of blinding as there was no sham therapy given. Potentially those not allocated to CPAP could have altered their behaviour or increased their compliance with anti-hypertensives which could have influenced the estimated effect of

CPAP on blood pressure. Caffeine increases blood pressure, particularly in those with hypertension. Caffeine consumption was not measured and could have been greater in those not randomised to CPAP, which could have biased study findings (75).

1.10. OSA and cardiovascular disease

OSA has been shown to be associated with cardiovascular events and mortality from cardiovascular causes (9)(76). In 2005, Marin *et al.* published data from an average of 10 years follow-up including over 1000 male patients with OSA of differing severity, 377 male snorers and 264 healthy men (9). This estimated an approximately threefold increased risk of fatal and non-fatal cardiovascular events in patients with severe untreated obstructive sleep apnoea when compared to healthy control subjects. Similarly, 18-year follow-up data from the Wisconsin sleep study of over 1500 patients estimated a five-fold increased risk of cardiovascular death in patients with severe OSA compared to those without OSA. Both the Marin study and the Wisconsin follow-up study estimates were adjusted for likely confounders. Adjustments were made for multiple potential confounders if they were significant ($p<0.10$) in univariate Cox proportional hazard model analysis; age, BMI, presence of cardiovascular disease, hypertension, diabetes, hyperlipidaemia, smoking, alcohol use, anti-hypertensive use, lipid lowering medications and anti-diabetic medication in the Marin study and age, gender and BMI in the Wisconsin follow-up study. Furthermore, Marin *et al.* found that the risk of cardiovascular events was similar when comparing patients on treatment for severe OSA to healthy control subjects. This may suggest that the link between OSA and cardiovascular disease is causal but there are likely to be other important differences between individuals who accept CPAP and those who reject CPAP.

Untreated patients with severe OSA who reject CPAP may not be as compliant with medical therapies or lifestyle advice and these other factors, and not usage of CPAP, may be responsible for the observed differences in cardiovascular disease. RCTs are necessary to provide evidence that treating OSA is of benefit in reducing cardiovascular disease. I will explore the evidence from RCTs (section 1.15) after considering the potential underlying causes for cardiovascular disease in OSA (sections 1.12, 1.13, & 1.14).

1.11. OSA and its associations with other comorbidities

OSA is associated with numerous other conditions. Most evidence of disease associations comes from uncontrolled and observational studies. Other than the effects of CPAP on blood pressure there is little evidence that CPAP reduces the incidence or recurrence of other potential complications. In this section I will consider the evidence for associations between OSA and some of its potential complications, particularly those related to cardiovascular disease.

Atrial fibrillation (AF) is associated with OSA. AF is a major risk factor for stroke (77). OSA may lead to AF by changes in ventricular modelling due to raised blood pressure, increased left atrial filling, or increase sympathetic activity (78). There is evidence that increasing severity of sleep apnoea is associated with increased long-term risk of developing new onset AF (79). This study included 6841 sleep apnoea patients with and without AF, 58 of these had dominantly central sleep apnoea and 832 were using CPAP treatment. Removing those with central sleep apnoea, and those who were treated with CPAP, was reported to have

negligible effect on this relationship, but the results of this further analysis were not reported.

There is also an increased recurrence of AF following cardioversion therapy in patients with OSA not on treatment, compared to those with OSA on treatment and those without OSA (80). This study was an uncontrolled study and patients who reject CPAP treatment are likely to differ from those who accept it which may bias the results. In addition, those in the control group did not have a sleep study so may have asymptomatic sleep apnoea.

Changes in cardiac repolarization have been demonstrated with return of OSA in the CPAP withdrawal model (81). The CPAP withdrawal model will be discussed in more detail later (section 1.16). In contrast there were no changes in cardiac repolarization or AF arrhythmia risk markers in a traditional RCT design in patients with minimally symptomatic OSA (82), and no changes were observed in the actual number of dysrhythmias in patients with moderate or severe OSA treated with CPAP (83). In a large trial of CPAP for secondary prevention of cardiovascular disease there was no difference in the incidence of new onset AF between CPAP and control (49).

One of the complications of Type 2 diabetes mellitus (T2DM) is cardiovascular disease with increased risk of myocardial infarction and stroke (84). There are both increased rates of T2DM in patients with newly diagnosed OSA (85), and increased rates of OSA in patients with T2DM (86), independent of obesity. In non-diabetic, overweight and obese subjects,

small improvements in blood glucose control have been shown with eight hours per night of CPAP compared to control, in a very controlled hospital setting (87). There are also small improvements in insulin sensitivity with CPAP in both non-diabetic patients with OSA (88), and in patients with T2DM and OSA (89). The standard way of assessing long-term glycaemic control is by measuring the glycosylated haemoglobin concentration (HbA1c) (90). One small RCT showed a slight benefit in average HbA1c with CPAP therapy (91). However most RCTs (92)(93), and a meta-analysis (89), have shown no benefit of CPAP in terms of HbA1c.

There are numerous associations between OSA and eye conditions. Perhaps the most established link is with diabetic maculopathy where the severity of OSA is an independent predictor of disease severity (94). A small non-randomised pilot study also suggested an improvement in visual acuity in patients with moderate or severe OSA and diabetic retinopathy with clinical significant macular oedema who used CPAP for over 2.5 hours/night (95). The results of an appropriately powered RCT exploring the effects of CPAP on diabetic retinopathy are awaited (ISRCTN 95411896). In addition, there are associations between OSA and risk of open-angle glaucoma, normal pressure glaucoma (96)(97), and non-arteritic ischaemic optic neuropathy (NAION) (98). However, these associations are from unadjusted analyses and did not account for potential confounders. It is not clear if OSA has a causal role in the development of glaucoma and NAION, or if OSA is simply a marker of other conditions such as obesity. Furthermore, the effects of CPAP on glaucoma and NAION have not been assessed in RCTs.

Along with the associations discussed above, OSA has also been linked to other conditions including aortic aneurysms, cancer, and to cognitive impairment. The prevalence of thoracic aortic aneurysm seems to be increased in OSA populations, particularly in those with severe OSA. Large intrathoracic pressure swings that occur during apnoeic events are a potentially important mechanism in the pathogenesis of aortic aneurysm (see section 1.14). There is also an association between OSA and abdominal aortic aneurysm so it is likely that other factors are also involved in the link between OSA and aortic disease (99). The association between OSA and cancer has not been consistently observed and is far from certain. In addition, the role CPAP treatment for OSA has in preventing cancer or delaying cancer progression has not been adequately assessed (100). Hypoxia is important in cancer pathogenesis but whether the intermittent hypoxia seen in OSA contributes to the development or acceleration of cancer is not known (101). OSA is associated with impaired cognitive function, causing impaired memory, inattention, impaired processing speed, and reduced executive functioning. Cognitive impairment in OSA is probably the result of both sleepiness and underlying structural brain changes possibly due to vascular damage (102). The extent to which cognitive function is treatable with CPAP is not fully established.

1.12. Intermittent hypoxia

1.12.1. Intermittent hypoxia, hypertension, and sympathetic activation

Hypertension is one of the main risk factors for cardiovascular disease and the leading cause of mortality from stroke and ischaemic heart disease (103). As discussed already, OSA is associated with increased blood pressure (68), and with increased diagnoses of hypertension (67). CPAP treatment, in patients with OSA, reduces both systolic and diastolic blood

pressure by approximately 2 to 3mmHg (69)(70). The additional benefit of CPAP may be more marked in those with resistant hypertension on multiple medications.

Animal models of intermittent hypoxia have shown its potential importance in the development of hypertension in OSA. Fletcher's group elegantly showed in some species of rodents that intermittent hypoxia leads to a significant increases in blood pressure that are independent of hypercapnia (104). This was dependent on carotid chemoreceptors (105), the sympathetic nervous system (106), the renal artery (107), and the renin-angiotensin-aldosterone axis (108). Sustained rises in blood pressure have been shown to be secondary to upper airway occlusion and its consequences including intermittent hypoxia, and not recurrent EEG-defined arousals, in a canine model of OSA (109).

Exposure to hypoxia is known to lead to elevations in blood pressure in healthy individuals, both in those exposed to sustained hypoxia at altitude (110), and those exposed to intermittent hypoxia (111). Tamisier *et al.* exposed healthy individuals to 14 nights of intermittent hypoxia (111), and from the first night onwards, observed increases in mean and diastolic daytime blood pressure, but not heart rate. In addition, daytime sympathetic activity, measured by muscle sympathetic nerve activity, was increased by intermittent hypoxia. Interestingly they did not observe changes in overnight blood pressure. It is likely that this relates to methodological limitations; They used standard ambulatory blood pressure monitoring which can disturb sleep and raise blood pressure (112), and not

continuous non-invasive blood pressure monitoring. Equally it could be that other mechanisms, and not intermittent hypoxia, determine blood pressure overnight.

Acute blood pressure rises overnight are likely to be related to arousal and not intermittent hypoxia. Blood pressure rises accompany arousal in healthy individuals with simulated apnoeas (113), and following spontaneous apnoeas in patients with OSA (114). Blood pressure rises are similar when arousals are induced by apnoeas with or without supplemental oxygen in patients with OSA (115), with arousals from an auditory stimulus in patients with OSA (114), or with arousals from a combined auditory and vibratory stimulus in healthy volunteers (116). Hypoxia without arousal does not lead to acute blood pressure rises (114). Furthermore, the blood pressure response to auditory induced arousals in healthy individuals were not different during normoxia compared to hypercapnic hypoxia (117). It is therefore plausible that night-time blood pressure in OSA is mainly determined by acute arousal driven rises in blood pressure.

Intermittent hypoxia in OSA may have a modulatory effect on sympathetic activation (118), and sustained hypoxia increases sympathetic activation for days following descent to sea level (119). The combination of OSA and hypoxia induced by travel to altitude in lowlanders causes elevations in blood pressure compared to levels prior to ascent, and this increase is somewhat mitigated by acetazolamide (120). Acetazolamide improves both mean oxygen saturations as well as the AHI at altitude so it is not clear if the effect on blood pressure is

due to improvements in hypoxia or other mechanisms such as reduced arousal mediated sympathetic activity.

Intermittent hypoxia may be important in leading to increased blood pressure in OSA via sustained increases in sympathetic activation. Therefore, it seems a reasonable hypothesis that supplemental oxygen in OSA, by attenuating intermittent hypoxia, may lead to similar reductions in blood pressure as CPAP. To date there have been two randomised controlled trials looking at the longer-term effect of supplemental oxygen on blood pressure in OSA (121)(122). Although supplemental oxygen was shown to reduce urinary noradrenaline, suggesting a reduction in sympathetic activation over two weeks (122), supplemental oxygen had no effect on daytime blood pressure over two weeks (122), or twelve weeks (121). Both of these studies have limitations; low flow rates of oxygen were used (2 or 3 l/min), in one study patients with severe OSA or severe hypoxaemia were excluded (121), and CPAP had only a small treatment effect on blood pressure (1.9mmHg). Blood pressure was not optimally measured; one study used standard ambulatory blood pressure monitoring to record 24-hour blood pressure rather than non-invasive measures (121), whilst the other used office recordings of blood pressure (122). Therefore, these have not definitively assessed the role of overnight intermittent hypoxia in the observed elevated blood pressure seen in patients with OSA.

1.12.2. Intermittent hypoxia and oxidative stress

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and antioxidant mechanisms. Intermittent hypoxia is thought to lead to oxidative stress by decreasing antioxidant mechanisms in periods of hypoxia and increasing reactive oxygen species production during periods of reoxygenation; termed an ischaemia-reperfusion injury (123). Oxidative stress is thought to be an important mechanism in the development of cardiovascular disease (124). Oxidative stress may lead to hypertension via increased brain nuclei sympathetic activation and increased angiotensin II (125), and endothelial dysfunction which is thought to be a precursor of atheroma formation (126), and will be discussed later in section 1.12.3. Whilst obesity and diabetes are more established risk factors for oxidative stress (127), the role of intermittent hypoxia in OSA is less certain.

Animal experiments have shown tissue specific increases in oxidative stress following intermittent hypoxia, for example; in the heart (128), in the brain (129), and in the mesenteric arteries (130). In human experiments, where healthy individuals were exposed to intermittent hypoxia during the daytime, some blood biomarkers of oxidative stress have been found to increase (131).

Patients with OSA exhibit increased levels of ROS production from monocytes and granulocytes, when compared to control subjects (132). In addition, endothelial cells harvested from forearm veins of patients with OSA show signs of increased inflammation

and oxidative stress; the degree of oxidative stress correlates to the degree of endothelial dysfunction (126). A novel breath analysis technique has highlighted a family of compounds associated with OSA, that are linked to oxidative stress (133). However, elevated oxidative stress is not a universal finding in OSA, with others reporting no increases in systemic markers of oxidative stress in OSA (134)(135).

Work by Prabhakar's group shows the importance of oxidative stress in the carotid body in modulating the chemoreceptor response to intermittent hypoxia in rodents (136)(137). Intermittent hypoxia upregulates hypoxia-inducible factor (HIF) -1 alpha activation and downregulates HIF-2alpha, leading to increased ROS formation in the carotid body. This is hypothesised to underlie increases in carotid body chemoreceptor responses, in sympathetic activation and hypertension in OSA.

Oxidative stress and its relationship to cardiovascular disease is tissue specific (138). Whilst blood is readily accessible, changes in traditional blood biomarkers of oxidative stress may not accurately reflect levels of oxidative stress in the coronary arteries or elsewhere in the cardiovascular system. Novel approaches are required to establish the role that tissue specific oxidative stress plays in the development of cardiovascular disease in OSA.

1.12.3. Intermittent hypoxia and endothelial dysfunction

An important role of the endothelium is in sensing changes in blood flow and releasing substances that regulate arterial calibre in response to these flow changes, described as

endothelial function. It has been recognised that impairment of endothelial function occurs in both hypertension (139), and in patients with coronary artery atherosclerosis (140), Therefore endothelial dysfunction is thought to be an early stage in the development of cardiovascular disease. Endothelial function is commonly assessed by measuring flow mediated dilatation at the brachial artery (141), and this non-invasive measurement is used as a surrogate marker of endothelial function elsewhere, such as in the coronary arteries.

Animal models suggest that intermittent hypoxia leads to endothelial dysfunction. Intermittent hypoxia has been shown to impair endothelial dependent vasodilation compared to normoxia, and this effect is somewhat mitigated by the xanthine oxidase inhibitor allopurinol (142). Using apoE deficient mice, only mice with early pre-atherosclerosis, and not mice fed a high fat diet leading to advanced pre-atherosclerosis, had impaired endothelial function following intermittent hypoxia compared to control mice exposed to normal or high fat diet respectively (143). Endothelial dysfunction under conditions of intermittent hypoxia may be dependent on inflammation and oxidative stress, as the anti-inflammatory drug infliximab and the antioxidant drug L-glutathione, both blocked this impairment (144). However, in a different mouse model (the C57BL/6J mouse which is susceptible to diet-induced obesity and atherosclerosis), whilst markers of oxidative stress were increased by intermittent hypoxia regardless of diet, intermittent hypoxia only led to endothelial dysfunction in mice when combined with a high fat diet (145).

In vitro studies, exposing endothelial cells to microvesicles from the blood of individuals exposed to intermittent hypoxia, showed adverse effects on endothelial cell function (146). Experimental exposure to intermittent hypoxia in healthy human volunteers, whilst leading to rises in blood pressure, may not cause the same effect on endothelial function as observed in animal and in vitro studies (147).

There is clear evidence of endothelial dysfunction in patients with OSA (148), and this is improved by treatment with CPAP (149)(150). There is contrasting evidence from animal experiments, in vitro studies, and experimentally induced intermittent hypoxia in healthy individuals, as to the role of intermittent hypoxia in OSA in causing endothelial dysfunction. As will be discussed later, endothelial dysfunction occurs following the return of OSA in the CPAP withdrawal model (148) (section 1.16). CPAP treatment improves endothelial function (149)(150), but this could be due to changes in sympathetic activity or other physiological aspects of OSA.

1.12.4. Intermittent hypoxia and inflammation

Another mechanism linked to intermittent hypoxia that potentially causes cardiovascular disease is systemic inflammation. Activation by hypoxia of both the nuclear factor kappa B, and the hypoxia-inducible factor-1 pathways are proposed mechanisms linking intermittent hypoxia and inflammation (151). Any or all of the carotid body, the endothelium or adipose tissue could be involved in intermittent hypoxia activating inflammation. The interaction between obesity and intermittent hypoxia may also be important. Both obesity and

intermittent hypoxia have been suggested to induce a “pro-inflammatory” state in adipose tissue. The evidence of increased inflammation in OSA is mixed. A meta-analysis of cohort studies showed increased levels of tumour necrosis factor – α (TNF- α), and both C-reactive protein (CRP) and high sensitivity C-reactive protein (hsCRP) (152). However, in a randomised control trial, CPAP did not reduce inflammatory markers, including hsCRP and adiponectin, which is a hormone linked to the “pro-inflammatory” adipose tissue hypothesis (153). It is questionable as to the role of these biomarkers in intermittent hypoxia mediated inflammation in OSA as one might expect an improvement on CPAP. However, it may be that one month of CPAP may be inadequate to reverse these changes as animal models of intermittent hypoxia have only shown partial reversal of an inflammatory state following restoration of normoxia (154). The extent of the contribution of inflammation to cardiovascular disease in OSA remains unknown.

1.13. Cortical and “autonomic” or “sub-cortical” arousal from sleep

Early observations by Remmers *et al.* (155), suggested that almost all obstructive events in OSA were terminated by cortical arousals leading to airway opening. This was thought to be a necessary protective mechanism to restore ventilation and restore blood-gas derangements. However, the patients studied by Remmers *et al.* would now all be classified as having severe OSA and mainly as having obesity hypoventilation syndrome, with most having daytime hypercapnia. Recently it has been observed that whilst arousals are frequently coincident with airway opening, often airway opening occurs prior to arousal or without arousal at all (156). Thus, it has been proposed that in most patients, unlike the severe patients described by Remmers *et al.*, cortical arousal may have a deleterious effect.

Cortical arousal may lead to overshoot in ventilatory drive, perpetuating further obstructive events and therefore worsening sleep fragmentation (157). The relative importance of this to cardiovascular disease is uncertain as the critical factor in determining cardiovascular risk is not known. If it is arousal induced sympathetic activation, then propensity to easy arousal may worsen cardiovascular risk by increasing the number of arousals. Alternatively, if hypoxia is the main determinant of cardiovascular risk then late arousal leading to long events and prolonged hypoxia may increase cardiovascular risk.

"Autonomic" or "sub-cortical" arousals are the concept of brainstem arousal at the termination of an obstructive event with restoration of airway patency. The heart rate rise seen at the end of respiratory events is thought to be a marker of "autonomic" or "sub-cortical" arousal. Heart rate rises, blood pressure rises, the AHI and the ODI, are all moderately correlated with EEG/cortical arousals in patients undergoing investigation for OSA (11). Occasional heart rate rises occur without EEG/cortical arousal in OSA and following an auditory stimulus in health individuals (158). This raises the possibility of "autonomic" or "sub-cortical" arousals occurring without cortical arousal. Autonomic responses tend not to habituate to repeated stimuli and so heart rate rises persist with repeated apnoeas (159). Repeated "autonomic arousals", occurring without EEG changes have been shown to cause daytime sleepiness (160). However, the concept of sub-cortical arousals is not universally supported. Azarbazin *et al.* showed that experimentally induced obstructive events that were terminated by CPAP without arousal were still associated with heart rate rises (161). They argue against the idea of "autonomic" or "sub-cortical" arousals and instead suggest that heart rate rises are more of a physiological reflex response. Supporting this is the

observation that the events capable of producing heart rate rises without EEG arousal were well below the threshold expected to cause an arousal. However, others have argued against this. Jordan *et al.* carried out similar experiments inducing hypopnoeas with incremental drops in the CPAP setting below a therapeutic CPAP setting, and observed heart rate rises both with and without cortical arousal. Crucially events occurring without arousal were associated with tensor palatini, a pharyngeal dilator muscle, activation (162). Tensor palatini muscle activity is strongly influenced by sleep-wake state, with activity levels much decreased during sleep. They argue that the activation of the tensor palatini muscle that coincides with airway opening in events without cortical arousal suggest that they represent "sub-cortical" arousal.

As discussed in section 1.12.1 the acute rises in blood pressure seen in OSA are likely to be due to arousal rather than intermittent hypoxia. Furthermore, increasing numbers of arousals per hour of sleep are associated with increased sympathetic activity, although it is not clear that this is entirely independent of the degree of hypoxia (163). Therefore, autonomic activation induced by arousal, is a potential mechanism by which hypertension and cardiovascular disease may occur in OSA.

1.13.1. Disruption of sleep architecture and sleep fragmentation

Patients with OSA have disruptions to normal sleep architecture. OSA tends to increase the percentage of time in non-REM stage 1 sleep, the number of arousals from sleep, REM sleep latency, and tends to reduce sleep latency, the time spent in slow wave, and REM sleep

(164). Short sleep duration is linked with increasing levels of obesity, diabetes and— probably secondarily— increased cardiovascular mortality (165). In healthy individuals, sleep deprivation to 4 hours/night, along with increasing daytime sleepiness, has been shown to increase daytime blood pressure and reduce nocturnal blood pressure dipping (166). It is possible that sleep deprivation with reduced time spent in certain sleep stages may cause cardiovascular disease in OSA.

1.14. Intrathoracic pressure swings

Intra-thoracic pressure swings are a part of the normal physiology of breathing. Inspiratory muscles generate small increases in thoracic negative pressure necessary to enable the movement of air during inspiration. During normal breathing much of expiration is passive, relying on elastic recoil in the lungs and not active expiratory muscles. During OSA the combination of upper airway obstruction and increased respiratory drive lead to much larger increases in negative intrathoracic pressure during inspiration. Large intra-thoracic pressure swings have been postulated to place shear stress on the heart and great vessels, and alter haemodynamics, both of which could contribute to the development of cardiovascular disease in OSA (167).

It is difficult to solely model the effects of these large intra-thoracic pressure swings on cardiovascular parameters in OSA. The Mueller manoeuvre has been used as a way of attempting to do so. In the Mueller manoeuvre an individual first exhales forcibly and then makes an inspiratory effort via an occluded mouth-piece. Individuals are awake so this does

not lead to changes in sleep-wake state/arousal or hypoxia. Bradley *et al.* showed that this leads to large negative intra-thoracic pressures, reductions in blood pressure, and reductions in cardiac output, in both healthy individuals and patients with congestive cardiac failure (168). The haemodynamic effects of this "simulated apnoea" were more marked and more prolonged in patients with congestive cardiac failure. The Mueller manoeuvre has also been shown to lead to changes that may promote arrhythmias. The Mueller manoeuvre alters the likelihood of atrial premature beats, prolongs ventricular repolarisation (169), and prolongs atrial conduction (170). These electrical changes suggest that the large intra-thoracic pressure swings in OSA may have a causal link with the increased propensity to AF.

The role of large intra-thoracic pressure swings in haemodynamic changes observed during apnoeas is not certain. Experiments in anaesthetised pigs with simulated apnoeas suggest that intermittent hypoxia and sympathetic activation may be more important (171)(172). Similar haemodynamic changes were observed in pigs with simulated obstructive apnoeas induced by endo-tracheal tube obstruction and with simulated central apnoeas induced by succinylcholine (171). In addition, hyperoxia and chemical sympathetic denervation with hexamethonium blocked haemodynamic effects induced by simulated apnoeas (172).

It seems likely that there are effects on cardiac conduction secondary to large intra-thoracic pressure swings, but the haemodynamic effects of large intra-thoracic pressure swings are less certain. Large intra-thoracic pressure swings, by potentially promoting arrhythmias such

as AF, are another potential independent mechanism by which OSA might lead to cardiovascular disease.

1.15. Randomised control trials of CPAP for cardiovascular disease in OSA

As already discussed there is an association between OSA and cardiovascular disease. In uncontrolled longitudinal observational studies, severe untreated OSA has been shown to be a risk factor for cardiovascular disease (9). Whilst adjustments are made for known confounders such as obesity in longitudinal studies, they cannot account for unknown confounders such as compliance with anti-hypertensives and statins. Randomised control trials are helpful in providing further insight into whether OSA has a causal role in the development of cardiovascular disease.

CPAP has been shown to improve surrogate cardiovascular endpoints, such as blood pressure (69)(70), and endothelial function (150), both of which are putative mechanisms for the development of cardiovascular disease. Early RCTs assessing the role of CPAP in cardiovascular disease focussed on minimally symptomatic patients (46), and patients with moderate to severe OSA but without daytime sleepiness (47). In minimally symptomatic patients, CPAP did not improve a cardiovascular risk score over six months (46), although it did improve endothelial function in this group (149), and there was a suggestion of reduced cardiovascular events with CPAP for those who completed 2 year follow-up (173). CPAP did not reduce the incidence of new cardiovascular events in patients with moderate to severe OSA without daytime sleepiness over an average of 4 years follow up (47). Two further

studies then focussed on CPAP in secondary prevention populations and showed no reduction in new cardiovascular events in patients with prior cardiovascular disease (174), or prior stroke (175). Those with prior stroke randomised to CPAP had a longer time to new cardiovascular events.

The SAVE trial was the first large RCT to look at the long-term effect of CPAP on cardiovascular events (49). This trial randomised approximately 2500 patients at multiple sites to either CPAP or standard care. CPAP did not reduce the occurrence of new cardiovascular events. This study was powered to detect a difference in cardiovascular events with a moderate compliance with CPAP of, on average, 3.3 hours/night. The conclusions that can be drawn from this study are limited to secondary prevention of cardiovascular disease; it may be that younger patients without prior cardiovascular disease might derive a benefit from CPAP (176). In addition, the SAVE study did not include those most sleepy (patients with an Epworth sleepiness score or ESS >15 were excluded), nor those with severe hypoxaemia (patients with >10% of their sleep study time with oxygen saturations <80% were also excluded).

The severity of hypoxaemia may be of relevance in determining risk cardiovascular risk. In the Sleep Heart Health Study the severity of oxygen desaturations caused by SDB was found to predict the likelihood of a coexisting cardiovascular disease (10). The relationship between SDB and cardiovascular disease was lost when considering hypopnoeas with oxygen desaturations <4%. This suggests that the development of cardiovascular disease in

OSA is only observed in those with more significant intermittent hypoxia. It may be that patients with more significant hypoxaemia than those included in the SAVE trial would have benefitted from CPAP.

The results of the SAVE study suggest there is no additional benefit in reduction of cardiovascular risk in secondary prevention for OSA in patients without severe sleepiness or hypoxia (49). CPAP may reduce cardiovascular risk for those more sleepy or more severe patients with OSA, who would receive CPAP treatment as standard practice for symptoms (177). Future work is required to explore whether other therapies, potentially including supplemental oxygen, may be beneficial in reducing cardiovascular risk in OSA.

1.16. CPAP withdrawal as a model of cardiovascular disease in OSA

CPAP withdrawal is an experimental way of modelling the short-term consequences of OSA. CPAP withdrawal can be used to assess the physiological effects of OSA without the confounding effects seen in cohort studies or the issues of low CPAP usage in conventional RCTs. Patients with known OSA who have been established on CPAP, typically for over 1 year, are randomised to 2 weeks of sham CPAP (with return of significant OSA) or continued therapeutic CPAP (control group). This model produces a significant return of OSA occurring from the first night off CPAP, with the largest between groups difference in AHI of +33.4 /hour (95% confidence interval +22.3 to +44.5) at the 14^{th} night off CPAP. Concerns have been raised over how representative patients undergoing CPAP withdrawal are of patients with OSA as many patients are unable to tolerate stopping their CPAP therapy. This raises

the possibility that those able to tolerate CPAP withdrawal might have little sleep fragmentation and therefore not develop the typical features of OSA. However, CPAP withdrawal leads to return of sleepiness with a between groups rise of 2.6 points in ESS (95% confidence interval 1.1 to 4.3) after 14 nights of CPAP withdrawal (148), a 9mmHg rise in systolic, and 7.8mmHg rise in diastolic home morning blood pressure (178), increased sympathetic activity with increased urinary normetadrenaline, and impaired endothelial function (148). Whilst blood and urine biomarkers of oxidative stress are not increased by CPAP withdrawal (135), sophisticated analysis of exhaled breath shows an increase in compounds associated with oxidative stress with CPAP withdrawal (133). Whilst CPAP withdrawal has demonstrated many short-term consequences of OSA, it did not show any effect on maximal myocardial perfusion capacity, renal microvascular function, or dermal microvascular function (179). The potential mechanisms of cardiovascular disease in OSA are summarised in *Figure 1.1* (reproduced with permission (167)).

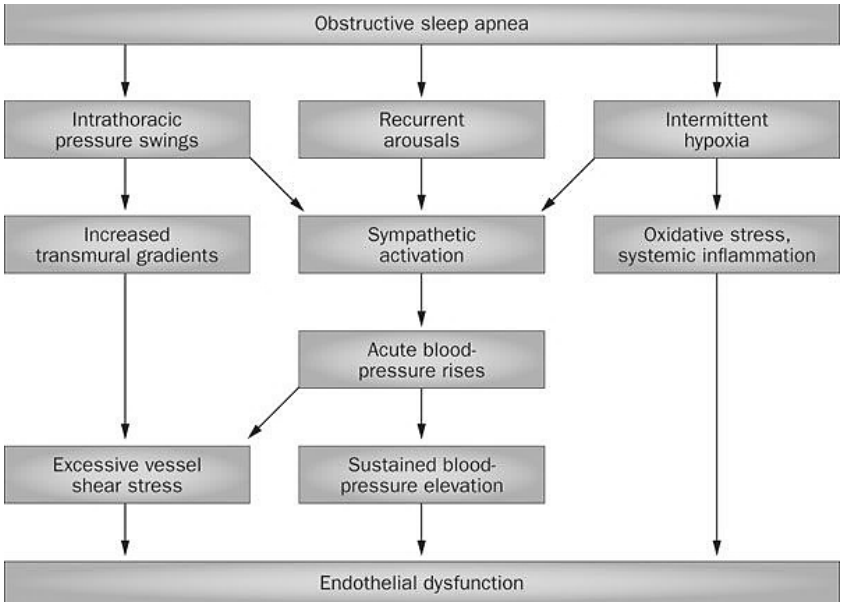


Figure 1.1: Potential mechanisms of the development of cardiovascular disease in OSA (reproduced with permission from (167)*). The CPAP withdrawal model has been used to*

demonstrate sympathetic activation, sustained blood pressure elevation, and endothelial dysfunction at the brachial artery, in association with returning OSA. The effects of the return of OSA during CPAP withdrawal on oxidative stress and systemic inflammation are less certain.

CPAP withdrawal can also be used to assess new therapies. The *Provent* device, a nasal expiratory positive airway pressure (EPAP) device, was postulated to decrease severity of OSA. EPAP involves inserting a nasal device with a one-way valve that opens during inspiration but provides resistance during expiration. This was thought to potentially reduce OSA severity either by slightly increasing blood carbon dioxide levels and therefore increasing respiratory drive, or by resistance to exhalation opening the upper airway. Rossi *et al.* conducted a CPAP withdrawal trial over two weeks and randomised patients with significant OSA (ODI>10) to either continued CPAP, *Provent* device, or sham *Provent* device. There was significant return of OSA with the *Provent* device which was similar to the sham *Provent* device (180). The number of patients needed to show this lack of benefit was 67 and involved only a two-week intervention, which demonstrates how CPAP withdrawal can be a fast and efficient way of initially assessing potential new therapies.

CPAP withdrawal is therefore a powerful experimental design to further explore the physiological changes in OSA as well as assessing potential new treatments.

1.17. Summary (Chapter One)

OSA is a common disorder and it is associated with increased cardiovascular risk. There are several potential mechanisms by which OSA may lead to cardiovascular disease including intermittent hypoxia, recurrent arousals, and intra-thoracic pressure swings. These processes lead to increased sympathetic activation, endothelial dysfunction, sleep fragmentation, shear stress on the heart and great vessels, and possibly oxidative stress and systemic inflammation, which all could play a role in the development of cardiovascular disease. In the SAVE trial, CPAP did not show any improvement in secondary prevention of cardiovascular disease over standard care, albeit in patients from cardiovascular clinics and not in those presenting to the sleep clinic. CPAP may be of benefit in reducing cardiovascular risk in patients without prior cardiovascular disease, or with more significant hypoxia or sleepiness. A greater understanding of the underlying pathophysiology of OSA in the development of cardiovascular is needed to direct new therapies when CPAP is not tolerated, for example in non-sleepy individuals with OSA and resistant hypertension. The CPAP withdrawal model is a promising way of efficiently assessing both the physiological effects of OSA and of assessing potential new therapies.

1.18. Thesis aims

The aims of this thesis are to understand the relative contributions of intermittent hypoxia versus recurrent arousals in the development of elevated daytime blood pressure in OSA. I aim to explore pathways including sympathetic activation, endothelial dysfunction, and inflammation, and how these may potentially contribute to intermittent hypoxia mediated effect on blood pressure. The main way in which I will explore the role of intermittent

hypoxia in OSA is using an experimental model using supplemental oxygen in patients with OSA and I also aim to examine the secondary effects of supplemental oxygen on OSA severity, blood gasses, and daytime sleepiness.

2. Chapter Two: Methods

2.1. Abstract (Chapter Two)

This chapter contains the methodology for the supplemental oxygen during CPAP withdrawal trial, blood cardiovascular markers during CPAP withdrawal trial, urinary isoprostanes during CPAP withdrawal, combined CPAP withdrawal methodology, and the development of modifications to the OSLER test in MOSAIC and Supplemental Oxygen trial patients. Detailed methodology, where not discussed here, will be covered in each of the results chapters.

2.2. Supplemental oxygen during CPAP withdrawal

As discussed in Chapter 1 the role of intermittent hypoxia in daytime blood pressure rises in OSA is controversial. Data from animal and human intermittent hypoxia experiments showed clear increases in diurnal and daytime blood pressure respectively. However, RCTs utilising overnight supplemental oxygen, which has been shown to attenuate intermittent hypoxia in OSA, did not reduce blood pressure compared to sham. These previous RCTs were limited by their methodology; only utilising low flow rates of oxygen, excluding patients with the most severe OSA and the most severe hypoxia, variable compliance with therapy, and utilising office of ambulatory blood pressure monitoring. In the supplemental oxygen trial I designed a protocol to administer supplemental oxygen without previous trials methodological limitations. This would therefore allow me to test the hypothesis that intermittent hypoxia in OSA causes daytime elevations in blood pressure.

I choose a CPAP withdrawal methodology as this has been shown to lead to large changes in daytime blood pressure, without the problems of compliance seen in traditional RCTs. Therefore with recruiting a relatively small number of patients I would be able to definitively assess whether intermittent hypoxia affected daytime blood pressure in OSA. I choose a cross-over design as this should allow patient retention and allow comparison of the same patients under different experimental conditions, thereby removing inter-individual variability. Cross-over and period effects are particularly concerns in cross-over trials and therefore a two week washout period back on CPAP therapy would be used between treatment arms. Control of OSA occurs rapidly on CPAP therapy and previous experiments showed that blood pressure increases return to normal levels within days of normoxia

overnight. I choose to use a high flow-rate of supplemental oxygen as previous trials showed only partial attenuation of intermittent hypoxia with flow-rates of 3l/min (Percentage time with oxygen saturations <90% from 5.0 ± 2.4% pre-oxygen to 2.5 ± 1.3% post) (122). Methods of monitoring blood pressure include home blood pressure monitoring, office blood pressure and 24-hour ambulatory blood pressure monitoring. I choose morning blood pressure as office blood pressure measurements were less increased in previous CPAP withdrawal trials (178), and ambulatory monitoring has been shown to cause arousals from sleep which elevate blood pressure acutely and may increase daytime blood pressure (112). As previous trials have failed to show any effect on blood pressure in patients with mild-moderate OSA (with mean AHI of ~25 based on a hypopnoea definition including 3% desaturations) I choose only to study patients with an $ODI_{\geq 4\%}$ >20 off of CPAP to ensure studying patients with more severe OSA than Gottlieb *et al* (121).

2.2.1. Trial design, registration and ethics

The Supplemental Oxygen during CPAP withdrawal (SOX) trial was a single-centre, double blind, cross-over trial with randomised treatment order. It was prospectively registered (ISRCTN 17987510) and approved by local ethics committee (South Central - Oxford B Research Ethics Committee, REC Reference 15/SC/0007).

2.2.2. Patients

Patients were recruited from a single centre, the Oxford Sleep Unit, which is part of the Oxford University Hospitals NHS Foundation Trust. The Oxford Sleep Unit has over 10,000 patients who are treated with CPAP therapy on its clinical database. The database was screened for patients with over 1 year of CPAP usage, a previous average CPAP usage of >4

hours/night, and an original diagnostic ODI of >20/hour, and home address close to the Oxford Sleep Unit. Suitable patients were invited to participate by postal invitation jointly from the clinical and research teams, including an ethically approved patient information leaflet. Patients were given the option of returning an opt-out slip by post. Three weeks were allowed from sending out the information to receipt of an opt-out slip, or for the patients to contact the trials unit by phone. If patients had not made contact by this point in time they were phoned to discuss the trial further by a member of the research team.

Patients who were interested were invited to attend the Churchill Hospital in Oxford, to give written informed consent and initiate screening. At the screening visit patients were verbally presented with the information in the patient information sheet. Patients were free to discuss the trial further with their GP or others. Following written informed consent screening was commenced.

2.2.3. Trial flow diagram

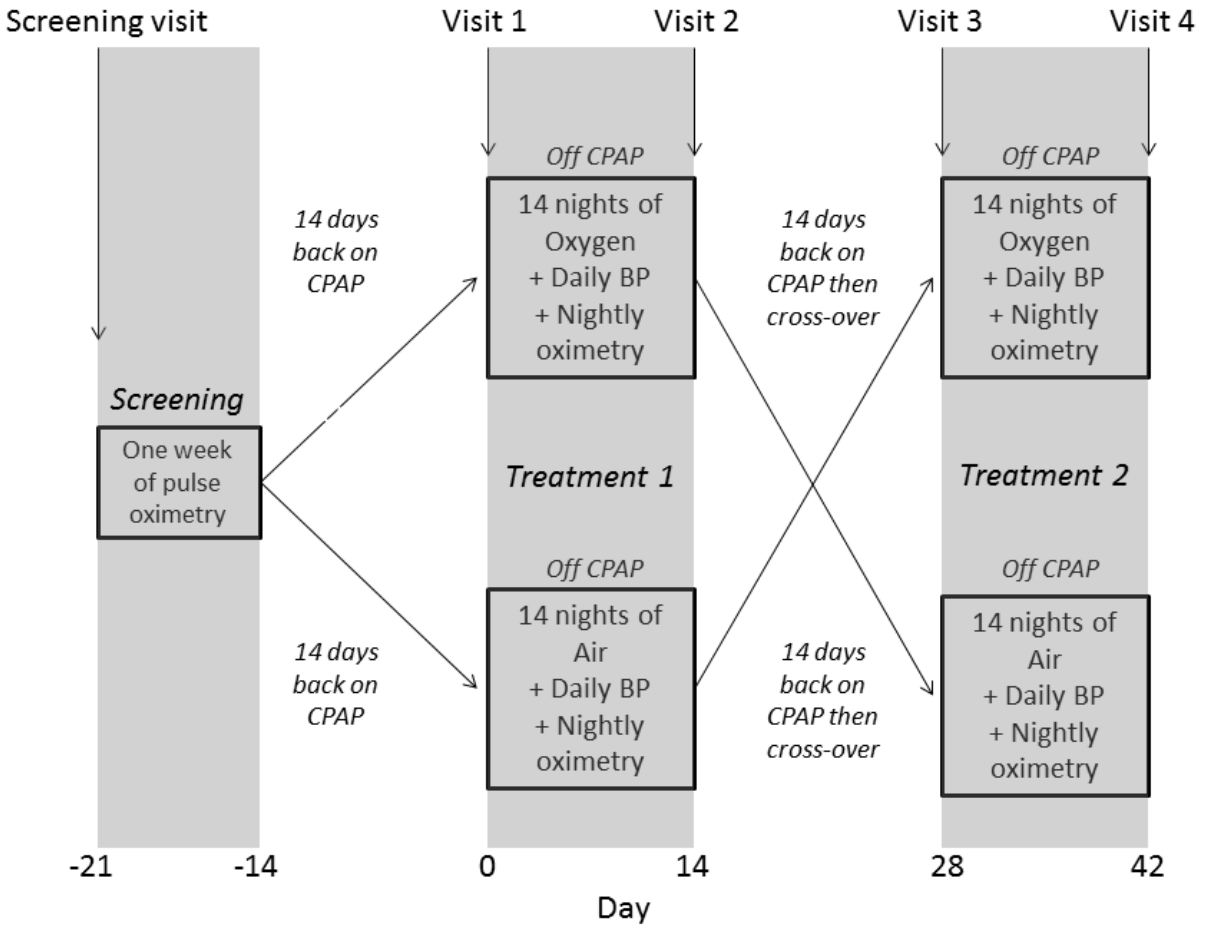


Figure 2.1: SOX trial flow diagram. Patients initially attend a screening visit where, following written informed consent, one week of overnight screening pulse oximetry was commenced. After completing screening, and following on from 14 days back on CPAP, patients were randomised for the order of interventions. Patients then attended four trial visits (Visits 1, 2, 3, and 4). At Visit 1 patients were supplied with their allocated concentrator, either oxygen of air, to use instead of their CPAP. Two weeks later patients then attended for follow-up assessments. Patients then had a washout period of at least two weeks back on their CPAP. At Visit 3 patients crossed over and received the alternate concentrator, either oxygen or air, instead of their normal CPAP. Following a further two weeks, patients then attended a final follow-up visit, Visit 4.

2.2.4. Screening

Patients' notes were screened to assess for inclusion and exclusion criteria (see sections 2.2.4.3 and 2.2.4.4). In addition, patients' CPAP usage was assessed by downloading data from their CPAP secure digital card (SD card) using ResScan software (ResMed, Abingdon, UK). Patients with a mean usage of CPAP of >4 hours/night (averaged over the last 6 months) were eligible to participate in the trial. Where available the machine-generated AHI was also recorded (available for patients using ResMed Autoset machines but not for most patients who used fixed pressure devices).

2.2.4.1. Medical history

At the screening visit a detailed medical history was noted. Patients' medications were recorded, past medical history and comorbidities were recorded. Patients' smoking status and smoking history in pack years (years of smoking multiplied by the number of cigarettes smoked on average and divided by 20) were recorded. The ODI and AHI from the patient's original sleep study were recorded along with the date that CPAP had originally been initiated.

2.2.4.2. Pulse oximetry screening

At the patient screening visit patients were supplied with a pulse oximeter (Konica Minolta 300i, Japan). This device is capable of storing up to 300 hours of data for both heart rate and oxygen saturations. The pulse oximeter has an internal clock and creates a new file every

time the device is turned on, enabling separate recordings of multiple nights. Patients were instructed to wear the pulse oximeter on their wrist and to turn it on just before they went to sleep, and to turn it off when they woke up; keeping the device switched on if they woke briefly during the night. Patients were issued with spare batteries and instructed when to replace the batteries. In addition, patients were given *Micropore*™ tape to secure the finger probe in place.

Patients were instructed to use the pulse oximeter every night for three nights whilst using their CPAP as per normal. Patients were only eligible if their $ODI_{\geq 4\%}$ during this period on CPAP was <10/hour on all nights, or if it was higher only at the Chief/Principle Investigator's discretion (i.e. if the calculated $ODI_{\geq 4\%}$ was higher due to artefact). This ensured adequate treatment of the patient's OSA at baseline.

Following on from this, patients were instructed to carry out a further four nights of pulse oximetry off CPAP therapy. Patients were eligible to participate if OSA returned sufficiently off CPAP such that their $ODI_{\geq 4\%}$ was > 20/hour on any one of the four nights. Thus, patients included were likely to subsequently have a return of OSA when their CPAP was stopped during the trial periods. It has been shown that 29% of patients withdrawing from CPAP do not get a return of OSA to above an $ODI_{\geq 4\%}$ of 10/hour within four nights off CPAP, and approximately 10% do not get a return of OSA after 14 nights off CPAP (181). Therefore, if this screening oximetry was not conducted, and all patients were included, the power of the

study would be reduced, as patients would be included who would be likely not to get a return of OSA during the two-week trial periods.

The reason(s) that OSA does not return in all individuals is/are not known. It is worth noting that only the ODI is used to assess return of OSA during screening for CPAP withdrawal trials. It is possible that OSA is returning but without associated hypoxia in the 29% of individuals who do not have a significant rise in their ODI off of CPAP. This could be due to alterations in individuals arousal thresholds (182). Initially on withdrawal of CPAP individuals who have been on long-term CPAP would be likely to have relatively normal arousal thresholds, meaning obstructive events will terminate at relatively normal intra-thoracic pressures and potentially before any oxygen desaturation has occurred. After these first few nights off of CPAP the arousal threshold may increase to levels comparable to previously untreated OSA patients leading to more prolonged obstructive events and more oxygen desaturations. Alternatively, slowly returning pharyngeal oedema or pharyngeal dilator muscle dysfunction may be responsible for OSA not reoccurring in some patients with OSA. Pharyngeal oedema is a feature of OSA and is reduced on CPAP (183), therefore it may take time to reoccur during CPAP withdrawal, meaning anatomically susceptibility to upper airway collapse might be lessened and stop OSA from returning. Pharyngeal dilator muscle fatigue has been shown in OSA and improves with CPAP therapy (184). Therefore, it may reoccur more slowly during CPAP withdrawal.

Pulse oximetry data was uploaded using VisiDownload (Stowood, Beckley, UK). Data was automatically scored to record the $ODI_{\geq 4\%}$, with manual editing of clear artefact only.

2.2.4.3. Inclusion Criteria

- Objectively confirmed obstructive sleep apnoea (at the time of original diagnosis) with an ODI of > 20/hour, and/or an AHI of >20/hour (this threshold will exclude subjects with borderline OSA, in whom there may be only a limited treatment effect from CPAP or its withdrawal)
- Current $ODI_{\geq 4\%}$ of >20/hour returning on any night during an ambulatory nocturnal pulse oximetry performed during four nights of CPAP withdrawal (again this threshold will exclude subjects with borderline OSA, in whom there may be only a limited treatment effect from CPAP or its withdrawal)
- Treated with CPAP for more than 12 months, minimum compliance 4 hours/night (averaged over all nights), AHI <10 with treatment (according to CPAP machine download where available) and an $ODI_{\geq 4\%}$ of <10/hour confirmed on CPAP during screening oximetry (to ensure any elevation in blood pressure caused by OSA is maximally treated prior to randomisation)
- Written informed consent

2.2.4.4. Exclusion Criteria

- Previous ventilatory failure (awake resting arterial oxygen saturation <93% or $PaCO_2$ >6.0 kPa) or severe respiratory disorder other than OSA (both so as to not include patients with a mixture of OSA and obesity hypoventilation syndrome who may have

different responses to supplemental oxygen in terms of blood pressure and so as to mitigate risk of precipitating hypercapnic respiratory failure)

- Unstable, untreated coronary or peripheral artery disease, severe arterial hypertension (>180/110mmHg), severe arterial hypotension (<90/60mmHg) (as if a patient's blood pressure were to increase from a high baseline of >180/100mmHg medical management would be unavoidable, which could potentially bias a treatment and as CPAP reduces preload which could worsen a low blood pressure during washout periods)
- Previously diagnosed with Cheyne-Stokes breathing
- Current professional driver or vigilance critical occupation (dependent on driving for livelihood)
- Any sleep related accident (as these individuals are either at increased risk of a driving related accident during CPAP withdrawal)
- Age <20 or >75 years at trial entry
- Mental or physical disability precluding informed consent or compliance with the protocol
- Non-feasible trial follow-up (for example, distance from follow-up centre, physical inability)
- Current smoker (concerns over oxygen therapy, smoking and fire risk)

Specifically, evidence of previous ventilatory failure was elicited from patient's clinical notes and from patient enquiry. A blood gas assessment was not required as a trial entry criterion.

2.2.5. Randomisation, intervention and blinding

Following the week of CPAP withdrawal for screening, patients were re-established on their CPAP therapy for at least 14 nights to ensure adequate treatment at study baseline (Visit 1). At Visit 1 patients were then randomised using online randomisation software (Sealed Envelope, London, UK). Patient's treatment order was randomly assigned 1:1 using permuted blocks of 6 to either A (oxygen) or B (sham or air). Block randomisation was used as there were 3 oxygen and 3 air concentrators available for use in this study. Patients and researchers carrying out study visits and outcome assessments were blinded to the study allocation, i.e. oxygen or sham (air) arm. Concentrators were labelled A and B by an unblinded member of staff not involved in the day to day running of the trial, with none of the research staff involved in the study aware of the coding. Thus, the study was double blinded.

Concentrators were identical in appearance and sham devices were prepared by the manufacturer (NewLife Elite, AirSep, Buffalo, USA). The sham devices had been produced without nitrogen filters. Devices were checked by an unblinded investigator to ensure that they delivered a suitable inspired oxygen fraction (FiO_2 0.21 for air, and FiO_2 >0.98 for oxygen; See *Appendix 8.1*). Anecdotally, this blinding was very effective and patients were not able to establish which therapy they had received.

At Visits 1 and 3, patients were shown how to use their allocated concentrator. Both the oxygen and air concentrators were set to a maximum flow rate of 5l/min and patients were

shown how to check that the flow rate remained at 5l/min during the trial. At Visits 1, 2, 3, and 4, the concentrator operation hours were noted to allow calculation of hours of usage by each patient during each treatment arm.

2.2.6. Oxygen delivery interface

Patients were given a choice of oxygen delivery interface. In a separate experiment with healthy volunteers the inspired and end-tidal CO_2, and end-tidal O_2 pressures were checked using different oxygen delivery interfaces, with supplemental oxygen and supplemental air (*Appendix* 8.1). As the end-tidal oxygen levels achieved with each oxygen delivery interface were comparable in these volunteers, patients were offered either a choice of a face mask or nasal cannulae to deliver the required 5l/min. Although the end-tidal carbon dioxide was slightly higher (1.4mmHg or 0.2kPa; see *Table 8.2*) with face mask oxygen delivery than with nasal cannulae, this was considered very unlikely to lead to significant hypercapnia. A maximum of 10m of tubing was supplied to patients and they were instructed to place the concentrator in a different room to their bedroom to minimize noise and to position the tubing so as to reduce the risk of tripping.

2.2.7. Outcome measures

The primary and secondary outcomes of the supplemental oxygen trial assess the differences between the two arms, supplemental oxygen and supplemental air therapy, mostly in the change of parameters during CPAP withdrawal.

2.2.7.1. Primary outcome

- The difference in the change of home morning blood pressure

Morning blood pressure was chosen as the primary outcome as it has been shown to be the period of time in the day when blood pressure is most effected by OSA (68). The nocturnal period was not compared as blood pressure overnight would also be affected by arousal mediated blood pressure rises (114).

2.2.7.2. Secondary outcomes

- The difference in the change of home morning heart rate
- The difference in the change of office morning blood pressure and heart rate
- The difference in the change of overnight urinary normetadrenaline and metadrenaline
- The difference in the change of home ODI and heart rate parameters overnight
- The difference in the AHI
- The difference in the change in subjective sleepiness
- The difference in the change in objective sleepiness

2.2.8. Procedures

2.2.8.1. Blood pressure recording

Patients were supplied with an automatic blood pressure monitor (M10, Omron, Kyoto, Japan). At the screening visit, patients were shown how to use the blood pressure monitor and were instructed to record their blood pressure and heart rate in triplicate at home every morning soon after awakening, for 5 days prior to the first and third study visits and for

every day of the trial periods. Patients were instructed to record their blood pressure from their left arm whilst seated after resting for at least 10 minutes. Blood pressure, heart rate, date and time of each recording were stored automatically on the blood pressure machine and downloaded at patient visits. In addition, patients were instructed to write each recording in their study diary as a back-up.

For the primary outcome measure, triplicate home morning blood pressure recordings were averaged on three days before Visits 1 and 3 (days -3, -2, and -1), and on days 11, 12, and 13 of each treatment arm in the run up to Visits 2 and 4. If data were missing, averages of available data were taken. Blood pressure data from the day of the trial visits was not included in these averages as it was thought that the patient's sleep and morning routine will have been somewhat disturbed by an overnight sleep study (on the night prior to Visit 2 and 4), the overnight urine collection, and attending for the study visit. The three nights prior to this were thought to be likely to be more representative of a patient's morning blood pressure (185).

In addition to home blood pressure recording, patient's office blood pressure and heart rate were recorded at Visits 1, 2, 3 and 4, following arrival at the hospital for the trial visits. Recordings were taken in triplicate whilst seated from the patient's left arm, and after at least 10 minutes rest, using an automatic blood pressure device (M7, Omron, Kyoto, Japan).

2.2.8.2. Trial overnight pulse oximetry

In addition to overnight pulse oximetry recordings during screening, as described above, patients were instructed to use a pulse oximeter (Konica Minolta 300i, Japan) on nights 1-13 of each treatment arm (following on from Visits 1 and 3). For the secondary outcome measures, comparing the effects of oxygen versus air on overnight oximetry and heart rate parameters, recordings were included in the study if they had over 4 hours of recording (after manual exclusion of clear periods of artefact). The $ODI_{\geq 4\%}$, mean oxygen saturations, percentage time with oxygen saturations below 90%, mean heart rate, standard deviation of the heart rate, and the heart rate rises >6bpm index were averaged from nights 8-13 during each arm of treatment. For the analysis, recordings during the first week were excluded to ensure that there had been adequate return of OSA; in previous withdrawal trials the $ODI_{\geq 4\%}$ only reached a plateau after approximately four nights (148). Thus, by only including data from the second week OSA should have reached this plateau.

2.2.8.3. Home sleep study

Home sleep studies (*BlackShadow* or *Greyflash*, Stowood, Beckley, UK) were performed on two occasions, on the night prior to Visits 2 and 4 (which was the 14^{th} night of each treatment period). These home sleep studies included pulse oximetry, nasal cannulae pressure transducer, body position, and sound recording. This enabled calculation of the apnoea hypopnoea index, as well as the separate apnoea and hypopnoea indices. Apnoea was defined as a reduction in airflow to less than 10% of baseline airflow for greater than 10 seconds relative to a baseline of the preceding 160 seconds. Hypopnoea was defined without an oxygen desaturation requirement, as inclusion of this would severely distort the

hypopnoea index during oxygen breathing, and thus prevent an assessment of any changes in the number of apnoeas and hypopnoeas. Hypopnoeas were defined as a reduction in airflow to below 50% of baseline airflow to minimise inclusion of trivial airflow limitation of no clinical consequence, given there was no requirement for an oxygen desaturation or an arousal. This differs from the most recent American Academy of Sleep Medicine 2012 manual (2), on scoring hypopnoeas, but is justified by the necessity of removing the error in scoring hypopnoeas in the oxygen arm. In addition, the $ODI_{\geq 4\%}$, mean oxygen saturations, percentage time with oxygen saturations below 90%, mean heart rate, standard deviation of the heart rate, and heart rate rises >6 bpm (HRR) index were also recorded. Overnight sleep studies were analysed manually, removing clear periods of wakefulness and artefact. Sleep studies were only considered when there was >2 hours of data.

2.2.8.4. Measurement of apnoea length

Previous studies have shown that oxygen therapy tends to increase the length of apnoeas (186), and that the ratio between apnoeas and the inter-apnoea period are related to the daytime CO_2 (187). Therefore, event lengths were measured to assess the impact of oxygen therapy, and to determine if it might cause alterations potentially leading to hypercapnia. Approximately 20 minute periods of the home sleep studies, with apparently stable sleep apart from the recurrent transient arousals, were identified manually. These periods were in one sleep position (sleep position assessed using the in-built accelerometer in the *Greyflash/BlackShadow* device) and contained periods of dominantly recurrent apnoeas without major arousals disturbing this repetitive pattern. Apnoeas were automatically scored with manual editing to ensure accurate length recordings (VisiDownload, Stowood,

Beckley, UK). Notepad files containing this stable period of sleep recording were then exported to a .csv file and opened using an Excel template. The average apnoea length, inter-apnoea length (time between apnoeas), and apnoea: inter-apnoea length ratio were all calculated. Full details are shown in the appendix (section 8.5).

2.2.8.5. Epworth sleepiness score

The Epworth sleepiness score (ESS) is a validated questionnaire to assess sleepiness. It was originally designed by Johns (39), as a way of identifying sleepiness in a population with sleep disorders compared to control subjects. Arbitrarily, an ESS of greater than 10 defines excessive daytime sleepiness, although, as previously discussed (Section 1.4), 14-21% of the general population have an ESS >10 (16)(17). Whilst there is wide inter-individual variability in ESS, the change over time in an individual is a useful way of assessing any treatment effect, with marked changes found in patients with OSA on CPAP treatment. Over one month in men with symptomatic, significant OSA, CPAP reduced mean ESS from 15.5 to 7.0, compared to reductions from 15.0 to 13.0 with sham-CPAP (5). Whilst the ESS is often considered a measure of longer-term sleepiness, changes have been shown during a relatively short two week CPAP withdrawal (mean difference in change of ESS, sham CPAP vs continuing therapeutic CPAP, +2.0, p=0.001, 95% CI +1.1 to +4.3 (148)).

The ESS is made up of 8 questions, which ask an individual to score their chances of nodding off as ‘never’ (0 points), ‘low’ (1 point), ‘moderate’ (2 points) or ‘high’ (3 points) (see Appendix 8.2). Scores can range from 0 to 24. The ESS was recorded at Visits 1, 2, 3 and 4.

2.2.8.6. Oxford Sleep Resistance (OSLER) Test

The Oxford Sleep Resistance (OSLER) test was developed as an alternative to the maintenance of wakefulness test (MWT), but without the requirement for continuous EEG monitoring. The OSLER test has been used to objectively assess daytime sleepiness in research studies (46)(62)(148)(188)(189). Previous studies have shown clear differences in OSLER test scores between patients with OSA and control subjects, and clear improvements in patients with OSA's scores following CPAP treatment (190). Patients lie in a dark room and listen to quiet white noise via headphones. They are then instructed to touch a sensor on a hand-held device, in response to a regularly flashing dim light. The light flashes regularly every 3 seconds, until 7 lights in a row are missed (termed the sleep latency 7). This equates to approximately one 20 second epoch on EEG scoring; when 7 lights are missed, 21 seconds will have past, and the patient is deemed to have fallen asleep. The time taken to 7 misses is recorded as the primary test result. The test lasts until 7 sequential lights have been missed, or until 40 minutes have past. If the patient does not miss 7 lights in a row, within the 40-minute time-period, the results is recorded as 2400 seconds (40 minutes).

2.2.8.7. Modified analysis of the OSLER test

Studies have shown that fewer sequential missed lights than the original 7 are associated with micro-sleeps. The time to missing 6, 5 and 4 lights (termed Sleep Latency 6, 5, and 4) have all been proposed as more sensitive markers of objective sleepiness than the originally described Sleep Latency 7 (191). In addition, the average number of missed lights normalized over one hour (error index), and the absolute number of 1-2 light misses, have been

proposed as methods of assessing psychomotor vigilance (192)(193). In this current study, modified analysis of the original OSLER test was performed by exporting data from VisiDownload (Stowood, Beckley, UK) as a spreadsheet DIF file. An Excel template was then used to calculate the sleep latency 6, sleep latency 5, sleep latency 4, number of misses 1-2, number of misses 3-6, and the error index. I had previously developed this modified analysis on a different set of patients (for methods for this development set see section 2.6).

2.2.8.8. Overnight and early morning urine collection

Patients collected both overnight, and early morning urine samples, on the night prior to, and the morning of, Visits 1, 2, 3, and 4.

For the overnight urine collection patients were instructed to empty their bladder at around 8pm and mark the exact time down as the start time in their patient diary. Patients were then instructed to collect any urine passed overnight into a large non-acidified urine container. Patients were instructed to empty their bladder once more after waking into the overnight collection bottle, and then mark this time down as the urine collection end time in their diary. Upon arrival to the hospital the volume of urine in the overnight collection was recorded and measured as were the urine collection start and end times. A 20ml sample was taken from the overnight collection to be sent to the biochemistry laboratory to measure the urine creatinine concentration. A further 10ml sample was then put into a falcon tube and spun at 2500rcf at room temperature. Two 1.8ml aliquots from this overnight spun non-acidified urine were stored at -80^{o}C. The remainder of the overnight urine collection

was then placed into an acidified bottle for at least 15 minutes. Two 10ml samples of overnight unspun acidified urine were then taken and were also stored at -80^{o}C for later measurement of normetadrenaline and metadrenaline.

In addition to the overnight urine sample, patients were instructed to collect an early morning urine sample the second time they passed urine in the morning in a universal container. A 10ml sample was then put into a falcon tube and spun at 2500rcf at room temperature. Two 1.8ml aliquots from this overnight spun non-acidified urine were stored at -80^{o}C.

2.2.8.9. Urinary creatinine concentration

As described above, urinary creatinine samples were sent at each patient visit to the local biochemistry laboratory (Oxford University Hospitals NHS Foundation Trust, Oxford, UK). Samples were analysed as per standard laboratory protocols.

2.2.8.10. Urinary normetadrenaline and metadrenaline

After collection of all samples, frozen unspun acidified overnight urine samples were sent to St Bartholomew's Hospitals (London, UK) for analysis. Samples from the same patient were run in the same batch to minimise any batch effect. Samples were analysed by high performance liquid chromatography to ascertain the concentration of urinary metadrenaline (adrenaline metabolite) and normetadrenaline (noradrenaline metabolite) (194)(195)(196). Urinary normetadrenaline and metadrenaline results were corrected by the rate of urine

production (total millilitres produced overnight/total hours of collection) and by urinary creatinine concentration.

2.2.8.11. Serum high-sensitivity CRP

High sensitivity CRP (hsCRP) was measured from frozen stored serum samples from each of the four patient visits using immunoturbidometry (Architect c16000, Abbott, Illinois, USA). HsCRP was measured as a marker of systemic inflammation. In cohort studies it has been shown to be elevated in OSA (197), but increases have not been shown in the CPAP withdrawal model so far (148). Elevated hsCRP levels have been linked to increased risk of cardiovascular disease (198). Both intermittent hypoxia and hyperoxia have been hypothesised to increase oxidative stress and inflammation (131)(199). HsCRP was measured to determine if oxygen therapy either reduced or increased inflammation relative to air, and relative to baseline on-CPAP levels.

2.2.8.12. Venous blood gas assessment

Venous blood gas samples were collected from a subset of patients. Samples were collected during the morning Visits, 1, 2, 3 and 4. Samples were analysed using blood gas analyser (ABL90 Flex, Radiometer, Crawley, United Kingdom) and the base excess, and partial pressure of carbon dioxide (PCO_2) were recorded.

2.2.8.13. Height, weight, neck circumference and body mass index measurements

Height, weight, and neck circumference were measured at Visit 1. Height and weight were measured with the patient clothed and wearing their shoes. Neck circumference was measured with a tape measure at the level of the cricoid cartilage. Body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the square of the patient's height in metres.

2.2.9. Patient case records and database

Data was recorded on paper case record forms (CRFs) designed specifically for the trial. On the CRFs, patients were only identifiable by a unique trial identifier. CRFs were stored in the Oxford Respiratory Trial Unit (Churchill Hospital, Oxford), in a locked filing cabinet. Paper CRFs were then entered onto an online bespoke database (OpenClinica, OpenClinica LLC, Waltham, Massachusetts, USA), and hosted on a secure University of Oxford server. No patient-identifiable data is stored on this electronic database and it is kept in accordance with Good Clinical Practice standards.

2.2.10. Sample storage

Patient samples that were stored during the trial were kept in -80^{o}C freezer. Duplicate samples were stored in a separate freezer. Freezers were kept locked in accordance with the Oxford Respiratory Trials Unit (Churchill Hospital, Oxford) standard operating procedures.

These include monitored alarms with staff notification in the event of the freezer temperature being out of range due to a technical failure.

2.2.11. Travel expenses

Patients were paid travel expenses for mileage and parking incurred during the trial, or for second class travel on public transport. Payments were not made for participation in this trial to not coerce patients to participate or continue if it was not possible, or difficult, for them to do so, for example if they had a significant return of sleepiness likely to impair their driving.

2.2.12. Statistics

2.2.12.1. Number of participants

The number of participants required was calculated based on the size of the effect of sham CPAP compared to continued CPAP on blood pressure in previous CPAP withdrawal trials. In a meta-analysis, sham CPAP led to a +9.7mmHg (standard deviation (SD) 14, 95% confidence interval (95% CI) +6.3 to +13.0) rise in home morning systolic blood pressure when compared to continued CPAP **(178)**. It was estimated that difference between supplemental oxygen and air treatment would not be as large. The number of participants required was 24 in order not to miss a 6mmHg difference in home morning systolic blood pressure (approximately two thirds the size of the blood pressure rise seen between sham CPAP and continued CPAP), in a cross-over design using a paired t-test, assuming a standard deviation of the change of 10mmHg (approximately two-thirds of the size of the standard deviation in

blood pressure change between sham CPAP and continued CPAP), for a power of 80%, and a significance level of <0.05. In previous parallel withdrawal trials, there was a very low drop-out rate of <1% (1 in 110 patients). Therefore, to allow for increased drop-outs due to the more arduous cross-over design, a sample size of 30 was initially proposed. The protocol was amended part way through the trial as there was a significantly higher drop-out rate. Nine of the first 23 patients dropped-out (39%) and therefore the sample size was recalculated to 50 patients to allow up to 52% drop-outs and still provide 24 complete datasets for analysis (see section 3.5.3).

2.2.12.2. Outcome analysis

Outcome measures were assessed for normality. Where normally distributed, the primary and secondary outcome measures were assessed using paired t-tests, and where not normally distributed, using Wilcoxon rank tests. Due to the cross-over design of this trial a combination of unpaired t-tests, paired t-tests, and mixed effect models were used to assess for any order or period effect.

2.3. Common CPAP withdrawal trial methodology

2.3.1. Ethical considerations for withdrawal trials

The most important ethical consideration with the CPAP withdrawal model is any potential adverse consequence that return of OSA might cause. In the SOX trial, CPAP withdrawal was planned for a longer total time than previous withdrawal trials. With the cross-over design of the trial, patients were withdrawn from CPAP for two periods of 14 days as compared with

one 14-day period in previous trials. Appropriate concerns over CPAP withdrawal apply to all such trials using this methodology, but particularly the SOX trial.

The major concern with CPAP withdrawal is the return of excessive daytime somnolence, particularly whilst driving. Many patients do stop CPAP for short periods of time, for example when they have an upper respiratory tract infection or when they go on holiday. Not all patients experience immediate return of symptoms during this brief CPAP withdrawal, and any symptoms may not return to the full extent experienced originally. Professional driving or vigilance critical jobs were exclusion criteria. In addition, previous sleep-related road traffic collision was an exclusion criterion. Patients were informed of the potential risk of recurrence of sleepiness, and asked to immediately report sleepiness whilst driving during the trial. If this did occur, patients were withdrawn from the trial and placed back on their CPAP.

Given the increased incidence of cardiovascular events in patients with untreated OSA (9), and the observed increase in blood pressure with CPAP withdrawal (178), the occurrence of cardiovascular events was a potential concern. However, in over 100 patients undergoing CPAP withdrawal previously, there have been no serious adverse events. Patients awaiting coronary intervention, or with unstable hypertension/cardiovascular disease, were excluded.

All trials had appropriate ethics committee approval.

2.3.2. Adverse events

Adverse events were recorded according to standard operating procedures and Good Clinical Practice standards. Serious adverse events were defined as any adverse occurrence that: resulted in death, was life threatening, required inpatient hospital treatment or prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity, consisted of a congenital anomaly or birth defect. Serious adverse events were to be reported to the Research Ethics Committee granting a favourable opinion for the study if they were deemed by the chief investigator to be 'related' to the study and 'unexpected' within 15 working days of the chief investigator being notified.

2.3.3. Discontinuation/Withdrawal of Participants from Studies

Patients were free to withdraw at any time from any of the trials without giving a reason. Withdrawing from a trial would in no way affect a patient's ongoing clinical care. When withdrawing patients had three options for withdrawing their consent:

- *No further contact* – meant that the research team no longer contacted the patient directly, but still had their permission to use information, samples and to obtain further information from health records.
- *No further access* – meant that the research team no longer contacted the patient or obtained information from their health records, but still had permission to use the information and samples already collected.

- *No further use* – meant that the research team no longer contacted the patient or obtained further information, aimed to destroy all samples already collected (though tracing previously distributed samples may not always be possible), did not use either data or samples for further analyses, but were not able to remove data from analyses already carried out. Data already entered on to the database cannot be deleted, but would be excluded from analysis.

The reason for withdrawal, if known, was recorded in the CRF. If the participant was withdrawn due to an adverse event, the investigator would arrange for visits or telephone calls to collect follow-up information on the adverse event until the adverse event had resolved or stabilised.

2.4. Methods for blood markers of cardiovascular disease in CPAP withdrawal trial

2.4.1. Trial design

The study looking at blood protein markers during CPAP withdrawal used data from three previous and separate withdrawal trials that used similar methodology (135)(148)(179). All three trials randomised patients with known OSA, established on, and compliant with, CPAP therapy, to either continued therapeutic CPAP or sham CPAP (CPAP withdrawal) for 14 days. The trials were registered (controlled trials.com ISRCTN 93153804, ISRCTN 73047833, and clinicaltrials.gov NCT 01797653) and approved by the local ethics committee (University Hospital Zurich REC Ref EK-1600, NRES Committee South West – Exeter Ref 12/SW/0254, Zurich Cantonal EC Ref KEK-ZH-NR.2012-0511). The trials were conducted in accordance with the amended Declaration of Helsinki. Written informed consent was taken from all participants.

2.4.2. Patients

Patients with OSA were recruited from the sleep database of the Sleep Disorders Centre of the University Hospital Zurich, Switzerland and the Oxford Centre for Respiratory Medicine of the Churchill Hospital, UK. Patients were eligible if they were; aged 20-75 years, had OSA and an oxygen desaturation index ($ODI_{\geq 4\%}$) >10/hour (148), or 20/hour (135)(179), on their original sleep study, had been treated with CPAP for >12 months, and had an average CPAP usage of ≥4 hours/night.

Following written informed consent, patients underwent pre-trial overnight pulse oximetry during withdrawing CPAP for four nights to determine eligibility. In one trial, patients were included if their $ODI_{\geq 4\%}$ was >10/hour during the initial pre-trial period off CPAP (148). In the other two trials patients were included if their $ODI_{\geq 4\%}$ >20/hour during the initial pre-trial period off CPAP (135)(179). Patients were excluded if they had previous ventilatory failure, Cheyne-Stokes breathing, unstable coronary artery disease, unstable peripheral arterial disease, severe arterial hypertension (>180/110mmHg), severe arterial hypotension (<90/60 mmHg), were a professional driver, had a history of sleep related accidents, or had an acute upper respiratory tract infection.

2.4.3. Intervention, randomisation and blinding

After the initial pre-trial screening week, patients returned onto CPAP therapy for at least 7 days in one trial (148), and at least 14 days in the other two (135)(179). Following baseline

assessments patients were then randomised in a 1:1 ratio to either continue therapeutic CPAP or to sham CPAP for 14 days (CPAP withdrawal). Outcome assessors and patients were blinded to treatment allocation.

2.4.4. Therapeutic CPAP and sham CPAP

Those randomised to therapeutic CPAP had received a new trial CPAP machine delivering a therapeutic pressure. Patients allocated to sham CPAP received a device set to the lowest pressure (4 cmH_2O), the delivered pressure was lowered further by inserting 6 extra holes in the mask end of the CPAP tubing and fitting a flow restricting device at the machine end of the tubing. The mask pressure delivered by sham CPAP was ≤1cmH_2O.

2.4.5. Trial outcomes: blood cardiovascular markers

The main outcomes were the change in the following cardiovascular blood markers: adrenomedullin (ADM), endocan, endothelin-1 (ET-1), resistin, and vascular endothelial growth factor (VEGF). Changes in erythropoietin (EPO) levels were assessed as a comparator that is known to be hypoxically regulated. Changes from baseline to follow-up were compared, sham CPAP versus therapeutic CPAP. Blood samples were collected in the early morning at baseline and at 14-day follow-up and the samples were stored at -80^{O}C for later analysis.

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for analysis: ADM (Caltag Medisystems #E-EL-H0275), ET-1 (R&D-Systems #QET00B), endocan

(Aviscera #SK00318-01), EPO (R&D Systems #DEP00), resistin (BioVendor #RD191016100) and VEGF (R&D Systems #DVE00). Ethlylenediaminetetraacetic acid (EDTA) plasma was used for all tests except for VEGF, which was performed on serum. Samples were measured in duplicate, except for VEGF which was measured in triplicate.

2.4.6. Other outcome measures

Other outcomes measures were the change in ODI, the change in ESS, the change in blood pressure, and the change in heart rate.

2.4.7. Statistical methods

Data were assessed for normality and presented as mean ± standard deviation where normally distributed, and median (1^{st} quartile, 3^{rd} quartile) when not. Treatment effects (Sham CPAP *versus* therapeutic CPAP) on follow-up blood protein levels were modelled using linear regression (SPSS, Version 20: IBM, Portsmouth, UK), controlling for baseline blood protein level, age, gender, BMI, smoking status, recruiting centre and comorbidities. Change in blood marker levels were approximately normally distributed where baseline and follow-up blood marker levels were not. As secondary analyses, the correlations between the change in blood protein levels and the change in ODI, and the change in blood pressure were explored using Pearson's correlation coefficient.

2.5. Methods for urinary isoprostanes study

Samples from a previous withdrawal trial were utilized (135). This trial initially reported data from two centres, Oxford and Zurich. In the data presented here I only utilized samples from the Oxford patients and this included 9 further patients who were not included in the original publication.

Patients all had an original $ODI_{\geq 4\%}$ >20/hour, and had been treated with CPAP for over 1 year with a compliance of over 4 hours/night. Patients were screened and had an $ODI_{\geq 4\%}$ of <10/hour on CPAP therapy three nights, and an $ODI_{\geq 4\%}$ of >20/hour on at least one of four nights off CPAP. Patients then went back onto their CPAP therapy for at least 14 nights prior to randomisation. Patients were randomized to either two weeks of sham CPAP (set-up as described in section 2.4.4) or two weeks of auto-titrating CPAP which would deliver a therapeutic CPAP setting.

Patients completed urine collections on the night prior to their baseline visit and the final night of their treatment period. Both acidified and non-acidified urine was collected as described in section 2.2.8.8.

Urinary F2-isoprostanes were measured by ELISA technique (Abcam, Cambridge, UK, Cat# ab175819) and urine creatinine was measured from the same samples. The treatment effect of CPAP withdrawal versus control on two week urinary isoprostanes levels was modelled using multivariable linear regression, adjusting for the baseline urinary

isoprostanes levels, age, gender, BMI, smoking status, antihypertensive usage, and statin usage. Data are expressed as mean ± standard deviation, median (1^{st} quartile, 3^{rd} quartile) or number (%).

2.6. Methods for development of modified OSLER analysis

I wished to develop a technique for a modified OSLER analysis in the supplemental oxygen in OSA trial to try and improve its sensitivity to any change in sleepiness that might occur. I did this by utilizing data from patients in a previous randomized control trial, the Multicentre Obstructive Sleep Apnoea Interventional Cardiovascular (MOSAIC) trial (46). This trial assessed the effects of six months of CPAP versus control on co-primary outcomes of the ESS and the Pocock cardiovascular risk score in patients with minimally symptomatic OSA and $ODI_{\geq 4\%}$ of greater than 7.5/hour. There was a significant improvement in the ESS (-2.0 in ESS, CPAP compared to control, 95% CI -2.6 to -1.4, $p<0.0001$) but not the cardiovascular risk score. There were significant improvements in the OSLER test (44% reduced odds of 7 misses within 40 minutes in the CPAP group, compared to control, 95% CI -1 to -68%, $p=0.045$). However, given that patients had minimally symptomatic OSA, many had ceiling sleep latency 7 times of 40 minutes, and therefore I wished to also test the hypothesis that modification to the OSLER test would improve its performance.

I extracted original OSLER data from VisiDownload (Stowood, Beckley, UK) to a custom designed Excel template. This template calculated the time to 6, 5 and 4 missed lights in addition to the original 7 missed lights. I also calculated the normalized error rate (events/hour), and the number of misses between 3-6. I then calculated the Cohen's d effect

size to assess if any of these tests had a larger effect size than the original OSLER time to seven misses.

3. Chapter Three: The effects of supplemental oxygen on blood pressure during CPAP withdrawal in OSA

3.1. Abstract (Chapter Three)

OSA is associated with transient elevations of blood pressure at night, diurnal hypertension, and cardiovascular risk. It is not known whether intermittent hypoxia is the dominant cause of elevated daytime blood pressure in OSA, or whether other mechanisms, such as recurrent arousals or sleep fragmentation, are responsible. Previous randomised control trials assessing the effects of supplemental oxygen on daytime blood pressure have had methodological limitations. I tested the hypothesis that supplemental oxygen, by attenuating intermittent hypoxia, would reduce the rise in morning blood pressure seen during CPAP withdrawal. I conducted a single-centre, double-blind, cross-over, randomised control trial assessing the effects of supplemental oxygen versus air, during two weeks of CPAP withdrawal on morning awake blood pressure. Treatment order was randomised. Throughout the trial, patients monitored their blood pressure in the morning at home. Twenty-five patients completed the supplemental oxygen trial. In the oxygen arm there were no significant increases in either systolic or diastolic blood pressure. Supplemental oxygen had a marked effect of attenuating the rise in morning systolic (-6.6mmHg, p=0.008, 95% CI -11.3 to -1.9) and diastolic (-4.6mmHg, p=0.006, -7.8 to -1.5) blood pressure during CPAP withdrawal compared to air. During CPAP withdrawal, supplemental oxygen therapy was effective in attenuating the blood pressure rise, compared to air. These results suggest that overnight intermittent hypoxia is the key pathophysiological process in provoking the rise in awake morning blood pressure seen in OSA.

3.2. Introduction

3.2.1. Hypertension in OSA

Hypertension is one of the major risk factors for cardiovascular disease (103). OSA is associated with increased blood pressure (68), and increased diagnoses of hypertension in cross sectional studies (15)(67). Furthermore in prospective longitudinal studies the presence of SDB is associated with increased risk of developing hypertension (66). The efficacy of CPAP in reducing blood pressure is further evidence of a causal relationship between OSA and hypertension (69)(70). Increased blood pressure could contribute to the increased cardiovascular risk seen in untreated patients with OSA (9). There are several potential pathways by which OSA could lead to increases in blood pressure. These include arousal-induced sympathetic activation, sleep fragmentation, and intermittent hypoxia (also acting via sympathetic activation as well as other potential pathways; see *Figure 3.1*).

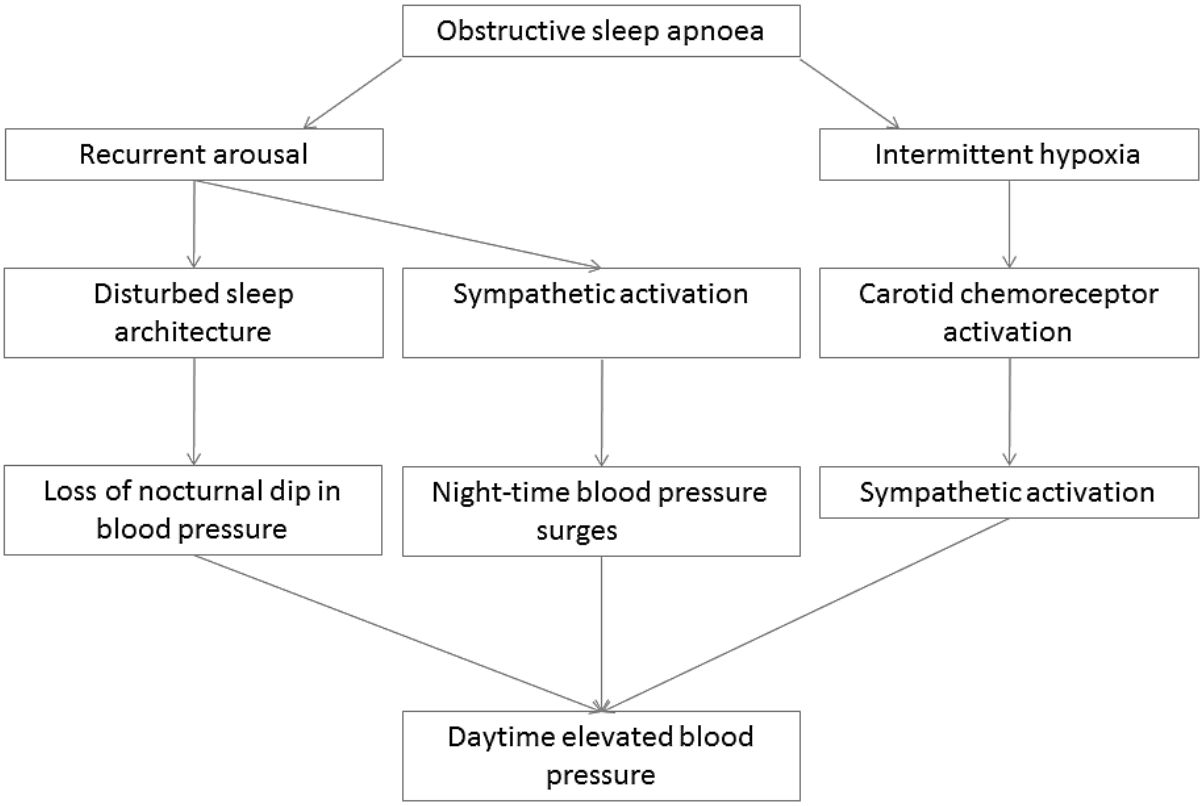


Figure 3.1: Schematic diagram showing some of the potential mechanisms by which OSA could lead to elevated daytime blood pressure.

3.2.2. Acute blood pressure rises and arousal

Acute rises in blood pressure overnight have been shown to be due to arousal and are not dependent on hypoxia (113)(114)(115)(116). These experiments demonstrate similar acute blood pressure rises in response to arousal induced by either auditory stimuli or apnoeas. Acute blood pressure rises were not seen with intermittent hypoxia alone. It is therefore the arousals, and not the attendant intermittent hypoxia, which cause the acute repetitive blood pressure rises in OSA overnight. The autonomic nervous system is activated during arousal and shows little habituation to repeated stimuli (159). Therefore, the recurrent arousals in OSA lead to increased sympathetic activity and acute blood pressure rise is part of this autonomic response. Increases in sympathetic activation are demonstrated by the finding of

elevated overnight urinary normetadrenaline in OSA (148), and increased peroneal nerve sympathetic activity during apnoeas (118). Therefore, arousal-mediated increases in sympathetic activity overnight could contribute to the increased daytime elevations in blood pressure in OSA.

3.2.3. Sleep restriction and blood pressure

Short sleep duration is associated with increased diagnoses of hypertension in cross sectional studies (200). This is thought to be due to reductions in slow wave sleep (201). In healthy individuals, blood pressure is reduced during slow wave sleep, and reductions in slow wave sleep lead to less of a nocturnal dip in blood pressure. In a mouse model, REM sleep deprivation led to hypertension in middle-aged, but not in young mice (202). Whilst this suggests that REM sleep may also be important in determining daytime blood pressure, this model did not directly measure sleep. The observed blood pressure effect could have been due to secondary changes in slow wave sleep. This study does raise the possibility that age is a factor in determining blood pressure response to sleep deprivation, as young mice had no change in blood pressure with sleep deprivation. However, sleep restriction to 4 hours/night causes increases in daytime blood pressure with a nocturnal "non-dipping" blood pressure pattern in relatively young healthy human adult volunteers (mean age 32±2 years) (166). OSA leads to alterations in sleep architecture with reductions in slow wave sleep (164). OSA is also associated with a "non-dipping" blood pressure pattern, with REM-predominant OSA being linked to this blood pressure pattern (203). Therefore, the reductions in slow wave sleep seen in OSA (204), or interruptions in REM sleep due to OSA (203), have been postulated as mechanisms underlying blood pressure increases in OSA.

3.2.4. Intermittent hypoxia and blood pressure

There is evidence from animal experiments (104), and from experiments in healthy volunteers (111), that intermittent hypoxia leads to increases in daytime blood pressure. As already discussed, Fletcher's group have shown that in some species of rodents, intermittent hypoxia leads to significant rises in blood pressure that are dependent on the carotid body, the sympathetic nervous system, and the renin-angiotensin-aldosterone system (104)(105)(107)(108). Tamisier *et al.* showed that in healthy human volunteers, intermittent nocturnal hypoxia causes elevations in daytime, but not night-time, blood pressure (111). They used ambulatory 24-hour blood pressure monitors which have been shown to cause arousals and thus raise night time blood pressure (112). The technique used by Tamisier *et al.* could have potentially masked any effect of intermittent hypoxia on night-time blood pressure. Tamisier *et al.* also showed increases in daytime muscle sympathetic nerve activity, reductions in baroreflex control of sympathetic output, and increased blood pressure rises in response to hypoxia. This suggests that intermittent overnight hypoxia increases daytime sympathetic activity, increases blood pressure gain in response to hypoxia (sensed by the chemoreceptors), and decreases baroceptor sympathetic gain. Prabhakar and colleagues argue that intermittent hypoxia leads to hypertension by causing an oxidative injury in the carotid chemoreceptors, leading to an altered chemoreceptor response (136). However, the experimental exposure to intermittent hypoxia, in both animal and human experiments, tends to be more severe than that observed in patients with OSA, and often does not model the accompanying transient hypercapnia seen in OSA. Randomised controlled trials using

supplemental oxygen in patients with OSA are an alternative way of assessing the impact of intermittent hypoxia on diurnal blood pressure on OSA.

3.2.5. Effects supplemental oxygen on blood pressure in OSA

Two randomised controlled trials have looked at the effect of supplemental oxygen therapy on blood pressure in OSA (121)(122). Whilst showing improvements in intermittent hypoxia, they did not show improvements in blood pressure. One study was significantly underpowered and had important methodological limitations - giving only 3l/min of oxygen overnight, and using office blood pressure recordings (122). Office blood pressure recordings are less increased by returning OSA in the CPAP withdrawal model, and tend to be recorded later in the day (178). The second study was well conducted and its results are reproduced with permission in *Figure 3.2* (121). There are some important limitations to consider. First, those with the most severe hypoxia (those with >10% time with oxygen saturations <85%), and the most severe OSA (those with an AHI of >50 /hour, with hypopnoeas defined with a >3% desaturation criterion) were not included as the investigators had ethical concerns about not treating these patients with CPAP for the twelve week follow-up period. Second, the flow rate of oxygen was low at 2 l/min via nasal cannulae and this may not have been sufficient to prevent desaturation during apnoeas. However, reductions in the ODI were similar between supplemental oxygen and CPAP (Personal correspondence, Appendix 8.4). Third, the average usage of supplemental oxygen therapy was modest with a mean usage of 4.8 ± 2.4 hours/night. It has been argued that usage of CPAP in this range would not treat REM OSA and thus potentially leave individuals still exposed to increased cardiovascular risk (205). Finally, ambulatory blood pressure recordings were measured every fifteen minutes

with an automatic blood pressure machine. This technique of monitoring blood pressure causes arousals from sleep and increases blood pressure (112). This will cause increases in blood pressure overnight and may also cause raised daytime blood pressure by reducing slow-wave sleep.

Table 2. **Effect of Treatment on 24-Hour Blood Pressure.***

Variable	CPAP (N=90)	NSO (N=94)	HLSE (N=97)	CPAP vs. HLSE	NSO vs. HLSE	CPAP vs. NSO
24-Hr mean arterial blood pressure						
Baseline	89.5±8.6	88.6±10.0	87.7±9.3			
12 Wk	87.8±8.1	90.2±11.1	89.0±11.2	−2.4 (P=0.04)	0.4 (P=0.71)	−2.8 (P=0.02)
24-Hr mean systolic blood pressure						
Baseline	124.7±13.5	125.3±16.9	123.6±14.3			
12 Wk	123.4±12.8	126.9±16.5	124.7±16.4	−1.9 (P=0.25)	1.2 (P=0.45)	−3.1 (P=0.06)
24-Hr mean diastolic blood pressure						
Baseline	72.0±7.7	70.8±8.3	69.6±8.6			
12 Wk	69.8±7.5	71.7±9.8	70.9±10.1	−2.8 (P=0.005)	−0.1 (P=0.95)	−2.8 (P=0.006)

* Plus–minus values are means ±SD. The between-group differences are the mean differences at 12 weeks, adjusted for study site, presence or absence of coronary artery disease, and blood pressure as measured at baseline. CPAP denotes continuous positive airway pressure, NSO nocturnal supplemental oxygen, and HLSE healthy lifestyle and sleep education.

Figure 3.2: *Table 2 from the Gottlieb study showing primary outcome data. Reproduced with permission from (Gottlieb et al. 2014 NEJM 370:2276-85* (121)*), Copyright Massachusetts Medical Society).*

A further randomised cross-over study, designed to look at the efficacy of oxygen treatment versus CPAP in mild OSA, monitored blood pressure as an unpowered exploratory secondary outcome (206). Eight patients with OSA received one month of oxygen or air, followed by the reverse, before finally receiving CPAP for the third and final month. By modern definitions these patients on average had moderate OSA, and two would fulfil criteria for severe OSA. There was a non-significant tendency to lower systolic, but not diastolic blood pressure with both oxygen and CPAP as compared to air. It is difficult to know exactly how to

interpret these data for several reasons. First, baseline values were only recorded at trial entry and there was a requirement for BP to be >150 mmHg systolic or ≥95 mmHg diastolic at trial entry. The lower values observed with air, oxygen and CPAP treatment are likely to be in part due to regression to the mean. There was no washout period between treatments and it is not clear if there was any order effect. However, the tendency towards lowering systolic blood pressure with oxygen therapy suggested that there might be an effect of oxygen treatment on blood pressure in OSA.

3.2.6. CPAP withdrawal as a model to explore blood pressure changes in OSA

The return of OSA in the CPAP withdrawal model causes large awake blood pressure rises of approximately 9mmHg in systolic and 7.8mmHg in diastolic blood pressure (178). This is associated with a rise in urinary normetadrenaline overnight, indicating increased sympathetic activation. This provides a powerful model to explore the effects of supplemental oxygen therapy in OSA on blood pressure in the short term. Due to the relatively short nature of two weeks CPAP withdrawal, compared to 12 weeks follow-up in the study by Gottlieb *et al.* (121), patients with very severe OSA or very severe hypoxaemia could be safely included. Therefore, I aimed to test the hypothesis that supplemental oxygen therapy would reduce the rise in blood pressure compared to supplemental air (sham) during two weeks withdrawal of CPAP.

3.3. Methods

Methodology for the supplemental oxygen trial can be found in Chapter 2 section 2.2. Details of screening and randomisation can be found in sections 2.2.3 and 2.2.5. In this chapter, further details of the statistical methods used in assessing the effect of supplemental oxygen on blood pressure and heart rate in the morning are included.

3.3.1. Statistical methods

3.3.1.1. Baseline characteristics in patients who withdrew (section 3.4.5)

Baseline characteristics were compared between patients who completed all four trial visits as per protocol, and patients who withdrew following randomisation and receiving the trial intervention for at least one night. Patients who withdrew before randomisation, or having not received either trial intervention, were not included in this analysis. Continuous variables were compared by unpaired t-tests where normally distributed, and Mann-Witney U tests where non-normally distributed. Categorical variables where compared using Chi-squared tests.

3.3.1.2. Primary outcome: home morning blood pressure (3.4.6)

The primary outcome measure was the effect of supplemental oxygen treatment on home systolic and diastolic blood pressure compared to air treatment during CPAP withdrawal. Primary outcome data were analysed as per a pre-specified statistical analysis plan (see Appendix 8.3). Triplicate home blood pressure recordings were taken for each day for five days prior to Visits 1 and 3 and during the 14 day intervention periods using an automated

blood pressure machine as previously described. Blood pressures from days -3, -2 and -1 prior to Visits 1 and 3, and from days 11, 12, and 13 following Visits 1 and 3, were averaged to give a mean blood pressure recording at baseline before Visit 1, at run-in before Visit 3 and following oxygen or air treatment. Home blood pressure measurements from the day of trial visits were not included as these were thought likely to be less representative of "true" home blood pressure as the patients had to perform an overnight urine collection, sleep studies, and attend study visits on these days. In previous CPAP withdrawal trials home blood pressure was similarly measured on the penultimate morning on each arm, and this may be one reason why CPAP withdrawal had a greater effect on home rather than office blood pressure in previous trials (178). Mean values from triplicate recordings in three day periods were used, except for one patient's baseline recordings where recordings from the day of their baseline visit were used as days -3, -2 and -1 were missing. The change in blood pressure was calculated by subtracting each patient's mean baseline/run-in blood pressure from their corresponding follow-up blood pressure for both systolic and diastolic blood pressure. This allowed calculation of the change in both systolic and diastolic blood pressure from baseline/run-in to follow-up for both oxygen and air treatment arms. The effects of supplemental oxygen versus air therapy on the change in home blood systolic and diastolic blood pressure during CPAP withdrawal were compared using paired t-tests. A mixed effect model was used to adjust for potential order effect and potentially modifier baseline characteristics. Details of the mixed effect models are discussed in sections 3.3.1.4 and 3.3.1.5.

3.3.1.3. Secondary outcome: home morning heart rate (3.4.7)

Home morning heart rate was recorded by the automated blood pressure machine in addition to home blood pressure. The same methodology was used to compare the change in home morning heart rate between oxygen and air therapy during CPAP withdrawal.

3.3.1.4. Assessment of potential cross-over effect (3.4.8)

Any potential cross-over effect (order effect or period effect) was assessed in three ways. First, blood pressures and heart rate at baseline (before Visit 1) were compared with run-in (before Visit 3) for each treatment order separately (oxygen-air or air-oxygen) using paired t-tests, to assess if the washout on CPAP after each order was adequate (carry over effect). Second, any potential period effect was assessed by summing and separately subtracting the changes in blood pressures and heart rate; change with supplemental oxygen plus air and change with supplemental oxygen minus air. These sum and difference of the blood pressure changes were compared between both treatment orders using unpaired t-tests. Third, mixed effect models were constructed. Mixed effect models were chosen as they would account for the paired nature of the data increasing statistical power whilst, which linear regression would not. In addition, linear regression requires that observations are independent where mixed effect models do not. These modelled systolic blood pressure, diastolic blood pressure or heart rate as the dependent variables. The treatment (oxygen=1 or air=0), visit (baseline/run-in=0 or follow-up=1), randomisation (oxygen-air=1 or air-oxygen=0) and sequence of treatment (1^{st}=0 or 2^{nd}=1 treatment received) were modelled as fixed effects. The subject trial number was included as a random effect and the interaction between visit and treatment represented the treatment effect (visit x treatment; baseline visit, either

treatment and follow-up visit with air treatment all = 0, follow-up visit and oxygen treatment = 1). Potential order effects and potential modifiers were included in a mixed effect model to determine the adjusted primary outcome.

3.3.1.5. Potential modifiers (3.4.9)

Potential confounders of the primary outcome were assessed as fixed effects in a mixed effects model with the change in either systolic BP, diastolic BP or heart rate as the dependent variables. Potential biological confounders modelled as fixed effects were age, gender, BMI, use of anti-hypertensive medications, and the severity of OSA (ODI) at screening. All potential confounders were entered in this mixed effect model to assess any potential effect and to provide the most conservative estimate of the treatment effect. Patient trial number was modelled as a random effect.

3.3.1.6. Secondary outcome: office blood pressure and heart rate (3.4.10)

Office blood pressures and heart rate were recorded in triplicate at each of the four trial visits. Recordings were averaged and the baseline/run-in means were subtracted from the follow-up means to give the change in systolic blood pressure, diastolic blood pressure and heart rate for both oxygen and air therapy. The change in systolic blood pressure, diastolic blood pressure and heart rate were separately compared between oxygen and air using paired t-tests.

3.3.1.7. Sensitivity analysis exploring the change in blood pressure in patients who withdrew following randomisation (3.4.11)

A post-hoc sensitivity analysis of the effect on blood pressure in those patients who withdrew was conducted. Criteria for inclusion in this sensitivity analysis were specified prior to data analysis. Patients were included in this analysis if they had recordings for blood pressure on at least three mornings out of the first five days of the trial. Baseline blood pressures were averaged over days -3, -2 and -1 and the follow-up blood pressures were averaged over the first five days of the trial. The changes in systolic and diastolic blood pressure were calculated by subtracting the baseline mean from the follow-up mean. The changes in blood pressure were compared between patients who completed the trial and those who did not separately for both oxygen and air using unpaired t-tests.

3.3.1.8. Blood pressure analysis by grouped trial day (3.4.12)

Systolic and diastolic blood pressures were averaged in three day grouped blocks of days for days -3 to -1, days 1 to 3, days 4 to 6, days 7 to 9 and days 10 to 12, for both oxygen and air treatment. The mean difference in blood pressure, oxygen minus air, was also calculated for each of these three day groups. Repeated measure ANOVA tests were used to assess if there were differences between these grouped days regarding the difference between oxygen minus air. Where statistically significant, between group differences were identified using Fisher's least squares differences post-hoc test.

3.3.1.9. CPAP and concentrator usage (3.4.13)

CPAP usage was assessed by download of the patient's CPAP SD card at Visits 1 and 3. Mean baseline CPAP usage was calculated by dividing the total hours of CPAP usage over 6 months prior to Visit 1 and dividing by the total number of days. The mean CPAP usage during the washout period, between Visits 2 and 3, was calculated by dividing the total hours of CPAP usage by the total number of days. The mean usage of CPAP at baseline and during the washout period was compared between treatment orders using unpaired t-tests.

Concentrator clock recordings were taken at each trial visit. The hours of concentrator operation was calculated for oxygen and air by subtracting the Visit 1 or 3 concentrator clock recording from the Visit 2 or 4 concentrator clock recording. The total hours of concentrator operation was divided by the total days for each treatment arm. The mean hours of concentrator operation/day were compared between oxygen and air using a paired t-test for patients with paired data.

3.4. Results

3.4.1. Pre-screening

There were 1081 patients' notes assessed for eligibility, 530 patients were excluded on the basis of meeting one or more exclusion criteria, or not meeting all of the inclusion criteria. Patients address was verified using hospital and GP secure online databases. Invitation letters and study patient information sheets were sent to 551 patients. There were 478 patients who declined to participate, could not be contacted, or were excluded as they met one or more exclusion criteria following invitation. Most patients who declined to participate

did so as they either felt unable to attend for five research visits or to come off of CPAP for 4 weeks and 4 days. Seventy-three patients attended a screening visit, 16 patients withdrew or were excluded at this point, prior to completing any study procedure. There were therefore 57 patients who consented and underwent screening pulse oximetry. Further details of pre-screening are shown in *Figure 3.3*.

This shows that many patients with OSA on CPAP treatment are either not eligible or are not able to participate in the CPAP withdrawal model. Therefore, those patients enrolled are a highly selected group of patients and it may be that they do not represent general patients with OSA. However, previous CPAP withdrawal trials have shown the physiological changes of interest in this study, most notably rapid return of OSA and a large rise in morning awake blood pressure (178).

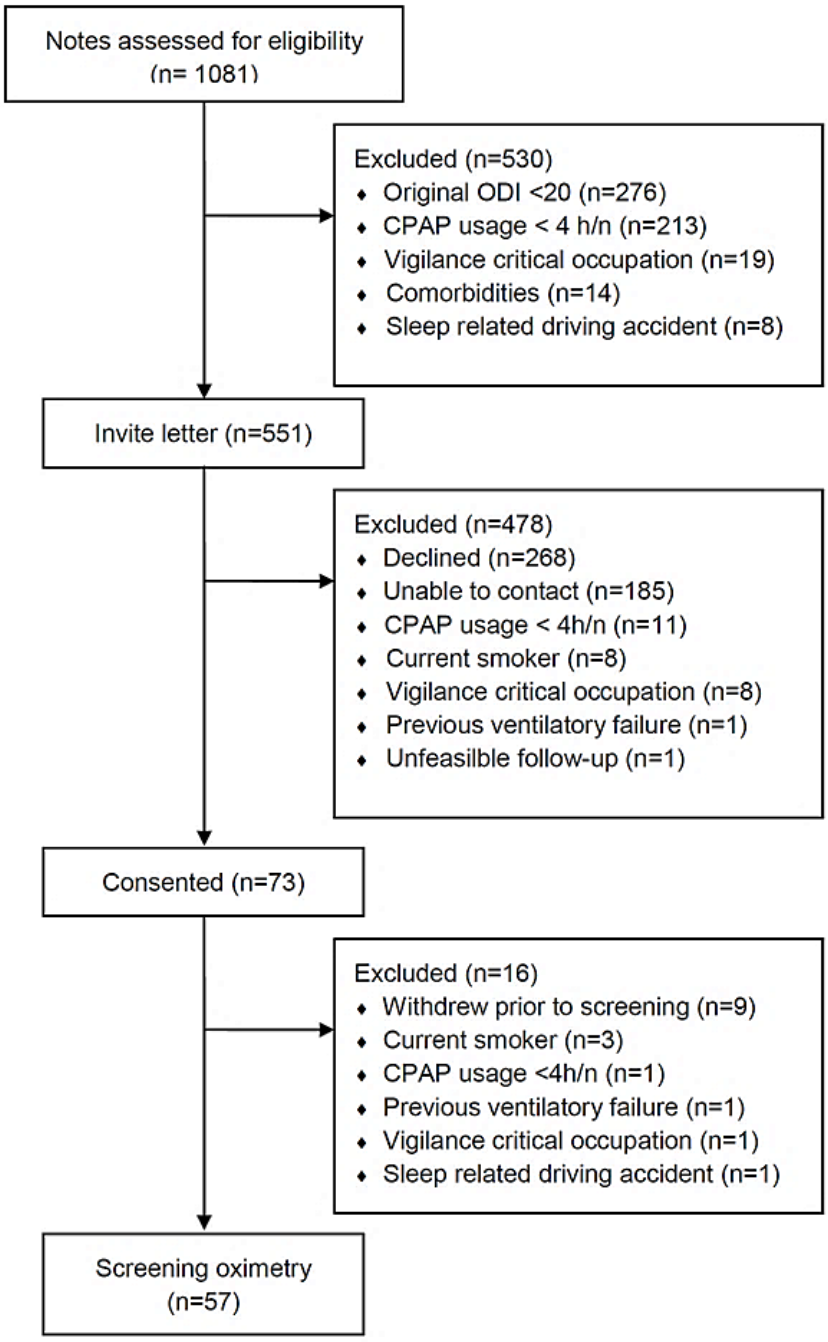


Figure 3.3: Pre-screening for the Supplemental Oxygen in OSA Trial. 1081 patients' notes were assessed for eligibility in pre-screening with 57 of these patients undergoing screening pulse oximetry.

3.4.2. Screening

Fifty-seven patients identified during pre-screening underwent screening pulse oximetry. Twelve patients were excluded following screening pulse oximetry due to not meeting the inclusion criteria; eight patients did not have a return of OSA to a sufficient extent (ODI<20 off CPAP), three patients did not have sufficiently controlled OSA on CPAP (ODI>10 on CPAP), and one patient had both an ODI<20 on four nights off CPAP and an ODI>10 on CPAP. A further seven patients withdrew their consent after screening but before receiving either trial intervention. Further details of screening are shown in *Figure 3.4*.

3.4.3. Randomisation

Following screening 38 patients were randomised. Nineteen patients were randomised to treatment order AB (oxygen followed by air) and 19 patients were randomised to treatment order BA (air followed by oxygen). One patient randomised to receive air and then oxygen, after discussion with Chief Investigator, received treatments in the alternate order of oxygen and then air, due to non-availability at the required time of one of the three air concentrators. Blinding was unaffected and as this was a cross-over trial with a physiological primary outcome this is very unlikely to have influenced the results. This meant that 20 patients received treatment order oxygen-air and 18 patients received treatment order air-oxygen.

During the first arm of treatment, four patients withdrew after having received oxygen as their first treatment, and seven patients withdrew having received air as their first

treatment. These eleven patients withdrew their consent as they were unable to tolerate being off of their CPAP therapy. One further patient, who received air first, withdrew during their washout period back on CPAP due to a prolonged hospital admission unrelated to the trial.

In the second arm of treatment, one patient was withdrawn having received oxygen as their second treatment and no patients withdrew when receiving air as their second treatment. This patient was withdrawn from the trial due to experiencing sleepiness whilst driving, which was essential to them as they drove for their commute to work. Upon notification of the trial team they were immediately withdrawn from the trial and recommenced their CPAP therapy. In summary, after receiving trial intervention, there were seven patients who withdrew whilst receiving supplemental air, five patients who withdrew whilst receiving supplemental oxygen, and one patient who withdrew during the washout period whilst on CPAP due to an unrelated illness. Further details of randomisations and withdrawals are shown in the CONSORT diagram in *Figure 3.4*.

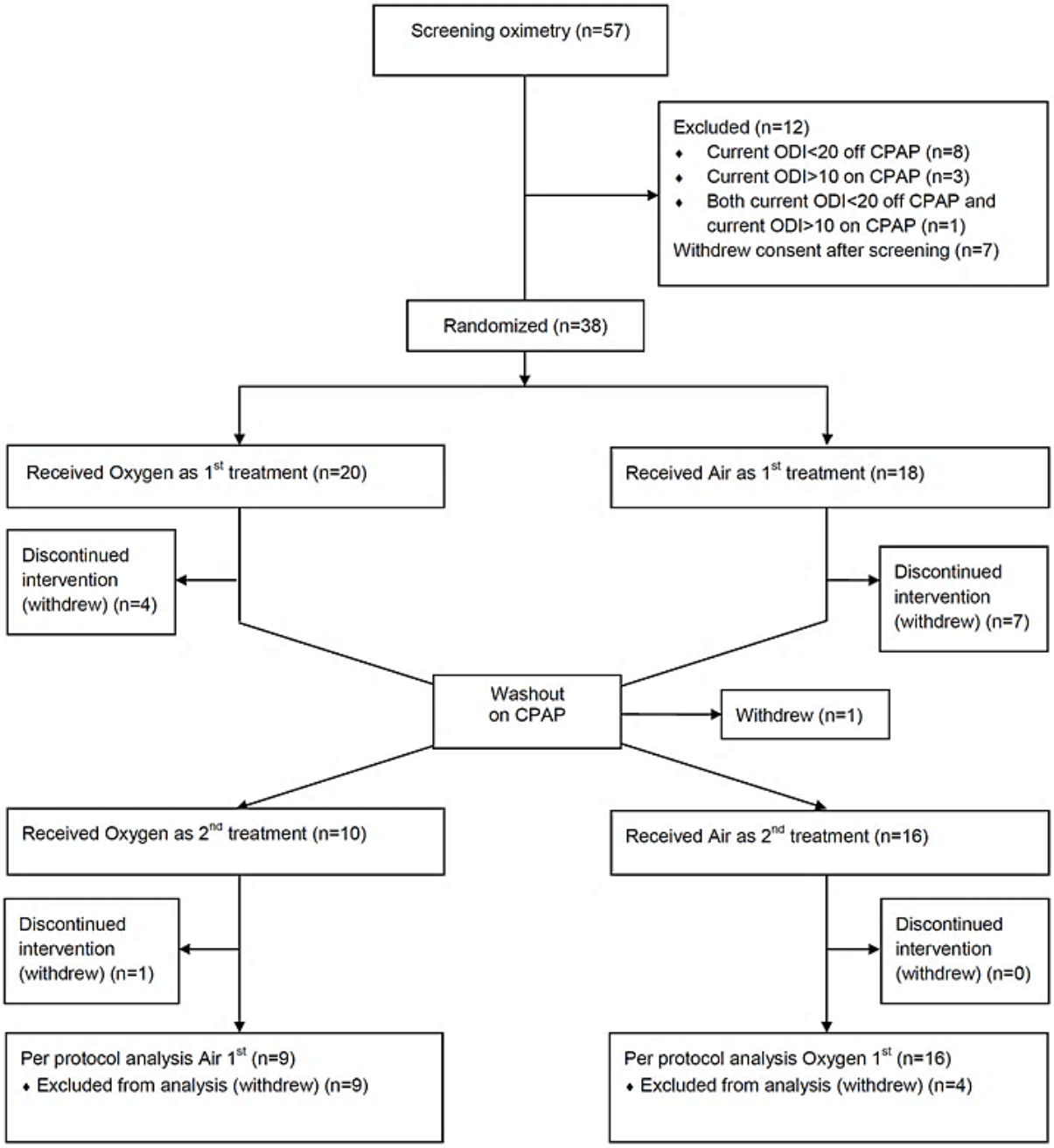


Figure 3.4: CONSORT diagram showing the flow of the 57 patients who entered screening and the 38 patients who were randomised in the Supplemental Oxygen in OSA Trial.

3.4.4. Baseline characteristics

The baseline characteristics for the patients who completed the study are shown in *Table 3.1*. The baseline characteristics for each order of treatment are also shown. Patients

allocated to the treatment order air-oxygen had a lower median (IQR) ODI during pre-trial screening of 26.7/hour (25.2, 34.3), compared to those allocated to the oxygen-air (median 42.7/hour (30.5, 54.3)). This imbalance in baseline characteristics is very unlikely to have an effect on the result due to the cross-over design of the trial, however, both of these factors were included in mixed effect models to assess for any effect on the treatment effect. Otherwise the two treatment orders were well matched.

	Mean ± SD, Median (IQR) or Number (%)		
	All Patients (n=25)	*Treatment Order Oxygen-Air (n=16)*	*Treatment Order Air-Oxygen (n=9)*
Age (years)	62.7 ± 6.9	61.0 ± 7.4	65.8 ± 4.8
Male gender	21 (84%)	14 (88%)	7 (78%)
BMI (kg/m^2)	35.3 ± 6.7	36.1 ± 7.1	34.0 ± 6.3
Neck circumference (cm)	44.2 ± 4.1	45.3 ± 3.7	42.4 ± 4.2
ODI at diagnosis (/hour)	48.0 (25.3, 68.2)	50.0 (26.1, 70.1)	45.7 (24.1, 65.7)
$ODI_{\geq 4\%}$ off CPAP in screening (/hour)	34.5 (26.3, 46.5)	42.7 (30.5, 54.3)	26.7 (25.2, 34.3)
CPAP usage (minutes)	389 ± 61	396 ± 71	377 ± 38
On regular anti- hypertensives	16 (64%)	11 (69%)	5 (56%)

Table 3.1: Baseline characteristics for patients who underwent randomisation and completed the trial. Characteristics are shown for all patients and divided into groups by treatment order.

3.4.5. Baseline characteristics in patients who withdrew

Baseline characteristics for patients who withdrew following randomisation compared with those who competed are shown in *Table 3.2*. Patients who withdrew following randomisation were significantly more obese, had significantly larger neck circumferences, and had increased baseline CPAP usage. Patients who withdrew also had a tendency towards higher median ODI values, both at diagnosis and during pre-trial screening, whilst neither of these results were statistically significant they are likely clinically meaningful. This suggests that in general, and unsurprisingly, it was the more severe patients who were less able to tolerate CPAP withdrawal.

	Mean ± SD, Median (IQR) or Number (%)		
	Patients who completed (n=25)	*Patients who withdrew after receiving intervention (n=13)*	p
Age (years)	62.7 ± 6.9	60.1 ± 9.9	0.34
Male gender	21 (84%)	12 (92%)	0.47
BMI (kg/m^2)	35.3 ± 6.7	40.2 ± 6.2	**0.04**
Neck circumference (cm)	44.2 ± 4.1	47.8 ± 3.4	**0.01**
ODI at diagnosis (/hour)	48.0 (25.3, 68.2)	57.9 (40.6, 83.6)	0.15
ODI off CPAP in screening (/hour)	34.5 (26.3, 46.5)	45.1 (31.1, 56.1)	0.29
CPAP usage (minutes)	389 ± 61	448 ± 48	**0.04**
On regular medication for hypertension	16 (64%)	8 (62%)	0.88

Table 3.2: Baseline characteristics for patients who underwent randomisation. Characteristics are shown for patients who completed the trial and patients who withdrew following

randomisation. Continuous normally distributed variables were compared using unpaired t-tests, continuous non-normally distributed variables were compared using Mann-Witney U tests, and categorical variables were compared using Chi-squared tests.

3.4.6. Primary outcome: home morning blood pressure

Triplicate home early morning blood pressures were averaged the three days prior to the visit on each of Visits 1, 2, 3 and 4. Baseline/run-in averages and follow-up data from Visits 1 or 3, and 2 or 4 respectively for oxygen and air treatment were pooled. Baseline, follow-up and primary outcome data are shown in *Table 3.3*.

Blood pressure (mmHg)	*Oxygen*		*Air*		Difference in mean change, oxygen versus air (95% CI)	p
	Baseline/ run-in	Follow-up	Baseline/ run-in	Follow-up		
Systolic	129.6 ± 15.1	129.8 ± 13.6	129.2 ± 14.1	136.1 ± 14.9	-6.6 (-11.3 to -1.9)	**0.008**
Diastolic	79.3 ± 8.0	81.6 ± 8.0	78.3 ± 7.8	85.3 ± 9.6	-4.6 (-7.8 to -1.5)	**0.006**

Table 3.3: Primary outcome data showing the treatment of oxygen compared to air on the difference in the change in blood pressure from baseline/ run-in to follow-up during CPAP withdrawal. Baseline/ run-in and follow-up data are displayed as mean ± standard deviation. p values and 95% confidence intervals (95% CI) were calculated using paired t-tests.

In the oxygen arm, there were no significant changes from baseline to follow-up in either systolic (mean difference +0.3 mmHg, p=0.89, 95% CI -3.5 to +4.0) or diastolic blood pressure (mean difference +2.3mmHg, p=0.08, 95% CI -0.3 to +5.0). In the air arm there were

significant increases from baseline to follow-up in both systolic (mean difference +6.9mmHg, p<0.001, 95% CI +3.6 to +10.1) and diastolic blood pressure (mean difference +7.0mmHg, P<0.001, 95% CI +4.5 to +9.5).

In unadjusted analyses, oxygen treatment significantly reduced the change in both systolic -6.6mmHg (p=0.008, 95% CI -11.3 to -1.9) and diastolic -4.6mmHg (p=0.006, 95% CI -7.8 to -1.5) blood pressure, when compared to air treatment during CPAP withdrawal. Oxygen appeared to markedly attenuate the rise in BP, particularly the systolic BP. Whilst the confidence intervals for this change were relatively wide, even at the bottom end of the 95% confidence interval there was still a clinically meaningful difference in systolic (-1.9mmHg) and diastolic (-1.5mmHg) blood pressure, oxygen versus air.

3.4.7. Secondary outcome: home morning heart rate

Morning heart rate was recorded along with blood pressure at home and mean heart rate on the three days prior to each visit was recorded. Mean heart rate was comparable at baseline/run-in, with values of 61.9 ± 9.4bpm and 61.7 ± 8.3bpm for oxygen and air treatment respectively. Mean heart rate was higher in both groups at follow-up, with values of 64.1 ± 9.1bpm and 64.9 ± 8.9 for oxygen and air treatment respectively. The difference in the change in heart rate from baseline to follow-up, oxygen versus air, was not statistically significantly different, with a mean of -1.0 bpm (p=0.50, 95% CI -3.9 to +1.9). Results are shown in *Table 3.4*.

	Oxygen		*Air*		Difference in mean	

	Baseline/ run-in	Follow-up	Baseline/ run-in	Follow-up	change, oxygen versus air (95% CI)	p
Heart rate (bpm)	61.9 ± 9.4	64.1 ± 9.1	61.7 ± 8.3	64.9 ± 8.9	-1.0 (-3.9 to +1.9)	0.50

Table 3.4: Secondary outcome data showing the treatment of oxygen compared to air on the difference in the change in heart rate from baseline/run-in to follow-up during CPAP withdrawal. Baseline/run-in and follow-up data are displayed as mean ± standard deviation. p values and 95% confidence intervals (95% CI) were calculated using paired t-tests.

3.4.8. Assessment of potential cross over effect

Potential carry-over of blood pressure and heart rate changes from the first arm of treatment to the second arm of treatment following washout were assessed. Baseline (pre-Visit 1) and run-in (pre-Visit 3) blood pressures and heart rates for patients were compared using paired t-tests separately for both arms of the trial. Results are shown in *Table 3.5*.

Order AB n=16	*Baseline (pre-oxygen)* Mean ± SD	*Run-in (pre-air)* Mean ± SD	Mean difference in change of BP (95% CI)	p
Systolic (mmHg)	130.8 ± 13.1	131.4 ± 12.4	+ 0.6 (-3.2 to + 4.3)	0.75
Diastolic (mmHg)	79.1 ± 7.4	79.7 ± 7.6	+ 0.6 (-1.2 to + 2.4	0.50
Heart rate (bpm)	60.7 ± 10.1	61.4 ± 8.1	+ 0.7 (- 1.8 to + 3.2)	0.57
Order BA n=9	*Baseline (pre-air)* Mean ± SD	*Run-in (pre-oxygen)* Mean ± SD	Mean difference in change of BP	p
Systolic	125.4 ± 16.8	127.3 ±18.6	+1.9 (-3.5 to + 7.3)	0.43

(mmHg)				
Diastolic (mmHg)	75.9 ± 7.9	79.6 ± 9.4	+3.7 (-1.8 to + 9.2)	0.16
Heart rate (bpm)	62.1 ± 9.1	63.9 ± 8.1	+1.8 (-0.4 to + 4.1)	0.10

Table 3.5: Results of a comparison of the baseline and run-in blood pressures for both treatment arms using paired t-tests. No significant differences were observed but there was a trend towards higher run-in diastolic blood pressure and heart rate in patients who received air and then oxygen treatment.

There was no significant carry-over effect in patients receiving oxygen followed by air. Patients who received air and then oxygen had a trend towards higher diastolic (mean difference +3.7mmHg, p=0.16) and heart rate (+1.8bpm, p=0.10), but neither were significant, possibly due to the small sample size. This is somewhat surprising as the effect of intermittent hypoxia resolves with 5 days of normoxia in healthy volunteers (111). This carry over effect will be included in adjusted primary outcome analyses.

Potential effects of order on the magnitude of blood pressure and heart rate rises were assessed. The summed magnitude of blood pressure rises during the two treatment arms were compared between the two treatment orders using unpaired t-tests. Results are shown in *Table 3.6*. There were no significant differences.

	AB (*Oxygen-Air)*	BA (*Air-Oxygen)*	Mean difference in summed increases in BP (AB-BA)	95% CI of mean difference	p value
	Summed BP increases (V1toV2 + V3toV4)	Summed BP increases (V1toV2 + V3toV4)			
	Mean ± SD	Mean ± SD			
Systolic (mmHg)	7.7 ± 11.8	6.1 ± 14.2	+1.5	-9.4 to +12.5	0.78
Diastolic (mmHg)	10.2 ± 10.9	7.8 ±7.7	+2.4	-6.1 to +10.9	0.57
Heart rate (bpm)	7.4 ± 12.1	2.0 ± 8.4	+5.4	-4.1 to +14.8	0.25

Table 3.6: Results of a comparison of the summed changes in blood pressure during both treatment arms between those both treatment order allocations. Unpaired t-tests were used. No significant differences were observed.

The potential effect of treatment order was assessed by subtracting the change in blood pressure or heart rate with air treatment from the change in blood pressure or heart rate with oxygen treatment and comparing these differences between both treatment orders using unpaired t-tests. Results are shown in *Table 3.7*. There were no significant differences.

	AB	BA	Mean difference in differences increases in BP (AB-BA)	95% CI of mean difference	p value
	Difference in BP increases (V1toV2 minus V3toV4)	Difference in BP increases (V3toV4 minus V1toV2)			
	Mean ± SD	Mean ± SD			
Systolic (mmHg)	-7.8 ± 12.4	-4.5 ± 10.0	-3.3	-13.3 to +6.7	0.50
Diastolic (mmHg)	-5.5 ± 8.3	-3.1 ± 6.7	-2.4	-9.1 to +4.3	0.47
Heart rate (bpm)	+0.1 ± 7.7	-2.8 ± 5.5	+2.9	-3.2 to +8.9	0.34

Table 3.7: Results of a comparison of the difference in changes in blood pressure with oxygen treatment minus air treatment between both treatment order allocations. Unpaired t-tests were used. No significant differences were observed.

As a secondary check of any order effect, a mixed effect model was constructed in SPSS. These were constructed to estimate the most conservative treatment effect of supplemental oxygen on morning blood pressure. Therefore all order and potential confounders were included in the final adjusted models. The dependent variable was systolic blood pressure, diastolic blood pressure or heart rate. Subject trial number was a random effect factor in the model and randomisation order (Random: 0=BA and 1=AB), visit (Visit: 0=Baseline/Run-in and 1=Follow-up), treatment received (Treatment: 0=Air and 1=Oxygen), sequence of treatment (Sequence: 0=First and 1=Second treatment received) were all fixed effects. An interactive term (visit × treatment) was added and this was the adjusted treatment effect.

For the mixed effect model with systolic blood pressure as the dependent variable there was a significant effect of air treatment at either time point of +5.7mmHg (p=0.001, 95% CI +2.4

to +9.0) and a trend towards a lower systolic blood pressure in the first treatment arm of -2.1mmHg (p=0.09, 95% CI -4.5 to +0.3). The effect of oxygen treatment on systolic blood pressure compared to air was essentially unchanged after adjustments for order effects and was -6.6mmHg (p=0.005, 95% CI -11.2 to -2.0). The full results of the model are shown in *Figure 3.5*.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	23	2138.002	.000
Visit	1	71.000	9.612	.003
Treatment	1	71.000	3.968	.050
Visit * Treatment	1	71.000	8.259	.005
Random	1	23	.828	.372
Sequence	1	71.000	3.016	.087

a. Dependent Variable: Systolic.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	133.018021	3.611173	29.572	36.835	.000	125.638542	140.397500
[Visit=.00]	-.260000	1.623920	71.000	-.160	.873	-3.498005	2.978004
[Visit=1.00]	0[b]	0	.	.	.	.	.
[Treatment=.00]	5.682812	1.658097	71.000	3.427	.001	2.376661	8.988964
[Treatment=1.00]	0[b]	0	.	.	.	.	.
[Visit=.00] * [Treatment=.00]	-6.600000	2.296570	71.000	-2.874	.005	-11.179230	-2.020769
[Visit=.00] * [Treatment=1.00]	0[b]	0	.	.	.	.	.
[Visit=1.00] * [Treatment=.00]	0[b]	0	.	.	.	.	.
[Visit=1.00] * [Treatment=1.00]	0[b]	0	.	.	.	.	.
[Random=.00]	-5.134934	5.643489	23	-.910	.372	-16.809380	6.539511
[Random=1.00]	0[b]	0	.	.	.	.	.
[Sequence=.00]	-2.077257	1.196130	71.000	-1.737	.087	-4.462273	.307759
[Sequence=1.00]	0[b]	0	.	.	.	.	.

a. Dependent Variable: Systolic.

b. This parameter is set to zero because it is redundant.

Figure 3.5: Results of fixed effects from SPSS mixed effect model for systolic blood pressure.

For the mixed effect model with diastolic as the dependent variable there was a significant effect of air treatment at either time point of +2.9mmHg (p=0.02, 95% CI +0.4 to +5.4), a significantly lower diastolic blood pressure in the first treatment arm -2.7mmHg (p=0.003, 95% CI -4.5 to -0.9), and a trend towards reduced blood pressure at baseline/run-in visit -2.3mmHg (p=0.06, 95% CI -4.8 to 0.1). The effect of oxygen treatment compared to air on diastolic blood pressure remained significant and was essentially unchanged by these adjustments for potential order effects and was -4.6mmHg (p=0.009, 95% CI -8.1 to -1.2).

For the mixed effect model with heart rate as the dependent variable there was a trend toward a reduction in heart rate at baseline/run-in visits -2.3bpm (p=0.06, 95% CI -4.6 to +0.1), but none of the fixed effects modelled were significant. The adjusted effect of oxygen treatment on heart rate compared to air was -1.0bpm (p=0.57, 95% CI -4.3 to +2.4).

Adjustments were made for order effects and potential modifiers (section 3.4.9). Results of the adjusted outcomes along with the unadjusted outcomes are shown in *Table 3.8*. The effects of oxygen treatment compared to air on systolic and diastolic blood pressure were essentially unchanged following these order adjustments when compared with the unadjusted effects. The effect of oxygen treatment compared to air on heart rate remained non-significant.

	Unadjusted effect (95% CI)	p value	Adjusted effect (95% CI)	p
Systolic BP (mmHg)	-6.6 (-11.3 to -1.9)	**0.008**	-6.6 (-11.2 to -2.0)	**0.005**
Diastolic BP (mmHg)	-4.6 (-7.8 to -1.5)	**0.006**	-4.6 (-8.1 to -1.2)	**0.009**
Heart rate (bpm)	-1.0 (-3.9 to 1.9)	0.50	-1.0 (-4.3 to 2.4)	0.57

Table 3.8: The unadjusted and adjusted effect of oxygen treatment on blood pressure or heart rate, compared to air treatment. Paired t-tests were used for unadjusted analyses and mixed effect models were used for adjusted treatment effects including order and modifier variables as fixed effects.

The confidence interval for the adjusted effect of supplemental oxygen on diastolic blood pressure and heart rate were wider than the unadjusted effect. This is due to inclusion of all potential confounders to produce the most conservative estimate of treatment effect.

3.4.9. Potential modifiers

Age, gender, BMI, anti-hypertensive use and severity of OSA (ODI) at diagnosis were considered as potential modifiers. These were explored in mixed effect models in SPSS, with the change in blood pressure or heart rate as the dependent variable and subject trial number as a random effect. None of age, gender, BMI, anti-hypertensive use or severity of OSA at diagnosis significantly modified the difference in either systolic BP, diastolic BP or heart rate.

3.4.10. Secondary outcome: office blood pressure and heart rate

Office blood pressure and heart rate were measured in triplicate and a mean recording was taken for systolic blood pressure, diastolic blood pressure and heart rate at each trial visit. Baseline/run-in and follow-up means were calculated for both oxygen and air treatment. The differences in change in blood pressure and heart rate between oxygen and air treatment were compared using paired t-tests.

Blood pressure was recorded after the OSLER test and mean time for the OSLER to end was 10:03 ± 00:23 hours. Therefore office blood pressure recordings, on average, were taken after 10am.

Office blood pressure and heart rate recordings are shown in *Table 3.9*. There were significant differences in the change in both systolic (mean difference -8.3mmHg, p=0.02, 95% CI -15.3 to -1.3) and diastolic blood pressure (mean difference -6.3mmHg, p=0.01, 95% CI -11.0 to -1.6), oxygen versus air. Unexpectedly, there appeared to be a larger effect of oxygen therapy on office blood pressure than home blood pressure. However, there is significant overlap of the confidence intervals for the effect of supplemental oxygen on office and home blood pressure. There was no significant difference in the change in heart rate with a difference of -0.8bpm (p=0.61, 95% CI -4.1 to +2.5), oxygen versus air.

Office recordings	*Oxygen*		*Air*		Difference in mean change, oxygen versus air (95% CI)	p
	Baseline/ run-in	Follow-up	Baseline/ run-in	Follow-up		
Systolic BP (mmHg)	132.4 ± 16.7	130.9 ± 15.3	128.0 ± 13.7	134.8 ± 15.5	-8.3 (-15.3 to -1.3)	**0.02**
Diastolic BP (mmHg)	80.8 ± 9.4	79.6 ± 8.2	78.9 ± 9.9	84.0 ± 9.2	-6.3 (-11.0 to -1.6)	**0.01**
Heart rate (bpm)	66.3 ± 13.5	67.6 ± 13.0	65.6 ± 11.9	67.8 ± 14.0	-0.8 (-4.1 to +2.5)	0.61

Table 3.9: Secondary outcome data showing the treatment of oxygen compared to air on the difference in the change in office blood pressure or heart rate from baseline/run-in to follow-up during CPAP withdrawal. Baseline/run-in and follow-up data are displayed as mean ± standard deviation. p values and 95% confidence intervals (95% CI) were calculated using paired t-tests.

3.4.11. Sensitivity analysis exploring the change in blood pressure in patients who withdrew following randomisation

Three patients who withdrew whilst receiving air treatment and four patients who withdrew whilst receiving oxygen treatment had sufficient blood pressure data to be included in the blood pressure sensitivity analyses.

Baseline systolic blood pressures where higher for those patients who withdrew compared to those who completed the trial with a mean difference on +15.6mmHg (p=0.01, 95% CI +3.8 to +27.3). Baseline mean diastolic blood pressure was not statistically significantly

different between those who withdrew compared to those who completed (mean difference +4.2mmHg, p=0.18, 95% CI -1.9 to + 10.3).

With air treatment, the average increases in systolic blood pressure over the first five days of treatment were comparable; for the four patients who withdrew the increase in systolic BP was 2.3 ± 6.7mmHg, compared with an increase of 2.4 ± 8.9 mmHg for those who completed the trial. Owing to the small number of patients this comparison is under-powered. The mean difference of -0.1mmHg between those who withdrew and those who completed was not significant (p=0.98, 95% CI -9.8 to +9.4). With air treatment, the average increase in the diastolic blood pressure over the first five days of treatment was +1.0 ± 7.0mmHg for patients who withdrew and +3.4 ± 6.7mmHg for patients who completed the trial. Again, owing to the small number of patients this comparison is unpowered. The mean difference of -2.5mmHg between those who withdrew and those who completed was not significant (p=0.51, 95% CI -9.9 to + 5.0).

With oxygen treatment, the average increase in systolic blood pressure for the three patients who withdrew over the first five days was +0.3 ± 9.3mmHg whereas the average systolic blood pressure reduced for patients who completed the trial by -2.4 ± 9.4mmHg. The mean difference in change of systolic blood pressure between those who withdrew and those who completed was +2.7mmHg (p=0.65, 95% CI -9.2 to +14.5). With oxygen treatment, the average increase in diastolic blood pressure for the patients who withdrew was +4.9 ± 6.2mmHg compared with -0.9 ± 6.5mmHg for those who completed the trial. The mean

difference in change of diastolic blood pressure between those who withdrew and those who completed was +5.8mmHg (p=0.15, 95% CI -2.3 to +13.9).

Owing to the small number of patients with data available who withdrew the confidence intervals for all estimates are broad and this analysis is underpowered to detect clinically meaningful differences.

3.4.12. Blood pressure analysis by grouped trial day

The blood pressures were averaged over three day periods, with baseline days -3 to -1, then treatments days 1 to 3, 4 to 6, 7 to 9, and 10 to 12. Mean blood pressures for these grouped days are shown in *Figure 3.6* for systolic blood pressure, and *Figure 3.7* for diastolic blood pressure.

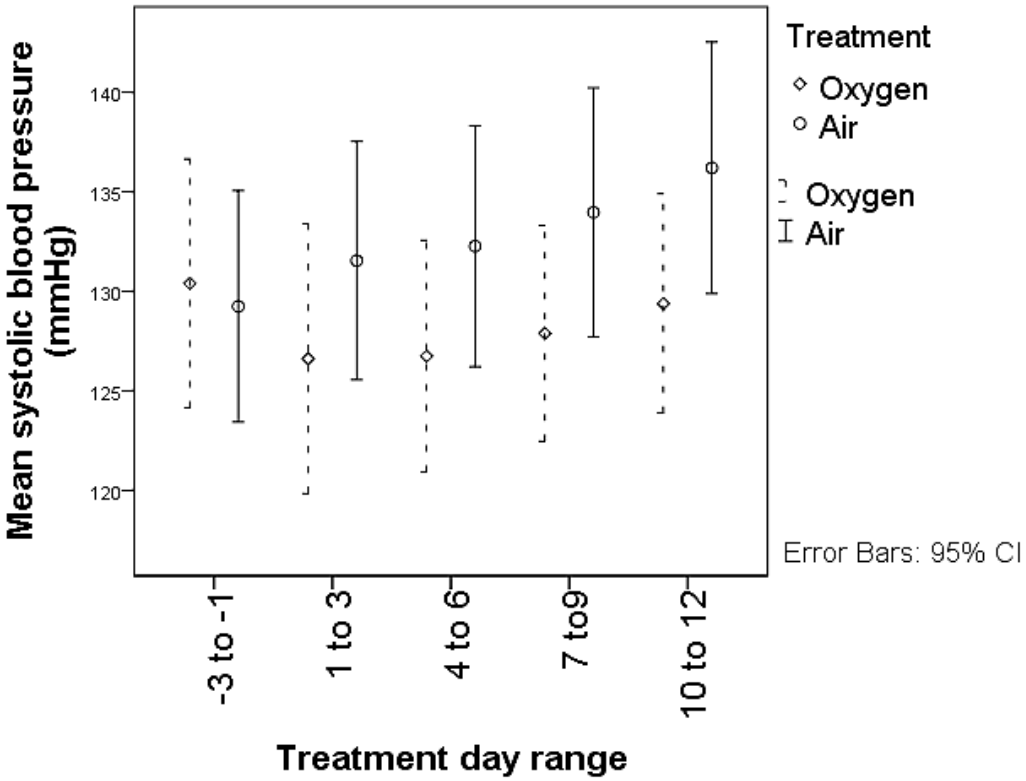


Figure 3.6: Mean systolic blood pressure grouped into three day blocks by treatment.

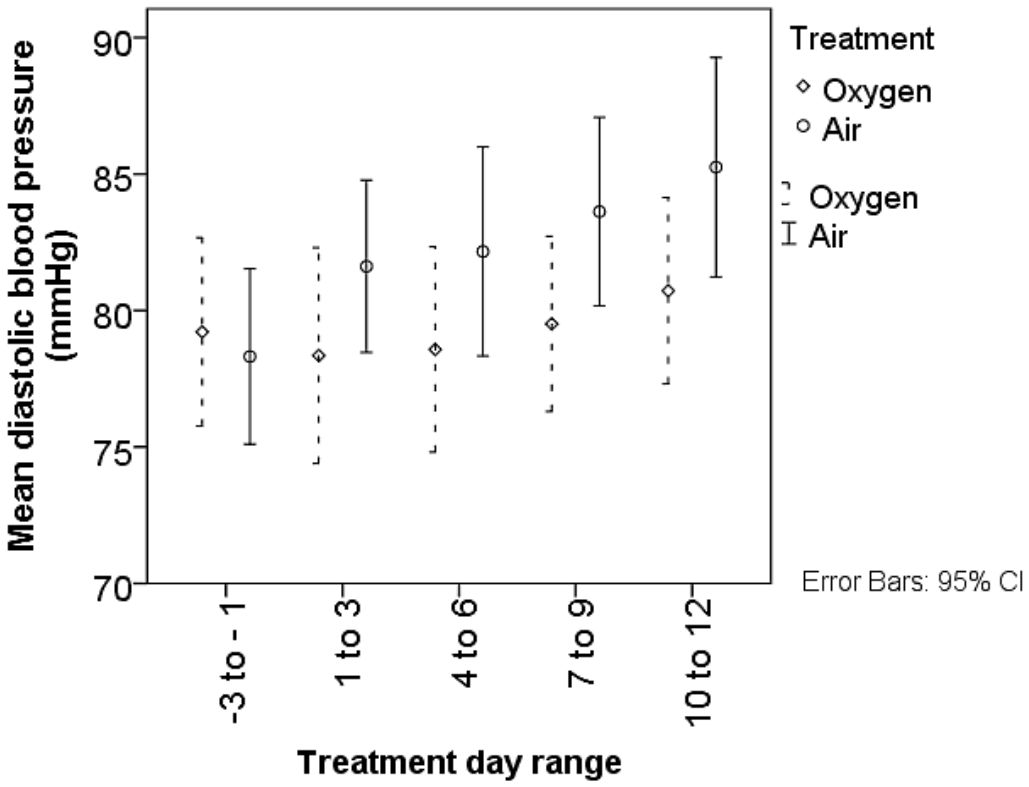


Figure 3.7: Mean diastolic blood pressure grouped into three day blocks by treatment.

The differences in blood pressure, oxygen minus air, were compared between the different grouped days. There were significant differences between groups in repeated measures for systolic blood pressure (p=0.005, F=3.93, degrees of freedom=4). Using Fisher's post hoc test there were significant differences between the baseline group and all treatment day groups. There were no statistically significant differences between the different treatment day groups. There were significant differences between groups in repeated measures for diastolic blood pressure (p=0.007, F=3.80, degrees of freedom=4). Using Fisher's least square difference post hoc tests there were differences between the baseline group and all

treatment day groups. There were no statistically significant differences between the different treatment day groups. Results are shown in *Table 3.10*.

Repeated measures ANOVA						
Blood pressure (mmHg)	Baseline -3 to -1	Days 1 to 3	Days 4 to 6	Days 7 to 9	Days 10 to 12	p value ANOVA
Systolic	-0.1 ± 6.8*	-5.3 ± 6.8	-5.9 ± 7.6	-6.3 ± 7.6	-6.9 ± 8.4	**0.005**
Diastolic	+0.5 ±4.8*	-3.6 ± 6.0	-3.8 ± 6.1	-4.2 ± 5.1	-4.7 ± 7.6	**0.007**
Fisher's post hoc test: Comparison to Baseline						
Systolic	-	-5.2 (-8.7 to -1.7) p=0.005	-5.8 (-10.4 to -1.2) p=0.02	-6.2 (-10.5 to -2.0) p=0.006	-6.8 (-11.4 to -2.2) p=0.005	
Diastolic	-	-4.0 (-6.6 to -1.4) p=0.004	-4.3 (-7.2 to -1.3) p=0.006	-4.6 (-7.4 to -1.8) p=0.003	-5.1 (-8.6 to -1.7) p=0.005	

*Table 3.10: Results of repeated measure ANOVA tests comparing the difference in the blood pressure, oxygen minus air, by day from baseline (days -3 to -1) and then in three day groups. *Denotes values that are significantly different using Fisher's post hoc test, no other between group comparisons were significant. Mean differences, p values and 95% Confidence intervals compared to baseline are quoted for all days.*

3.4.13. CPAP and concentrator usage

Baseline CPAP usage was 6:29 ± 1:01 h:min/night. CPAP usage at baseline was comparable for those allocated oxygen-air (6:36 ± 1:11 h:min/night) and those allocated to air-oxygen

(6:17 ± 0:38 h:min/night). CPAP usage during the washout period between Visit 2 and 3 was 7:06 h:min/night. The difference in CPAP usage during the washout period by treatment order was not significant (oxygen-air order compared to air-oxygen: mean difference=+0:11 h:min/night, p=0.75, 95% CI -0:59 to 1:20).

The number of hours that each concentrator was in operation between visits was recorded at each visit. Concentrator operation data was available for 21 patients for concentrator A (oxygen) and for 21 patients with concentrator B (air). Mean times that each concentrator was in operation were 7:12 ± 1:01 h:min/night and 7:22 ± 0:47 h:min/night for oxygen and air respectively. Operation hours were compared between oxygen and air concentrators using a paired t-test for the 19 patients who had paired data. There was no statistically significant difference in hours of operation (difference in mean usage -0:09 h:min/night for oxygen versus air, p=0.34, 95% CI -0:30 to + 0:11).

3.5. Discussion

In this chapter I have presented results of the effect of supplemental oxygen therapy compared to sham during two weeks of CPAP withdrawal on morning blood pressure and further explored the effect with a number of sensitivity analyses. Supplemental oxygen therapy markedly attenuates the rise in systolic blood pressure seen with short-term CPAP withdrawal. The effect of oxygen treatment on blood pressure was independent of the order of treatment, age, gender, BMI, severity of OSA, anti-hypertensive medication use and time

of day the blood pressure was recorded. In contrast, supplemental oxygen therapy does not change the rise in morning heart rate seen with CPAP withdrawal.

The confidence intervals for the effect of supplemental oxygen on blood pressure are wide (-11.3 to -1.9mmHg for systolic and -7.8 to -1.5mmHg for diastolic blood pressure). However, even at the lower end of this estimate there is still a clinically meaningful effect on both systolic and diastolic blood pressure, which is comparable to the effects of CPAP (70). This result was consistently observed, in both home and office blood pressure readings, and remained following adjustments for potential confounders. Lung function was not measured and therefore adjustments could not be made for end-expiratory lung volume. However, BMI was included in adjustments and given the cross-over design of the data, differences in lung function are very unlikely to have influenced the results. The effect of supplemental oxygen on morning blood pressure occurred within the first few days of CPAP withdrawal and was not diminished during the 14 days of CPAP withdrawal. Therefore this effect of supplemental oxygen is robust, consistent and sustained throughout 14 days of CPAP withdrawal.

The relationship between OSA and blood pressure is clear. OSA is associated with increased blood pressure and increased diagnoses of hypertension, and treating OSA with CPAP improves blood pressure (15)(66)(67)(68). However, the mechanism responsible for elevated blood pressure has not been fully established. My results support a dominant role of intermittent hypoxia in causing elevated daytime blood pressure in OSA.

3.5.1. Experimental intermittent hypoxia

Fletcher's experiments elegantly showed the effect of intermittent hypoxia in increasing blood pressure in a rodent model of OSA (104). They showed that this increase in blood pressure was dependent on the carotid body sensing hypoxia (105), that it was dependent on sympathetic activation via the renal arteries, as renal denervation reversed this effect (107), and on the renal angiotensin-aldosterone system, as blocking this system with losartan reversed the effect (108). The observation that anti-hypertensive medications did not interfere with oxygen attenuating the blood pressure rise in my study, may be due to differences in effective doses of medications, with much higher doses being used in animal experiments than used in clinical practice. In healthy human volunteers, intermittent hypoxia has been shown to increase daytime, but not night-time, blood pressure over 14 nights (111). This increase in blood pressure was also associated with increases in sympathetic activity, increases in chemoreceptor gain and decreased baroceptor gain.

3.5.2. RCTs of supplemental oxygen in OSA

Supplemental oxygen therapy has not been shown to reduce blood pressure in previous randomised controlled trials assessing its effect on blood pressure in OSA. Mills *et al.* carried out a small randomised control trial where 17 patients received supplemental oxygen therapy for two weeks which was probably underpowered (122). In addition, it had methodological limitations with office blood pressure being used rather than home early morning readings, and with low flow rates of oxygen 3l/min. Results of their observations on 24-hour ambulatory blood pressure from the same patients were reported separately. In this

subgroup only 13 patients had oxygen treatment and 24-hour ambulatory blood pressure. Whilst this was underpowered, they observed reductions in both night-time and daytime BP with CPAP, no change in daytime BP with oxygen therapy, and increases in both daytime and night-time BP with air therapy. The direction of effect of oxygen versus air was the same as I observed (207).

Gottlieb *et al.* conducted a moderate size randomised control trial, published in the *New England Journal of Medicine*, in which 106 treatment-naïve patients received supplemental oxygen therapy, versus CPAP, versus control (121). However, they excluded the most severely hypoxic patients, and the patients with the most severe OSA, due to ethical concerns about not treating these patients with CPAP over the longer follow-up duration of 12 weeks. The shorter duration of CPAP withdrawal in my trial allowed inclusion of these more severe patients. Again, I have concerns about ambulatory 24-hour BP monitoring used by Gottlieb *et al.,* which causes arousals from sleep and increases blood pressure (112). In addition to raising night-time blood pressure, arousals from sleep fragment sleep, and may therefore cause raised daytime blood pressure by reducing slow-wave sleep (201).

A small cross-over trial studied eight patients with mainly mild to moderate OSA and hypertension. As discussed earlier there were significant methodological limitations in this trial. This showed a trend towards oxygen and CPAP improving blood pressure but not diastolic blood pressure when compared to air treatment over one month of each treatment (206).

Whilst these trials were not definitive and had significant limitations, the direction of change in blood pressure in the two smaller trials was in favour of oxygen compared to air. The supplemental oxygen trial, in similarly severe patients, addressed many of these limitations. I used higher flow rates of oxygen more likely to attenuate intermittent hypoxia and used a cross-over design. Therefore, the supplemental oxygen trial has shown definitively in patients with more severe OSA that supplemental oxygen has a marked effect on daytime blood pressure.

The larger Gottlieb study may have produced different results for several reasons; the most hypoxic patients were excluded, the lower flow rates of oxygen not adequately treating the intermittent hypoxia, the duration oxygen therapy was used at night was modest, or it could relate to the duration of the intervention. My data, and data from the two smaller studies (122)(206), assessed the effects of oxygen over either 2 or 4 weeks, which is shorter than the Gottlieb study duration of 12 weeks (121). It is possible that the effect of oxygen therapy on blood pressure in OSA is not sustained. Alternatively the improved compliance with supplemental oxygen I achieved may explain this difference. In my study supplemental oxygen was used for a mean of 7:12 ± 1:01 h:min/night which was markedly longer than the 4.8 ± 2.4 h/night in the Gottlieb study. REM OSA has been linked to nocturnal “non-dipping” blood pressure patterns (203). Lower supplemental oxygen usage may be meaningful as it may not be treating intermittent hypoxia in REM sleep which is more common in the latter portion of the night (205), and is likely to represent periods of the most significant hypoxia.

Also the lower flow rates of oxygen may have failed to adequately treat intermittent hypoxia during REM sleep.

3.5.3. Limitations

There are limitations in this study. First, CPAP was only withdrawn for two weeks at a time. This is due to ethical concerns over, and patient tolerance of, withdrawing therapy for any longer. Therefore, the longer-term effect of supplemental oxygen therapy on blood pressure in OSA is not known. It is possible that the rise in blood pressure seen with return of OSA with CPAP withdrawal diminishes over time and is due to different mechanisms to the longer term hypertension seen in some patients. The magnitude of the blood pressure rise with CPAP withdrawal (178), is much more marked than the magnitude of the reduction in blood pressure seen with CPAP treatment (69). Therefore, the difference in blood pressure between supplemental oxygen therapy and sham may decrease over time. There did not seem to be any reduction in the effect of oxygen versus air in the last few days of CPAP withdrawal compared with the first few days (Section 3.4.12). It has been argued that the larger differences in blood pressure in CPAP withdrawal reflect patients' higher compliance with CPAP and the dose related effect of OSA on blood pressure (178); indeed the magnitude of blood pressure improvements with CPAP treatment are similar for compliant patients— 4.9mmHg improvement in mean blood pressure in those using CPAP >5h/night (69)— to the effect of CPAP withdrawal. Certainly the increased compliance, and higher oxygen flow rates more likely to adequately treat intermittent hypoxia, in my study may explain the difference in my results compared to the Gottlieb study.

A compensatory mechanism such as that seen on exposure to chronic hypoxia when travelling to altitude cannot be ruled out. Blood pressure increases on travel to altitude (4790m) from sea level, but returns to normal levels after one week at altitude (208). However, sympathetic activity seems to remain elevated, even in native highlanders, with prolonged exposure to chronic hypoxia (209). Lundby *et al.* took Danish sea level residents to 4100m altitude and measured their mean arterial blood pressure and muscle sympathetic activity at sea level, during one hour of acute hypoxia at sea level, at 10 days after travel to altitude, and following 50 days of travel to altitude. One hour of acute hypoxia did not increase either mean arterial blood pressure or muscle sympathetic nerve activation but both were markedly increased after 10 days at 4100m. After 50 days at 4100m, muscle sympathetic nerve activation remained similarly elevated, but the mean arterial blood pressure had partially recovered (210). This suggests that blood pressure can acclimatise to chronic hypoxia. It is possible that similar adaptation could occur to intermittent hypoxia in the longer-term and reduce the effect size of supplemental oxygen on blood pressure. However, no such adaption was seen with the 14 days of the trial (Section 3.4.12). The supplemental oxygen trial was not designed to address the longer-term effects of supplemental oxygen in OSA, and the longer-term effects need to be assessed in future research.

There was also a higher drop-out rate in this trial compared to previous withdrawal trials (13 of 38 vs 1 of 110 or 26% vs 1%). Two factors probably explain this increase in withdrawals after randomisation. First, patients were asked to go without the CPAP therapy for a longer period of time, with two periods of CPAP withdrawal in this cross-over study. Second,

patients in previous withdrawal trials had a 50% chance of continuing on CPAP therapy during the trial which they did not have here. Patients who withdrew after randomisation were more obese and had more severe OSA. It is possible that they may not have responded to supplemental oxygen therapy during CPAP withdrawal in the same way and the results are therefore not applicable to all patients with OSA.

There were more patients who withdrew whilst receiving supplemental air than when receiving supplemental oxygen (8 versus 5, NS). One of the patients who withdrew after receiving air did so during the washout period due to an unrelated prolonged hospital admission. Thus, seven receiving air and five receiving oxygen withdrew due to being unable to tolerate being off CPAP therapy. This potentially introduces bias into the results, however, this was addressed in my sensitivity analyses and did not change the results after adjustments for order and baseline characteristics. Whilst it is plausible that those who withdrew might have had more marked increases in blood pressure, and therefore there would have been a greater difference in blood pressure between oxygen and air therapy, this was not demonstrable in the sensitivity analysis as there were insufficient data from these patients. In addition to having more severe OSA and being more obese, patients who withdrew had increased systolic blood pressure at baseline. The acceptability of oxygen therapy to patients will have to be considered in future trials addressing the longer-term efficacy of oxygen on blood pressure in OSA.

Blood pressure was only recorded in the morning and the effect of oxygen therapy on 24-hour blood pressure profile is not known. Morning blood pressure was chosen as an outcome as OSA is associated with a "non-dipping" pattern in blood pressure with more marked increases in blood pressure overnight and in the morning, relative to control subjects (203). Standard 24-hour blood pressure monitoring techniques have limitations and have been shown to cause arousals from sleep and raise blood pressure (112). Non-invasive techniques for monitoring blood pressure can be used (211), but would not have been practicable for the entirety of the two-week trial. Changes in blood pressure were of similar magnitude at the later office visit, which was usually conducted shortly after 10am. This suggests the blood pressure rise is not isolated to a very brief period after waking. Even if the elevations in morning blood pressure were not sustained during the entire daytime, a "non-dipping" pattern of blood pressure is associated with increased mortality (212). Thus the observation of short-term attenuation of morning blood pressure with oxygen treatment in OSA warrants further investigation in future trials to see if this effect is sustained.

Supplemental oxygen is not thought to be effective in controlling apnoeic events leading to arousals from sleep (186), and therefore it is unlikely that oxygen would improve sleepiness. In patients with hypertension there is little additional benefit of CPAP for patients whose blood pressure can be controlled with a single anti-hypertensive agent (71), but in patients with resistant hypertension the effects of CPAP appear to be more marked (70). It is possible that oxygen is also more efficacious in patients with resistant hypertension. Supplemental oxygen therapy does not appear to be superior to CPAP in improving oxygenation in OSA (186), suggesting that it may be an alternative only in those who do not tolerate CPAP.

Therapeutic trials of supplemental oxygen in OSA are likely to only be applicable to patients where CPAP is intolerable, where patients are not sleepy, and in patients with resistant hypertension where medications have not adequately controlled blood pressure. If improvements in blood pressure are proven in these patients and supplemental oxygen is shown to be tolerable then it will also be important to show an effect on cardiovascular outcomes. This is particularly important as although CPAP has been shown to improve blood pressure, this has not been shown to translate into reduced cardiovascular events (49).

The sequence in which oxygen therapy was given was a predictor of blood pressure and there were trends towards slightly increased run-in blood pressures in patients who received air first, compared to their baseline (mean effect on systolic +1.9mmHg, 95% CI -3.5 to +7.3 and mean effect on diastolic +3.7mmHg, 95% CI -1.8 to +9.2). However, in mixed effect models, including adjustments for treatment order, a large significant effect of oxygen treatment on blood pressure remained and was essentially identical to unadjusted analyses.

Only four patients were female in this trial and therefore it is not known whether these results are generalizable to females. Approximately 80% of patients in the Gottlieb study (121), the Mills study (122), and my study were male. Females are thus under-represented in trials assessing the effect of oxygen treatment on blood pressure in OSA. In a meta-analysis looking at the effects of CPAP on blood pressure, females accounted for the minority of patients. However, 207 females (26%) could be included, and gender was not a significant predictor of blood pressure response to CPAP. This suggests that the effect of CPAP on blood

pressure is not different in females (70). However, owing to the small number of female patients in this study any difference in effect of oxygen treatment in females cannot be assessed. It is conceivable that hormonal driven differences in ventilatory responses (35), may influence the effects of oxygen on blood pressure in OSA.

3.5.4. Lack of effect of supplemental oxygen on morning heart rate

An interesting observation was that whilst increases in morning blood pressure were attenuated, the increase in morning heart rate was not. If supplemental oxygen attenuates blood pressure rises by attenuating sympathetic activation, similar attenuation in heart rate rises may be expected. However, when increases in blood pressure occur, these are sensed by the baroreceptors which lead to decreases in heart rate. Baroceptor stimulation leads to decreased sympathetic tone and increased vagal tone causing increased vasodilation, decreased cardiac contractility and decreased heart rate (213)(214). Intermittent hypoxia in the air arm may have increased sympathetic activation causing rises in blood pressure and heart rate. However, baroreceptor compensation for increased blood pressure may have caused compensatory decreases in heart rate and stopped rises in the heart rate with supplemental air over and above those observed with supplemental oxygen (which presumably occur via a different mechanism).

In addition, the mechanisms by which intermittent hypoxia/sympathetic activation lead to blood pressure rises and heart rate rises are different. Experiments by Fletcher and colleagues suggest that the changes in blood pressure in intermittent hypoxia are dependent

on the renin-angiotensin-aldosterone axis which, whilst increasing blood pressure, would not have a direct effect on heart rate (107)(108). Indeed, in these animal experiments, whilst blood pressure increased, heart rate did not change (104). Vagal tone is also important in determining heart rate and there is evidence of altered sympathetic/vagal balance with reductions in vagal modulation in OSA (215), which could be mediated by other mechanisms such as large intra-thoracic pressure swings (170). Studies of heart rate variability have shown reductions in high frequency power, representing decreased parasympathetic activity, both overnight (216), and during the daytime (215), in patients with OSA. However, animal intermittent hypoxia experiments have shown reductions in hypothalamic mediated vagal tone (217), suggesting that intermittent hypoxia may at least in part explain reductions in parasympathetic activity as well as increases in sympathetic activity.

The reasons why supplemental oxygen attenuates increases in blood pressure rises but not heart rate during CPAP withdrawal are thus not entirely clear. This disparity could represent baroreceptor mediated compensatory decreases in heart rate, heart rate increases may occur via apnoea/arousal induced mechanisms and not via hypoxia, or hypoxia-induced increases in heart rate may not be sustained in the daytime, in contrast to blood pressure.

3.6. Conclusions (Chapter Three)

In conclusion, in this chapter I have shown that supplemental oxygen therapy attenuates the rise in blood pressure seen with a two-week CPAP withdrawal, compared to air. The effect of oxygen on blood pressure during short term CPAP withdrawal is independent of age, gender,

BMI, OSA severity, treatment order and concomitant medication use. This result contrasts with previous study findings, particularly with the study by Gottlieb *et al.* Differences from previous studies are probably due to including patients with more severe hypoxia and more severe OSA, the higher flow rates of oxygen used, the increased compliance with therapy, the use of a power experimental design with treatment cross-over and CPAP withdrawal methodology, and the use of a more sensitive method to detect blood pressure. My results suggest that intermittent hypoxia is central to daytime increases in blood pressure seen during return of OSA during two weeks of CPAP withdrawal. The mechanism(s) by which supplemental oxygen attenuates blood pressure increases is/are not clear, and will be explored in the following chapters. Future studies are warranted to see if this effect is sustained, whether blood pressure reductions translate into a reduction in cardiovascular events, and whether supplemental oxygen therapy is an acceptable treatment option for some patients with OSA. 3.

4. Chapter Four: Secondary outcomes of the supplementary oxygen in OSA trial: physiological effects of supplemental oxygen

4.1. Abstract (Chapter Four)

Supplemental oxygen during CPAP withdrawal has a clear effect on daytime blood pressure. However, the mechanisms underlying supplemental oxygen's effect on blood pressure in OSA are not clear. Supplemental oxygen could attenuate blood pressure rises either direct, or indirect, by effects related to hypoxia. Supplemental oxygen, by attenuating intermittent hypoxia, could reduce carotid chemoreceptor mediated sympathetic activation, or could reduce systemic inflammation. Alternatively, supplemental oxygen could have indirect effects on sympathetic activation by reducing the AHI, and therefore reducing arousal mediated sympathetic activation. In this chapter, the effects of supplemental oxygen on intermittent hypoxia, the AHI, sympathetic activation, and inflammation, are examined. Furthermore, the effects of supplemental oxygen on apnoea length and venous blood gases are explored to measure the potential adverse consequence of hypercapnia. In the supplemental oxygen trial, there was a marked attenuation of intermittent hypoxia with oxygen therapy, with a small, non-significant effect on the AHI. There was a trend towards partial reduction in urinary normetadrenaline levels with supplemental oxygen. Supplemental oxygen caused a small but significant increase in venous carbon dioxide and base excess levels, and lengthened apnoeas. Intermittent hypoxia therefore leads to raised daytime blood pressure in OSA but it is not clear sympathetic activation underlies this. Future trials are needed to assess if supplemental oxygen may have a role in OSA, such as in the treatment of resistant hypertension. Derangements of blood gases need to be given careful consideration in future trials of supplemental oxygen therapy in OSA.

4.2. Introduction

The results of Chapter 3, showing the effect of supplemental oxygen on blood pressure, suggest that intermittent hypoxia is critical to the development of elevated daytime blood pressure in OSA. However, the mechanism(s) by which supplemental oxygen attenuates blood pressure rises in OSA is/are not clear. Supplemental oxygen therapy could potentially have influenced several different pathways in OSA, both those directly, and those indirectly influenced by intermittent hypoxia. Supplemental oxygen, by attenuating intermittent hypoxia, could have influenced sympathetic activation (105)(111), or inflammation (218). Alternatively, supplemental oxygen could have reduced the AHI (219), via an effect on ventilatory drive/loop gain (220), and thus reduced arousal mediated sympathetic activation. The potential effects of supplemental oxygen in OSA are shown in Figure 4.1. The effects of supplemental oxygen on intermittent hypoxia, the AHI, sympathetic activation and inflammation, in the supplemental oxygen trial will be discussed in this chapter.

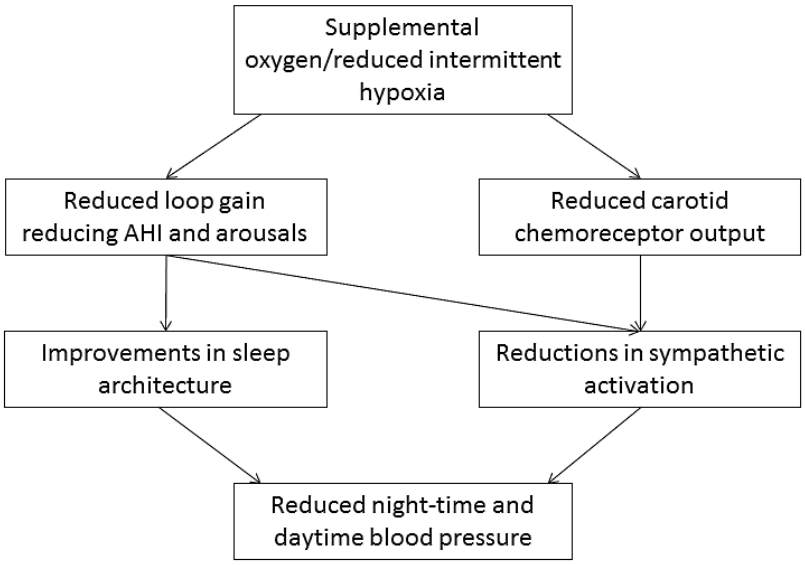


Figure 4.1: The potential mechanisms by which supplemental oxygen therapy in OSA could lead to reductions in daytime blood pressure

4.2.1. Supplemental oxygen, intermittent hypoxia and the AHI

In previous trials, supplemental oxygen has been shown to attenuate intermittent hypoxia (186). Supplemental oxygen and CPAP appear to have similar effects on intermittent hypoxia, but this is dependent on the flow rate of oxygen and the method of administration (121)(122)(186) . Ensuring adequate attenuation of intermittent hypoxia with supplemental oxygen was important in the supplemental oxygen trial, to be able to interpret whether such attenuation could explain the reduction in blood pressure observed in the supplemental oxygen trial.

Theoretically, oxygen therapy could lead to improvements in blood pressure by other mechanisms, and improvements in the AHI have been shown with supplemental oxygen therapy in combination with eszopiclone in patients with OSA (219). Eszopiclone is a non-benzodiazepine hypnotic medication commonly used for insomnia. In this trial it could either be effects of supplemental oxygen or eszopiclone on sleep that effected the AHI. Such reductions in the AHI could reduce arousal induced sympathetic activation, and improvements in the AHI with oxygen might thus have an indirect effect of oxygen therapy on daytime blood pressure. The length of apnoeas in OSA have previously been shown to be increased by supplemental oxygen in OSA (186), and this could decrease the AHI. Oxygen can also lead to changes in ventilatory drive in patients with OSA (220), and this probably underlies improvements in the AHI with oxygen in central sleep apnoea (221). Increases in the ratio between the length of apnoeas and the length of recovery time between apnoeas has been associated with increased risk of daytime hypercapnia (187). The effects of

supplemental oxygen on intermittent hypoxia, the AHI, the length of apnoeas and venous blood gases will be examined.

4.2.2. Sympathetic activation in OSA

Sympathetic activation is thought to be a key mechanism by which OSA leads to elevations in blood pressure (167). Increased sympathetic activation has been shown previously in the CPAP withdrawal model, with increases in overnight urinary normetadrenaline, which is a metabolite of the sympathetic hormone noradrenaline (148). In patients with OSA, whilst increases in noradrenaline metabolites have been consistently found (148)(222)(223)(224), reports of additional increases in adrenaline metabolites are rarer (225), and one small study reported increases in adrenaline metabolites alone (226). There are differences in the way adrenaline and noradrenaline are produced, with adrenaline being produced only by the adrenal medulla, and noradrenaline being both produced by the adrenal medulla and by sympathetic nerve endings. It has been argued that there are only increases in noradrenaline production from nerve endings in muscles and the kidneys in OSA (122). However, in animal intermittent hypoxia models both adrenaline from the adrenal medulla, and noradrenaline from sympathetic nerve endings, are important, as blocking either stops diurnal rises in blood pressure (106). The authors argue that adrenaline and noradrenaline act together to elevate blood pressure in this animal intermittent hypoxia model, perhaps by adrenaline facilitating the release of noradrenaline. Although the exact mechanisms of increased sympathetic activation in OSA are not clear, normetadrenaline is a useful biomarker of sympathetic activation in OSA as it is consistently increased. Potential causes for increased sympathetic activation in OSA include both arousals and intermittent hypoxia.

Arousals from sleep have been shown to be important in causing overnight acute increases in blood pressure, that are not dependent on hypoxia, as blood pressure rises are similar when arousals are induced by apnoeas with or without supplemental oxygen in patients with OSA, and from arousals produced by non-respiratory stimuli (115). Arousal mediated sympathetic activation does not show habituation during sleep (159), suggesting the importance of arousals in increases in sympathetic activation. However, animal intermittent hypoxia experiments have shown that diurnal blood pressure rises are dependent on the carotid chemoreceptors (105), and the sympathetic nervous system (106), and that these changes are potentially dependent on an oxidative injury in the carotid chemoreceptors (136). Intermittent hypoxia in healthy human volunteers causes both raised daytime blood pressure and sympathetic activation (111). These experimental intermittent hypoxia data support a dominant role for intermittent hypoxia in sympathetic activation and raised daytime blood pressure in OSA.

Mills *et al.* examined changes in noradrenaline excretion in patients with OSA treated with either CPAP, supplemental oxygen, or air (122). There were reductions in daytime noradrenaline excretion with both CPAP and supplemental oxygen compared to air. Night-time reductions in noradrenaline excretion were only observed with CPAP. Whilst this suggests that intermittent hypoxia is of some importance in sympathetic activation in OSA, it was not definitive. There were methodological limitations in the low flow rates of oxygen used (3l/min) and the technique used to monitor ambulatory blood pressure itself causing arousals (112), and therefore sympathetic activation. In addition, oxygen was only partially

effective in attenuating intermittent hypoxia (only a 2.5% mean reduction in time below saturations of 90%) when compared to CPAP (producing an 8.8% mean reduction in time below saturations of 90%). Supplemental oxygen also only partially reduced the ODI (from 34.8 ± 32.1 to 15.7 ± 24.8) compared to CPAP (38.3 ± 33.9 to 1.3 ± 1.9). It is not known whether it is the amount of time with significant hypoxia or the number of episodes of cyclical deoxygenation and reoxygenation that leads to hypertension in OSA.

4.2.3. Urinary volume in OSA

Nocturia is a common symptom of OSA and the frequency and volume of urine production overnight are reduced by CPAP (227)(228). OSA causes increased urinary losses of sodium and water (229). Increases in atrial natriuretic peptide are thought to be involved in the diuresis seen in OSA. Haemodynamic changes leading to increased atrial natriuretic peptide could either be triggered by hypoxia-induced vasoconstriction, or intra-thoracic pressure swings (230). In addition to changes in atrial natriuretic peptide, reductions in anti-diuretic hormone are also thought to be involved in the overnight diuresis observed in OSA (231). The effects of supplemental oxygen during CPAP withdrawal will be examined to explore whether intermittent hypoxia is important in overnight diuresis in OSA.

4.2.4. Intermittent hypoxia, hyperoxia and inflammation

Intermittent hypoxia is thought to lead to inflammation in OSA via both the hypoxia-inducible factor-1 and nuclear factor kappa B pathways (151). OSA has been shown to be associated with elevations in the inflammatory biomarker, highly-sensitive C-reactive

protein (hsCRP)(152). However, randomised controlled trials of CPAP therapy have not shown improvements in hsCRP levels (153), and changes have not previously been observed in the CPAP withdrawal model (148). The lack of effect of CPAP treatment or CPAP withdrawal could suggest that the intermittent hypoxia seen in OSA may not cause inflammation. Alternatively, it may be that inflammatory changes induced by intermittent hypoxia may not be fully reversible, as suggested by an animal intermittent hypoxia model (154). Furthermore, inflammation could be occurring in specific end-organs or tissue beds, without changes in systemic biomarkers such as hsCRP (218). Hyperoxia has been shown to induce inflammation *in vitro* in endothelial cells (232), and in an animal coronary artery bypass model (233). Therefore, it is important to examine the effects of supplemental oxygen on inflammation as oxygen therapy could plausibly either increase or decrease inflammation in OSA.

4.2.5. Aims

- To examine the effects of supplemental oxygen on intermittent hypoxia during CPAP withdrawal, compared with air
- To examine the effects of supplemental oxygen on the AHI during CPAP withdrawal, compared to air
- To examine the effects of supplemental oxygen on heart rate parameters overnight, compared to air
- To examine the effects of supplemental oxygen on urinary volume and dilution during CPAP withdrawal, compared to air

- To examine the effects of supplemental oxygen on sympathetic activation during CPAP withdrawal, compared to air
- To examine the effects of supplemental oxygen on inflammation during CPAP withdrawal, compared to air
- To examine the effects of supplemental oxygen on the length of apnoeas during CPAP withdrawal, compared to air
- To examine the effects of supplemental oxygen on blood gases during CPAP withdrawal, compared to air

4.3. Methods

4.3.1. Secondary outcome: overnight oximetry parameters (4.4.1)

Overnight pulse oximetry was recorded from the first 13 nights following Visits 1 and 3, as previously described. Oximetry traces were automatically scored and any file with less than 4 hours total recording time was discounted. Oximetry from nights 8-13 of both the oxygen and air treatment arms were averaged for the $ODI_{\geq 4\%}$, the mean oxygen saturations, and the percentage time with oxygen saturations <90%. The average oximetry values for nights 8-13 were compared between oxygen and air treatment arms using paired t-tests and Wilcoxon rank tests as appropriate.

4.3.2. Secondary outcome: overnight heart rate parameters (4.4.2)

Heart rate parameters were averaged from nights 8-13 and compared with t-tests and Wilcoxon rank tests as appropriate, using the methodology outlined in section 4.3.1 for

oximetry parameters. Heart rate parameters were the heart rate rises >6bpm index (number of heart rate rises greater than 6bpm normalised per hour of oximetry), the mean heart rate, and the standard deviation of the heart rate. Heart rate parameters were used as surrogate markers of arousal, as heart rate rises are part of the autonomic arousal response at the end of obstructive events (159).

4.3.3. Sensitivity analysis: the effect of oxygen/air delivery system (4.4.3)

At Visit 1 each patient chose either face mask or nasal cannulae to use with their allocated concentrator. They used the same oxygen/air delivery interface during their second treatment arm. The change in average oximetry parameters from nights 8-13 were compared between those who used nasal cannulae and those who used face mask using unpaired t-tests and Mann Witney U tests as appropriate.

4.3.4. Secondary outcome: overnight sleep studies (4.4.4)

Overnight sleep studies were recorded at home on the final night of each treatment arm as described in section 2.1.8.3. From these sleep studies the AHI, apnoea index (AI) and hypopnoea index (HI) were recorded. Hypopnoea was defined without an oxygen desaturation requirement, as inclusion of this would severely distort the hypopnoea index during oxygen breathing, and thus prevent an assessment of any changes in the number of apnoeas and hypopnoeas. Hypopnoeas were defined as a reduction in airflow to below 50% of baseline airflow so as to minimize inclusion of trivial airflow limitation of no clinical consequence, given there was no requirement for an oxygen desaturation or an arousal. This

differs from the most recent American Academy of Sleep Medicine 2012 manual (2), on scoring hypopnoeas, but is justified by the necessity of removing the error in scoring hypopnoeas in the oxygen arm. Parameters recorded on overnight pulse oximetry were also analysed from the sleep study nights ($ODI_{\geq 4\%}$, mean oxygen saturations, percentage time oxygen saturations <90%, heart rate rises >6bpm index, mean heart rate, and standard deviation of the heart rate). Comparisons were made using paired t-tests and Wilcoxon rank tests as appropriate, oxygen versus air.

4.3.5. Urine volume and creatinine (4.4.5)

The volume of urine and concentration of creatinine (mg/l) were measured from the overnight urine collection on the nights prior to Visits 1, 2, 3 and 4. Details of this urine collection are described in sections 2.2.8.8 and 2.2.8.9. The rate of urine production (ml/hour) was calculated by dividing the urine volume (ml) by the total length of urine collection (hours). Difference in urine volume, rate of urine production and urinary creatinine concentration were compared using paired t-tests and Wilcoxon rank tests as appropriate.

4.3.6. Urinary normetadrenaline and metadrenaline (4.4.6)

Urinary normetadrenaline and metadrenaline levels were measured from the overnight urine samples collected prior to Visits 1, 2, 3 and 4 (see section 2.2.8.8). Urinary normetadrenaline and metadrenaline levels were measured by high performance liquid chromatography and described in section 2.2.8.10. Changes in urinary normetadrenaline and

metadrenaline were compared using a combination of paired t-tests and Wilcoxon rank tests.

4.3.7. Serum hsCRP (4.4.7)

High sensitivity CRP (hsCRP) was measured from frozen stored serum samples from each of the four patient visits using immunoturbidometry (Architect c16000, Abbott, Illinois, USA). hsCRP was measured as a marker of systemic inflammation as described in section 2.2.8.11. Changes in hsCRP were assessed using a combination of paired t-tests, Wilcoxon rank tests, and mixed effects models.

4.3.8. The effect of oxygen therapy on apnoea length (4.4.8)

Apnoea length was measured manually as described in section 2.2.8.4. This was conducted to assess for risk of developing hypercapnia, as long apnoeas with brief interapnoea periods would be more likely to lead to hypercapnia (187). This assumes that hyperventilation is of similar magnitude during interapnoea periods. Hypopnoeas were not included in this analysis as the degree of hypoventilation cannot be easily assessed during hypopnoeas, whereas it is reasonable to assume that there is no ventilation during apnoeas.

This analysis was conducted manually as automated labelling of apnoeas is inaccurate, frequently marking events as either too long, or too short and splitting single events into two. Briefly, a single 20-minute period of sleep with recurrent apnoeas, without changes in body position or large movements, was identified for both air and oxygen treatments for

each patient. A short period was picked to ensure that the patient remained in one sleep position and that there were no periods of time included without events. Apnoeas were automatically identified with manual editing of both events and event length. During these periods the absolute magnitude of each heart rate rise (>6bpm) was manually scored and recorded. The oximetry channel was removed to blind treatment allocation. Information from Visi-Download (Stowood Software, Beckley, Oxford, UK), was saved and extracted as a Wordpad file. This Wordpad file was then opened as an Excel template. This Excel template allowed extraction of apnoea length, the length between apnoeas (apnoea interlength – long gaps of >60 seconds were excluded) and the ratio of apnoealength:apnoea-interlength. The median magnitude of heart rate rises was recorded, as was the mean and SD of the heart rate during this period. Data were assessed for normality and paired t-tests and Wilcoxon rank tests were used as appropriate. Details of this analysis are shown in Appendix (section 8.5).

4.3.9. The effect of oxygen therapy on venous blood gases (4.4.9)

Part way through the trial the decision was made to collect blood gases. Venous blood gases were measured by collecting an extra syringe from the existing blood draw and therefore avoiding adding an additional arterial puncture to the protocol. Venous blood gases were measured in a subset of patients as a safety measure to assess for potentially deleterious effects of oxygen therapy on ventilatory drive and thus carbon dioxide levels. The main outcome of interest was any changes in base excess. Base excess is an integrated measure of hypercapnia, reflecting PCO_2 levels particularly over the night before measurement, rather than a one-off morning measurement (234). In addition, PCO_2 values are not as reliable

when venous rather than arterial sampling is used (235). The change in PCO_2 and base excess levels from baseline to follow-up were compared, oxygen versus air. Values were also separately compared in the oxygen and air arms, follow-up versus baseline. Paired t-tests and Wilcoxon rank tests were used as appropriate.

Any potential effect of treatment order was compared using mixed effect models. The venous base excess and PCO_2 values were the dependent variables in separate mixed effect models. Fixed effects included the treatment (oxygen or air), visit (baseline or follow-up), treatment order (oxygen-air or air-oxygen), and treatment sequence (first or second treatment). The patient's study number was modelled as a random effect. The visit × treatment interaction was modelled as a fixed effect to estimate the treatment effect of oxygen versus air.

4.3.10. Correlations between physiological parameters and blood pressure (4.4.10)

The relationship between measures of OSA severity, intermittent hypoxia, heart rate rises and the blood pressure changes reported in Chapter 3 were explored. The correlations between overnight pulse oximetry, sleep study parameters, urinary normetadrenaline, hsCRP, venous base excess, and the changes in systolic and diastolic blood pressure were assessed using Spearman's rank correlations. Baseline measurements for the $ODI_{\geq 4\%}$ and the heart rate rises index on CPAP treatment varied between patients. To adjust for this variation in baseline measurements, the ODI and heart rate rises index were adjusted by subtracting the ODI and heart rate rise index recorded during screening from the average

from nights 8-13 during the treatment arms. Correlations were assessed for all observations (oxygen and air arms jointly).

4.4. Results

4.4.1. Secondary outcome: overnight oximetry parameters

Averaged oximetry parameters for nights 8-13 for oxygen and air treatment are shown in Table 4.1 along with comparison statistics. Oxygen therapy markedly attenuated all measures of intermittent hypoxia compared to air therapy.

	Supplemental air, median (IQR) or mean ± SD	Supplemental oxygen, median (IRQ) or mean ± SD	Median difference (IQR)	Mean difference (95% CI)	p
$ODI_{\geq 4\%}$ (/h)	32.5 (25.6, 47.0)	6.4 (4.0, 14.7)	-23.8 (-31.0, -16.3)	-	**<0.001**
% time <90%	14.3 (5.9, 21.2)	2.0 (0.3, 3.9)	-9.8 (-16.7, -4.3)	-	**<0.001**
Mean saturations (%)	92.6 ± 1.8%	96.9 ± 1.2%	-	+4.3% (+3.6 to +4.9)	**<0.001**

Table 4.1: Comparison of the effects of oxygen and air therapy on average oximetry parameters on nights 8-13. For ODI and percentage time with oxygen saturations <90% Wilcoxon rank tests were used. For the mean oxygen saturations a paired T-test was used.

4.4.2. Secondary outcome: overnight heart rate parameters

Average heart rate parameters for nights 8-13 for oxygen and air treatment are shown in Table 4.2 along with comparison statistics. There was a small but significant effect of oxygen treatment, reducing the heart rate rises >6bpm index by about 10% compared to air therapy, but no significant effect on mean heart rate or the SD of the heart rate.

	Supplemental air median (IQR)	Supplemental oxygen median (IRQ)	Median difference (IQR)	p
Heart rate rises > 6bpm index (/h)	31.9 (24.0, 44.3)	27.1 (21.4, 37.4)	-3.7 (-9.1, -0.8)	**0.006**
Mean heart rate (bpm)	62.0 (55.4, 65.1)	61.5 (53.5, 64.0)	-1.2 (-2.8, +1.1)	0.12
SD heart rate (bpm)	5.7 (5.0, 6.7)	5.9 (5.0, 6.4)	-0.0 (-0.3, +0.3)	0.88

Table 4.2: Comparison of the effects of oxygen and air therapy on average heart rate parameters on nights 8-13. Wilcoxon rank tests were used.

Data for the mean heart rate and the standard deviation of the heart rate were both non-normally distributed due to one patient with outlying values in both the oxygen and air arms. Excluding this single patient allowed comparison between oxygen and air therapy using paired t-tests for the mean heart rate and the standard deviation of the heart rate. There remained no significant differences for either the mean heart rate (mean difference oxygen minus air = -0.9bpm, p=0.16, 95% CI -2.1 to +0.4) or the standard deviation of the heart rate (mean difference oxygen minus air = -0.1, p=0.74, 95% CI -0.4 to +0.3), between oxygen and air therapy.

4.4.3. Sensitivity analysis: the effect of oxygen/air delivery system on oximetry and heart rate parameters

Seven patients used nasal cannulae and eighteen used face masks as their chosen delivery interface for both oxygen and air. The changes in oximetry and heart rate parameters between oxygen and air were assessed from normality, for some variables the change was normally distributed where the baseline values were not. There were no statistically significant differences in outcomes between nasal cannulae and face mask oxygen delivery. Whilst this analysis is exploratory and under-powered, there were also no apparent trends favouring one delivery interface over the other. Full results are shown in Table 4.3.

Parametric (oxygen minus air)				
	Nasal cannulae Mean ± SD	Face mask Mean ± SD	Diff in means (95% CI)	p
Difference in ODI (/h)	-24.1 ± 10.3	-24.9 ± 11.4	+0.7 (-9.5 to +11.0)	0.88
Difference in mean oxygen saturations (%)	+ 4.4 ± 2.1	+ 4.2 ± 1.4	+0.1 (-1.3 to +1.6)	0.84
Non-parametric (oxygen minus air)				
	Nasal cannulae Median (IQR)	Face Mask Median (IQR)	p	
Difference in % time <90% (%)	-9.8 (-23.0, -1.2)	-9.6 (-16.3, -5.3)	0.84	

Table 4.3: Comparison of the effects on the change in overnight oximetry parameters from overnight pulse oximetry recordings averaged over nights 8-13. Differences reported are oxygen minus air, and comparisons are between the two delivery interfaces. Unpaired t-tests were used to compare parametrically distributed changes and Mann Witney U tests were used to compare non-parametrically distributed changes.

4.4.4. Secondary outcome: overnight sleep studies

Seventeen patients had paired sleep studies available for comparison of AHI, HI and AI. The median AHI was 34.4/hour (22.7, 44.4) on air and 30.4/hour (23.6, 42.6) on oxygen therapy. The reduction in median AHI with oxygen minus air of -3.6/hour (-10.2, +10.1) was not significant (p=0.98). The median AI was 21.4/hour (9.6, 28.7) on air, and 18.7/hour (9.7, 29.7) on oxygen therapy. The reduction in median AI with oxygen minus air of -2.0/hour (-10.6, +6.9) was not significant (p=0.83). The median HI was 13.0/hour (8.5, 16.8) on air, and

15.2/hour (10.0, 18.4) on oxygen therapy. The change in median HI with oxygen minus air of +1.1/hour (-4.8, +5.6) was not significant (p=0.69).

Sleep study lengths were comparable, with a median sleep study length of 392 mins (364, 448) on air and 394 mins (377, 404) on oxygen (p=0.80).

Oximetry and heart rate parameters from the sleep studies were compared between oxygen and air therapy. Sixteen patients had paired sleep studies with pulse oximetry data for comparison. Similarly, to the results from the overnight pulse oximetry on days 8-13, oxygen had a large significant effect on the ODI, percentage time with oxygen saturations <90%, and the mean oxygen saturations. There was a small significant effect of oxygen therapy on the heart rate rises index but not on the mean heart rate of the standard deviation of the heart rate. Full results are shown in Table 4.4.

	Supplemental air median (IQR) or mean ± SD	Supplemental oxygen median (IRQ) or mean ± SD	Median difference (IQR)	Mean difference (95% CI)	p
ODI (/h)	31.4 (21.0, 49.2)	4.5 (1.1, 19.0)	-28.0 (-34.9, -16.7)	-	**0.001**
% time <90%	9.3 (1.4, 20.5)	0.1 (0.0, 2.1)	-7.8 (-16.9, -1.2)	-	**0.001**
Mean saturations (%)	93.7 (92.1, 95.2)	98.0 (96.6, 98.6)	+4.4 (+2.3, +5.7)	-	**0.001**
Heart rate rises >6bpm index (/h)	29.1 (23.3, 54.9)	25.4 (18.1, 49.6)	-4.3 (-12.6, +1.1)	-	**0.03**
Mean heart rate (bpm)	61.4 (55.1, 65.5)	59.0 (54.0, 65.1)	-0.3 (-3.6, +2.3)	-	0.50
SD of the heart rate (bpm)	5.3 ± 1.3	5.3 ± 1.3	-	+0.1 (-0.5 to + 0.7)	0.82

Table 4.4: Comparison of the effect of oxygen versus air therapy on oximetry and heart rate parameters from paired overnight sleep studies. Wilcoxon rank tests were used when data was non-normally distributed and a paired t-test was used when data was normally distributed.

4.4.5. Urinary volume and creatinine

Urine volume, rate of urine production, and urinary creatinine were compared between baseline and follow-up, for each arm separately. Baseline and follow-up values were not normally distributed and therefore were compared using Wilcoxon rank tests. There were significant changes in urine volume, rate of urine production and urinary creatinine from baseline to follow-up in the oxygen arm (p values of 0.01, 0.02 and 0.006 respectively). There were trends toward changes in urine volume and rate of urine production from baseline to follow-up in the air arm which did not quite reach conventional statistical significance (p values of 0.09 and 0.07 respectively). There was no significant difference in

the urinary creatinine between baseline and follow-up (p=0.14). Full results are shown in Table 4.5.

	Oxygen				Air			
	Baseline	Follow-up	Change	p	Baseline	Follow-up	Change	p
Urine volume (ml)	800 (535, 1125)	1000 (700, 1175)	+140 (0, +370)	0.01	800 (515, 1225)	1150 (650, 1400)	+150 (-100, 480)	0.09
Rate of urine production (ml/h)	66.7 (48.6, 95.8)	85.1 (59.0, 111.8)	+13.3 (0.0, +31.7)	0.02	66.7 (46.8, 105.1)	95.8 (56.3, 116.7)	+12.4 (-8.9, +73.4)	0.07
Urinary creatinine (mmol/l)	8.8 (7.1, 14.6)	6.6 (5.0, 9.4)	-1.7 (-4.6, +0.5)	0.006	8.1 (5.6, 14.2)	7.9 (4.9, 9.1)	-0.6 (-4.9, +1.9)	0.14

Table 4.5: Baseline and follow-up urine volumes, rate of urine production and urinary creatinine values from overnight urine collections in both the oxygen and air arms. All values are expressed as median (IQR). Comparisons were made using Wilcoxon rank tests.

The changes in urine volumes, rate of urine production and urinary creatinine in both the oxygen and air arm were normally distributed. Paired t-tests were used to compare the difference in the change in these values, oxygen versus air. There were no significant differences in the change in urinary volumes, rates of urine production or urinary creatinine between oxygen and air during CPAP withdrawal (p values of 0.88, 0.97 and 0.65 respectively). Full results are shown in Table 4.6.

	Oxygen Change baseline to follow-up on (Mean ± SD)	Air Change baseline to follow-up on (Mean ± SD)	Difference in change Oxygen minus Air Mean (95% CI)	p
Urine volume (ml)	+172 ± 305	+157 ± 491	+15 (-191 to +221)	0.88
Rate of urine production (ml/h)	+15.5 ± 27.4	+15.1 ± 41.8	+0.4 (-18.2 to +19.0)	0.97
Urinary creatinine (mmol/l)	-2.1 ± 3.6	-1.6 ± 5.2	-0.5 (-2.9 to +1.9)	0.65

Table 4.6: Mean change in urine volumes, rate of urine production and urinary creatinine from overnight urine collections oxygen versus air. Between group differences were compared using paired T-tests.

4.4.6. Urinary normetadrenaline and metadrenaline

Urinary normetadrenaline and metadrenaline were corrected both for the rate of production of urine and urinary creatinine concentrations. The rate of urine production is a direct measure of urinary flow whereas urinary creatinine concentration is only a surrogate marker of flow. However, if urinary collection is incomplete this could potentially influence rate corrected values and so creatinine corrected values were also used. Urinary normetadrenaline and metadrenaline levels were not normally distributed and so were compared using Wilcoxon rank tests.

First, changes in normetadrenaline and adrenaline levels were compared in each arm (oxygen and air) separately to ensure similar changes in these markers as previously

observed (148). Second, the effect of supplemental oxygen on normetadrenaline and metadrenaline was assessed, compared to air.

When correcting for the rate of urine production, there was a significant increase in urinary normetadrenaline levels from baseline to follow-up in the oxygen treatment arm ($p=0.007$). There were significant increases in both urinary normetadrenaline and metadrenaline from baseline to follow-up in the air arm ($p<0.0001$ and $p=0.04$ respectively).

When correcting for the urinary creatinine concentration, there was a significant increase in urinary normetadrenaline levels from baseline to follow-up in the oxygen treatment arm ($p=0.003$). There was a significant increase in urinary normetadrenaline ($p<0.001$) and a trend towards a significant increase in urinary metadrenaline ($p=0.09$) from baseline to follow-up in the air arm. Full results are shown in Table 4.7.

Rate of urine production corrected	*Oxygen*				*Air*			
	Base-line	Follow-up	Change	p	Base-line	Follow-up	Change	p
Normet-adrenaline (nmol/h)	71.0 (50.2, 98.2)	97.8 (57.9, 111.7)	+14.7 (-5.2, +28.4)	**0.007**	64.7 (52.1, 80.5)	94.5 (74.9, 128.6)	+32.2 (+6.8, +47.8)	**<0.0001**
Metadrenaline (nmol/h)	21.4 (17.4, 27.5)	22.2 (17.9, 27.5)	-1.1 (-3.8, +3.7)	0.99	21.2 (14.5, 28.2)	23.6 (16.6, 31.0)	+2.0 (-1.2, +9.3)	**0.04**
Urine creatinine corrected	*Oxygen*				*Air*			
	Base-line	Follow-up	Change	p	Base-line	Follow-up	Change	p
Normet-adrenaline (nmol/mmol)	121.7 (85.2, 145.3)	140.0 (116.8, 186.2)	+32.5 (-8.5, +57.1)	**0.003**	107.5 (79.7, 137.6)	134.7 (113.7, 199.7)	+36.8 (+24.4, +55.6)	**<0.001**
Metadrenaline (nmol/mmol)	34.1 (28.3, 44.6)	37.9 (27.1, 43.2)	+1.3 (-3.5, +4.1)	0.48	35.1 (24.8, 43.6)	37.3 (26.9, 46.8)	+4.2 (-2.3, +6.4)	0.09

Table 4.7: Baseline and follow-up urinary normetadrenaline and metadrenaline values corrected for rate of urine production from overnight urine collections in both the oxygen and air arms. Both rate of urine production and urinary creatinine corrected normetadrenaline and metadrenaline levels are displayed. All values are expressed as median (IQR). The changes in values from baseline to follow-up were compared for each arm separately using Wilcoxon rank tests.

The differences between baseline and follow-up urinary normetadrenaline and metadrenaline levels were not normally distributed. The changes in both normetadrenaline and metadrenaline levels from baseline to follow-up for oxygen treatment were not normally distributed. Figure 4.2 shows the change in normetadrenaline levels from baseline to follow-up and there were two outlying values in the oxygen arm, and one in the air arm.

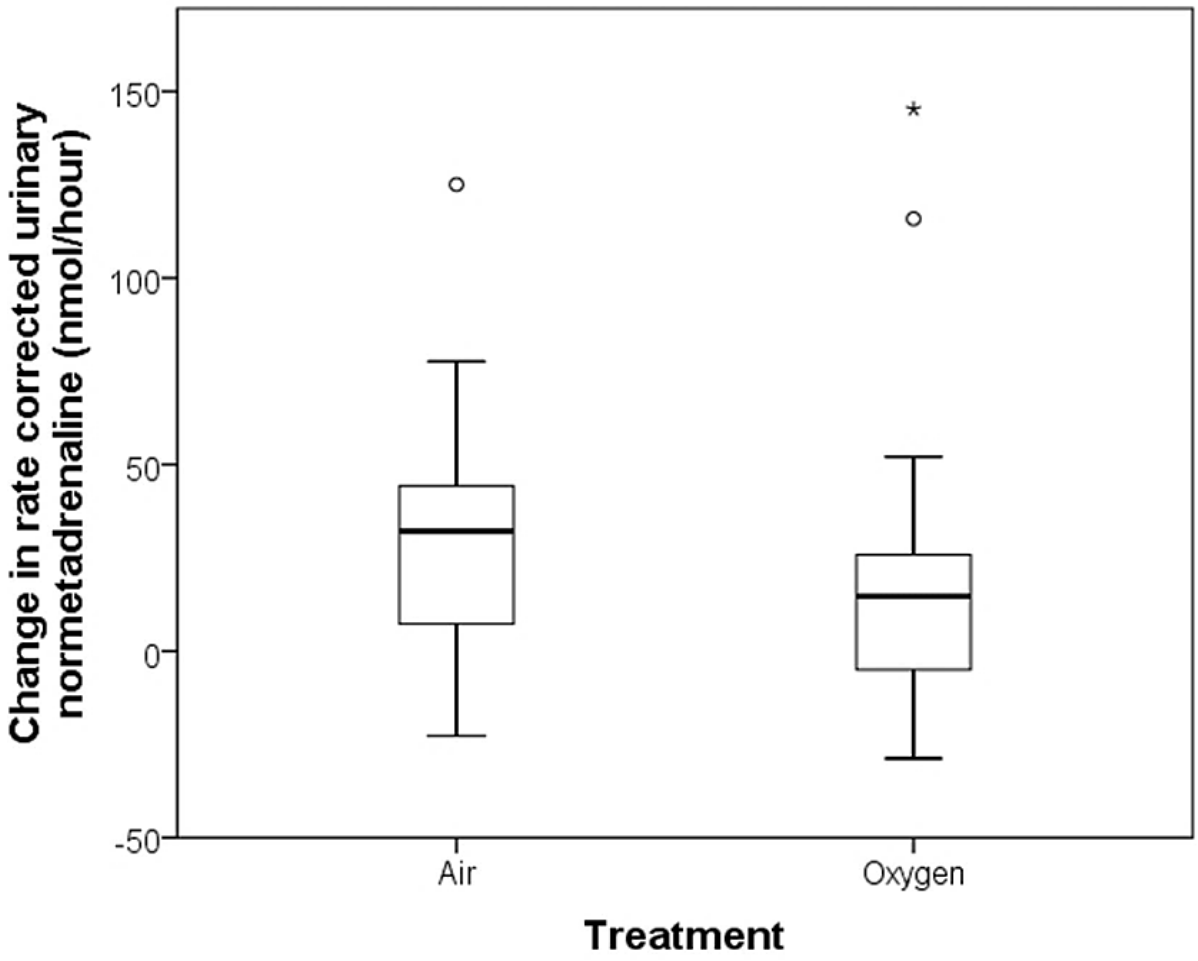


Figure 4.2: Boxplot of the change in rate corrected urinary normetadrenaline from baseline to follow-up for both oxygen and air treatment arms. The middle black lines represent the medians, the box edges represent the 1st and 3rd quartiles, and the whiskers/inner fences represent 1.5 times the IQR. Circles represent outliers which do not fall within the inner fences and the asterisk represents an extreme outlier which falls outside of 3 times the IQR from the edge of the box.

Correcting for rate of urine production, the difference in the changes of urinary overnight normetadrenaline and metadrenaline from baseline to follow-up, oxygen versus air was compared using Wilcoxon rank tests. There was a significant difference in the median

change in rate corrected overnight urinary normetadrenaline (p=0.05) but not metadrenaline (p=0.14) from baseline to follow-up, oxygen versus air.

Correcting for creatinine concentration, the difference in the changes of urinary overnight normetadrenaline and metadrenaline from baseline to follow-up, oxygen versus air was compared using a paired Wilcoxon rank test. There were no significant differences in the change in median creatinine corrected overnight urinary normetadrenaline (p=0.28) or metadrenaline (p=0.68) from baseline to follow-up, oxygen versus air.

Full results are shown in Table 4.8.

	Oxygen Change baseline to follow-up Median (IQR)	Air Change baseline to follow-up Median (IQR)	Difference in change Oxygen minus Air Median (IQR)	p
Rate corrected				
Normetadrenaline (nmol/h)	+14.7 (-5.2, +28.4)	+32.2 (+6.8, +47.8)	-18.3 (-43.9, +4.9)	**0.05**
Metadrenaline (nmol/h)	-1.1 (-3.8, +3.7)	+2.0 (-1.2, +9.3)	-4.0 (-8.4, +3.5)	0.14
Creatinine concentration corrected				
Normetadrenaline (nmol/mmol)	+32.5 (-8.5, +57.1)	+36.8 (+24.4, +55.6)	-5.3 (-47.6, +17.1)	0.28
Metadrenaline (nmol/mmol)	+1.3 (-3.5, +4.1)	+4.2 (-2.3, +6.4)	-0.1 (-7.3, +5.4)	0.68

Table 4.8: Median changes from baseline to follow-up in both the rate corrected and creatinine concentration corrected urinary normetadrenaline and metadrenaline for oxygen, air and oxygen minus air. Data are expressed as medians (IQR). The difference in changes in rate corrected urinary normetadrenaline and metadrenaline from baseline to follow-up were compared using Wilcoxon rank tests.

The relationship between change in the ODI and change in normetadrenaline are shown in Figure 4.3. This shows two patients with significantly outlying values for change in rate corrected normetadrenaline. These two outlying patients represent two of the three patients who still had significant intermittent hypoxia while on oxygen therapy (increase in ODI >20/hour whilst on supplemental oxygen from the screening ODI on CPAP).

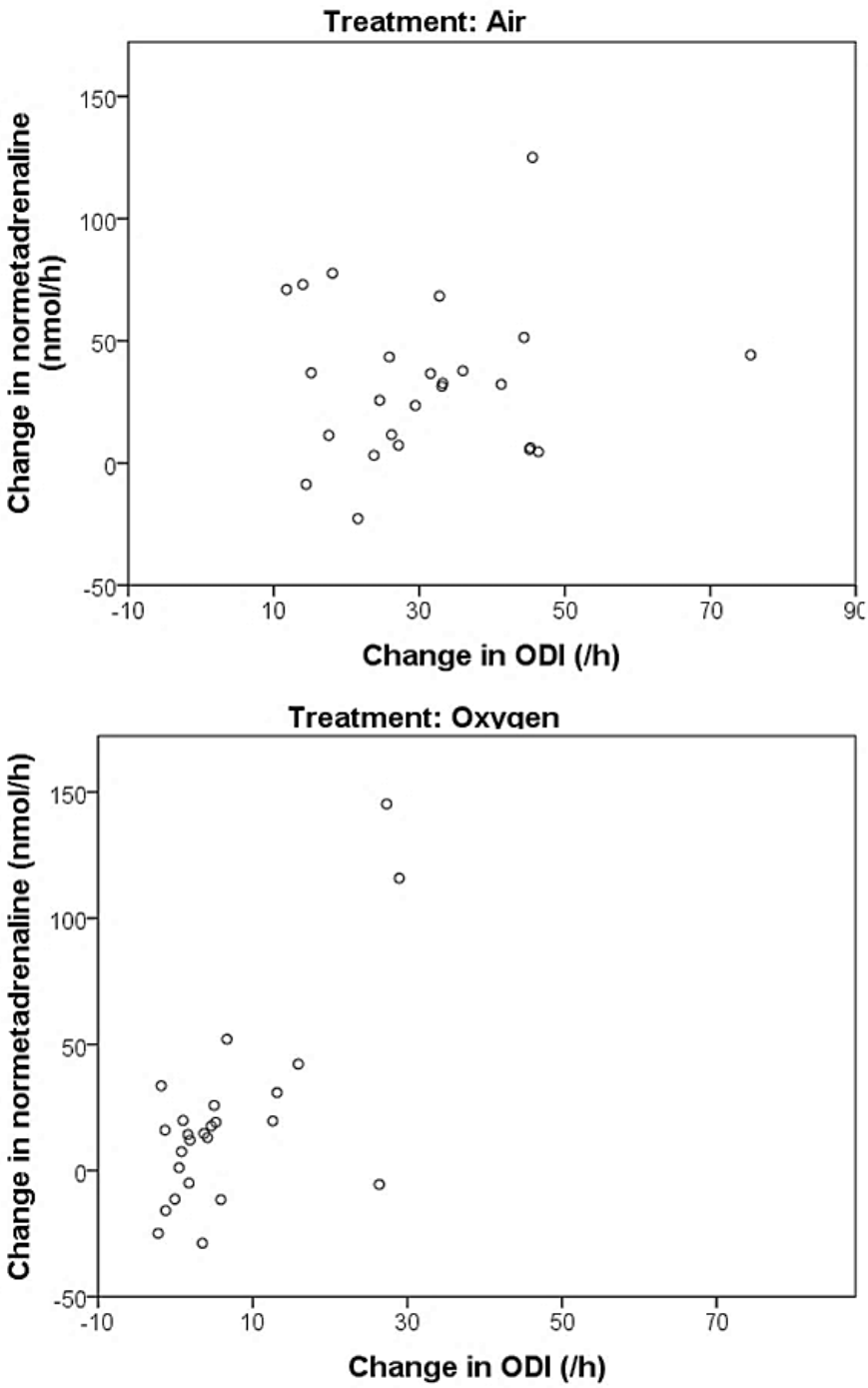


Figure 4.3: The relationship between the change in ODI and the change in rate corrected overnight urinary normetadrenaline, both from baseline to follow-up. Air treatment is displayed on the top graph with oxygen treatment displayed on the bottom graph with similar axes.

Changes in mean normetadrenaline and metadrenaline were checked in addition to changes in the median, oxygen versus air. Urinary normetadrenaline and metadrenaline levels were corrected in two ways; by rate of urine production and by urinary creatinine. Results are shown in Table 4.9.

Rate of urine production corrected (ml/h)				
	Oxygen Change baseline to follow-up on (Mean ± SD)	Air Change baseline to follow-up on (Mean ± SD)	Mean difference in change, Oxygen minus Air (95% CI)	p
Normetadrenaline (nmol/h)	+19.9 ± 38.9	+33.1 ± 32.4	-13.2 (-33.6 to +7.1)	0.19
Metadrenaline (nmol/h)	+1.1 ± 7.7	+3.8 ± 9.6	-2.7 (-7.4 to +2.0)	0.25
Urine creatinine corrected (mmol/l)				
	Oxygen Change baseline to follow-up on (Mean ± SD)	Air Change baseline to follow-up on (Mean ± SD)	Mean difference in change, Oxygen minus Air (95% CI)	p
Normetadrenaline (nmol/mmol)	+29.0 ± 41.5	+41.8 ± 38.7	-12.8 (-35.3 to +9.6)	0.25
Metadrenaline (nmol/mmol)	+1.4 ± 6.5	+2.0 ± 13.2	-0.6 (-6.9, +5.7)	0.84

Table 4.9: The difference in mean changes from baseline to follow-up, oxygen versus air. Results were compared using paired T-tests.

There were no significant differences in the changes of mean urinary normetadrenaline or metadrenaline, oxygen versus air. However, the direction of changes in corrected mean values was the same, and was of similar magnitude, to the significant change in median rate corrected normetadrenaline levels. Excluding the two patients with outlying values for changes in rate corrected normetadrenaline in the oxygen arm, there was a significant effect

of supplemental oxygen versus air (mean reduction in rate corrected normetadrenaline of -23.6 nmol/hour, p=0.004, 95% CI -38.8 to -8.3). These results suggest that supplemental oxygen reduces the increase in urinary normetadrenaline during CPAP withdrawal by approximately one third, compared to air.

4.4.7. High sensitivity CRP

Median (IQR) hsCRP values at baseline were 2.7 mg/l (0.9, 3.5) for the oxygen arm, and 1.1 mg/l (0.5, 2.8) for the air arm. The values at baseline were higher in the oxygen arm, although there seems to be no obvious explanation, given the random order of the treatments, and thus may have occurred by chance alone. Median hsCRP values at follow-up were 2.0mg/l (0.7, 3.8) for the oxygen arm and 1.7mg/l (0.9, 2.6) for the air arm. The median changes in hsCRP from baseline to follow-up were 0.0 mg/l (-1.8, 1.1) in the oxygen arm, and +0.4 mg/l (0.0, 1.6) in the air arm. The unadjusted difference in the change in hsCRP levels oxygen versus air was not significant (p=0.12). Results are shown in Table 4.10.

	Oxygen		Air		Diff in the change in medians Oxygen versus air	p
	Baseline	Follow-up	Baseline	Follow-up		
hsCRP (mg/l)	2.7 (0.9, 3.5)	2.0 (0.7, 3.8)	1.1 (0.5, 2.8)	1.7 (0.9, 2.6)	-0.4 (-3.7, +0.6)	0.12

Table 4.10: Unadjusted hsCRP levels at baseline and follow-up for oxygen and air. Differences in the change in medians were compared using a Wilcoxon rank test. There were significant differences between oxygen and air arms at baseline, which presumably occurred by random chance. Therefore, the effect of treatment order on the treatment effect was explored in mixed-effect models. Data are expressed as medians (IQR).

There seemed to be an order effect with higher hsCRP levels at baseline before oxygen compared to run-in levels before air in the oxygen-air arm (Table 4.11).

	Baseline (pre-oxygen)	Run-in (pre-air)	p
Order AB (n=16)	2.9 (0.9, 4.4)	1.1 (0.6, 3.0)	0.007
	Baseline (pre-air)	Run-in (pre-oxygen)	p
Order BA (n=9)	1.1 (0.5, 2.6)	1.9 (0.5, 3.3)	0.80

Table 4.11: Comparison of order effect of treatment arms on hsCRP levels using Wilcoxon rank tests. Data expressed as medians (IQR).

There were differences in hsCRP levels depending on treatment order, which presumably occurred by random chance. As treatment order affected hsCRP results, a mixed effect model to adjust for treatment order was constructed. HsCRP levels were not normally distributed and therefore were log-transformed to approximate a normal distribution for this analysis. In a mixed effect model the natural log-transformed hsCRP +1 (ln (hsCRP +1)) was the dependent variable. Visit (baseline=0, follow-up=1), treatment order (BA=0, AB=1), sequence (first treatment arm=0, second treatment arm=1), treatment (air=0, oxygen=1) and the interaction of the treatment × visit (which modelled the treatment effect) were entered as fixed effects. The patient identification number was a random effect. None of the fixed effects were significant. Adjusted for order there was no significant treatment effect of oxygen versus air (modelled mean effect on ln(hsCRP+1) of +0.3 arbitrary units, p=0.09, 95%

CI -0.0 to +0.6). Thus, the calculated effect of oxygen versus air on hsCRP would be +0.3mg/l (-0.0 to +0.9).

4.4.8. The effect of oxygen therapy on apnoea length

Sixteen patients had paired sleep study data from stable periods with airflow for analysis of apnoea length derivatives. Two patients had insufficient apnoeas to allow this analysis and others were missing paired sleep studies altogether. Fifteen patients had paired data to analysis heart rate derivatives; one patient was excluded from heart rate analysis as their stable sleep study period contained no heart rate data.

The mean apnoea length was 37.0 ± 14.9s with supplemental oxygen, and 29.4 ± 11.9s with supplemental air. Oxygen significantly increased the apnoea length (+7.6s, p=0.046, 95% CI +0.2 to +15.1). The mean apnoea-interlength was 24.1 ± 8.2s with supplemental oxygen, and 20.0 ± 7.9s with supplemental air. There was a non-significant trend towards increased apnoea-interlength with supplemental oxygen compared to air (+4.1s, p=0.09, 95% CI -0.8 to +8.9). There was no significant difference in the mean apnoea: apnoea-interlength ratio with supplemental oxygen compared to air (+0.1, p=0.66, 95% CI -0.4 to +0.7).

During the stable periods of the sleep studies the median (IQR) magnitude of all heart rate rises was 10.5 bpm (9, 17.5) with supplemental oxygen, and 10.8 bpm (9, 16) with air. There was no significant difference between oxygen and air (p=0.97) suggesting that oxygen did not attenuate the 'size' of the autonomic arousals, as represented by the accompanying

heart rate rise. The median of the mean heart rate was 59.3 bpm (55.0, 65.9) with supplemental oxygen, and 61.4 bpm (56.2, 67.9) with air; there was no significant difference between oxygen and air (p=0.65). The median of the SD of the heart rate was 4.0 bpm (3.1, 5.4) with supplemental oxygen, and 3.8 bpm (3.3, 5.7) with air; there was no significant difference between oxygen and air (p=0.28), again suggesting equal disturbance of sleep in the two arms.

4.4.9. The effect of oxygen therapy on venous blood gases

Venous blood gas samples were collected in a subset of twelve patients. Data is shown for PCO_2 and base excess for all four visits in these twelve patients. Average values are displayed below in Table 4.12.

	Oxygen		Air		Diff in change in means Oxygen vs. Air (95% CI)	p
	Baseline/run-in	Follow-up	Baseline/run-in	Follow-up		
PCO_2 (kPa)	5.9 (5.1, 6.0)	6.0 (5.5, 6.8)	6.0 (5.6, 6.7)	5.8 (5.1, 5.9)	+1.1 (+0.3 to+1.8)	**0.009**
Base excess (mmol/l)	+1.6 ± 2.5	+3.2 ± 2.2	+3.0 ± 2.3	+1.5 ± 1.9	+3.1 (+1.8 to +4.4)	**<0.001**

Table 4.12: Venous blood gas results at baseline and follow-up for both oxygen and air treatment. The differences in the changes in mean values were compared using paired t-tests.

There were significant increases in venous PCO_2 (mean difference = +0.5kPa, p=0.04, 95% CI +0.0 to +0.9), and venous base excess (mean difference = +1.6mmol/l, p=0.009, 95% CI +0.5 to +2.6) from baseline to follow-up, with oxygen treatment, implying the possibility of renal compensation for the raised PCO_2.

There were unexpected significant reductions in venous PCO_2 (mean difference = -0.6kPa, p=0.04, 95% CI -1.2 to -0.0), and venous base excess (mean difference = -1.5mmol/l, p=0.01, 95% CI -2.7 to -0.4) from baseline to follow-up, with air treatment.

Oxygen therapy significantly increased the change in venous PCO_2 (mean difference = +1.1kPa, p=0.009, 95% CI +0.3 to +1.8), and venous base excess (mean difference = +3.1mmol/l, p<0.001, 95% CI +1.8 to +4.4) from baseline to follow-up, compared with air treatment.

The potential effects of treatment order were explored. Mixed effect models were used with venous PCO_2 and base excess as the dependent variables in three separate models as described.

There were significant effects of treatment (oxygen = +0.5kPa, p=0.04, 95% CI +0.0 to +1.0), on venous PCO_2. There was trend towards reduced PCO_2 at baseline (baseline visit = -0.5kPa, p=0.06, 95% CI -1.0 to +0.0) but no effect of treatment order (oxygen-air = -0.2kPa, p=0.51,

95% CI -0.8 to +0.4) or sequence (first treatment = -0.0kPa, p=0.98, 95% CI -0.4 to + 0.4) on the venous PCO_2. The treatment effect of oxygen versus air on venous PCO_2 remained significant after these adjustments for treatment order (oxygen effect = +1.1kPa, p=0.003, 95% CI +0.4 to +1.8).

There were significant effects of treatment (oxygen = +1.7mmol/l, p=0.003, 95% CI +0.6 to +2.7) and visit (baseline = -1.6mmol/l, p=0.004, 95% CI -2.6 to -0.5) on venous base excess. There was no significant effect of treatment order (oxygen-air = -1.9mmol/l, p=0.11, 95% CI -4.5 to +0.5) or sequence (first treatment = 0.1mmol/l, p=0.75, 95% CI -0.6 to +0.9) on venous base excess. The treatment effect of oxygen versus air on venous base excess remained significant after these adjustment for treatment order (oxygen effect = +3.1mmol/l, p<0.001, 95% CI +1.6 to +4.5).

The unadjusted and adjusted effects of supplemental oxygen compared to air are shown in Table 4.13. Whilst venous PCO_2 and base excess tended to be higher in general with oxygen therapy and lower in general at baseline than follow-up, oxygen treatment significantly increased both PCO_2 and base excess, after adjustments for these effects.

	Unadjusted effect Diff in change in means Oxygen vs. Air (95% CI)	p	Adjusted effect Diff in change in means Oxygen vs. Air (95% CI)	p
PCO_2 (kPa)	+1.1 (+0.3 to +1.8)	**0.009**	+1.1 (+0.4 to +1.8)	**0.003**
Base excess (mmol/l)	+3.1 (+1.8 to +4.4)	**<0.001**	+3.1 (+1.6 to +4.5)	**<0.001**

Table 4.13: The unadjusted and adjusted effects of oxygen compared to air on venous blood gas results. The unadjusted effects were calculated using paired t-tests and the adjusted effects were calculated using a mixed effect model including adjustments for treatment order.

4.4.10. The relationships between overnight pulse oximetry and sleep study parameters and the primary outcome

The correlations between pulse oximetry parameters, the heart rate rises index, the AHI, urinary normetadrenaline, venous base excess, the hsCRP and the change in blood pressure were assessed using Spearman's rank correlation. Correlations were assessed for all observations. All oximetry parameters were significantly correlated with both the change in systolic and diastolic blood pressure, with similar correlation coefficients of 0.33 to 0.38 (Systolic: ODI r=0.38, p=0.007; % time saturations <90% r=0.35, p=0.01; mean saturations r=-0.33, p=0.02. Diastolic: ODI r=0.33, p=0.02; time saturations <90% r=0.36, p=0.01; mean saturations r=-0.34, p=0.01). Similarly, the normalised heart rate rises index was correlated with both the systolic (r=0.37, p=0.008) and diastolic blood pressure (r=0.29, p=0.04). The AHI was significantly correlated with the systolic blood pressure (r=0.37, p=0.03). The r value for AHI and diastolic blood pressure was not significant but was not much lower than other

values (r=0.25, p=0.15). The change in urinary normetadrenaline was correlated with both the systolic (r=0.31, p=0.03) and diastolic (r=0.29, p=0.04) blood pressure. The change in hsCRP was correlated with the systolic blood pressure (r=0.31, p=0.02). Although the correlation between hsCRP and diastolic blood pressure was not significant the correlation was of similar strength to other correlations (r=0.26, p=0.07). Full results are shown in the appendix 8.6.

There were significant co-correlations between different parameters and it is difficult to determine which factor is dominant in determining changes in blood pressure by this method. There are correlations of similar magnitude with blood pressure for: parameters that relate to arousal (heart rate rises, ODI, AHI), parameters that relate to cyclical hypoxia (ODI, AHI, heart rate rises), parameters that relate to the overall extent of hypoxia (mean saturations, % time with saturations <90%), parameters that relate to sympathetic activation (normetadrenaline, heart rate rises), and inflammation (hsCRP). The degree of co-correlation and sample size do not allow for the meaningful construction of multivariable models to further explore these relationships.

4.5. Discussion

Overnight supplemental oxygen causes marked attenuation of intermittent hypoxia with only a small reduction in the AHI and autonomic arousals. There was a trend towards supplemental oxygen partially reducing overnight production of urinary normetadrenaline, a metabolite of the sympathetic hormone noradrenaline, compared to air, after CPAP

withdrawal. However, this partial reduction does not fully explain change in blood pressure, and it remains unclear how intermittent hypoxia, in OSA, causes daytime blood pressure rises. The extent of hypoxia was correlated with the change in blood pressure, although there were also associations between markers of arousal, sympathetic activation and inflammation and the heart rate. Supplemental oxygen also caused increases in standard base excess and carbon dioxide levels, relative to air therapy, during CPAP withdrawal, which will be discussed further and needs careful monitoring in future trials.

4.5.1. Supplemental oxygen and markers of intermittent hypoxia

Previous trials have shown marked attenuation of intermittent hypoxia with oxygen. In the Gottlieb study, the $ODI_{\geq 3\%}$ and the time with oxygen saturations below 90% were comparably reduced by supplemental O_2 and CPAP (121) (Personal correspondence, appendix 8). In the Mills study the time with oxygen saturations less than 90% was reduced by both CPAP (0.07 ± 0.08%), and oxygen (2.5 ± 1.3%), compared with air therapy (6.2 ± 2.0%). CPAP seemed to more markedly attenuate intermittent hypoxia than supplemental O_2, but the difference was not significant (122). In a different publication using the same patients, there were significant differences in the post treatment ODI with CPAP (1.3 ± 1.9/hour) versus placebo (31.9 ± 33.0/hour). Again, whilst the difference was not significant, oxygen (15.7 ± 24.8/hour) seemed less effective in attenuating the ODI than CPAP (1.3± 1.9/hour) (207). In my study the ODI and time with oxygen saturations less than 90% were not normally distributed and the data are shown as medians but, even allowing for this, patients in my trial appeared to have more marked hypoxia in the air arm (median time <90% 14.3%, presumably due to more severe OSA), and to have more marked attenuation of

their hypoxia in the supplemental oxygen arm (median reduction in time <90% -9.8%). In my study (7:22 h:min/night) the usage of oxygen was similar to the Mills study (mean 6.6 h/night) (122), and both were higher than the Gottlieb study (mean 4.8 h/night) (121). Non-randomised trials of oxygen therapy for half to one night show almost universal improvements in mean oxygen saturations (186). Therefore, it seems clear that the oxygen improves intermittent hypoxia in both the short and medium term. Reductions in intermittent hypoxia depend on the flow rate of oxygen, the degree of hypoxaemia present prior to treatment, the length of the apnoeas, the functional residual capacity, and the hours of usage of oxygen therapy. Functional capacity was not assessed in this trial, but given the cross-over design this should not have biased the results. Including patients with higher ODIs, higher flow rates of oxygen, and the improved oxygen therapy hours in my study, probably explain the more marked magnitude of the reduction in intermittent hypoxia.

4.5.2. AHI and supplemental oxygen

In the supplemental oxygen trial there was a small (~10%) reduction in the AHI with oxygen versus air, but this was not significant. AHI was assessed without requirement for oxygen desaturation or arousal, and so represents flow limitation/obstructive events but not their consequences. Reported effects of oxygen therapy on the AHI are mixed, and are dependent on the physiological characteristics of the patients included, and on the length of intervention. In short term observational studies, generally with half or one night of supplemental oxygen, an overall reduction in the AHI has been shown (186). The Boston group have produced compelling work identifying non-anatomical traits for obstructive sleep apnoea, including a measure of ventilatory response (182). First, they characterise how

susceptible to collapse a patient's upper airway is. They do this by incrementally dropping the CPAP setting from a pressure holding the upper airway open whilst the upper airway dilator muscles are relaxed, to experimentally induce hypopnoeas. They measure ventilation during repeated induced hypopnoeas, over a range of pressures and extrapolate the pressure at which no ventilation would have occurred, termed the P_{CRIT}. They showed that approximately 20% of patients with OSA surprisingly do not have particularly collapsible upper airways; collapsing at subatmospheric pressures of less than -2cmH_2O. Many healthy individuals have similarly collapsible upper airways, without having OSA. These 20% of patients with OSA without particular anatomical susceptibility to upper airway collapse all have at least one of three non-anatomical traits predisposing to OSA. The three traits identified were impaired genioglossus muscle responsiveness, easy arousability/low arousal threshold, and high loop gain. Using their PALM (P_{CRIT}, arousal threshold, loop gain and muscle responsiveness) scale they propose that patients with OSA with moderate or low anatomic susceptibility to upper airway collapse and high loop gain may benefit from oxygen therapy.

Loop gain is the ratio of the ventilatory overshoot following termination of a hypopnoea (without arousal) to the ventilatory undershoot during a hypopnoea. Figure 4.4 is a schematic diagram showing ventilation following termination of a hypopnoea in an individual with low/normal loop gain (top) and an individual with high loop gain (bottom).

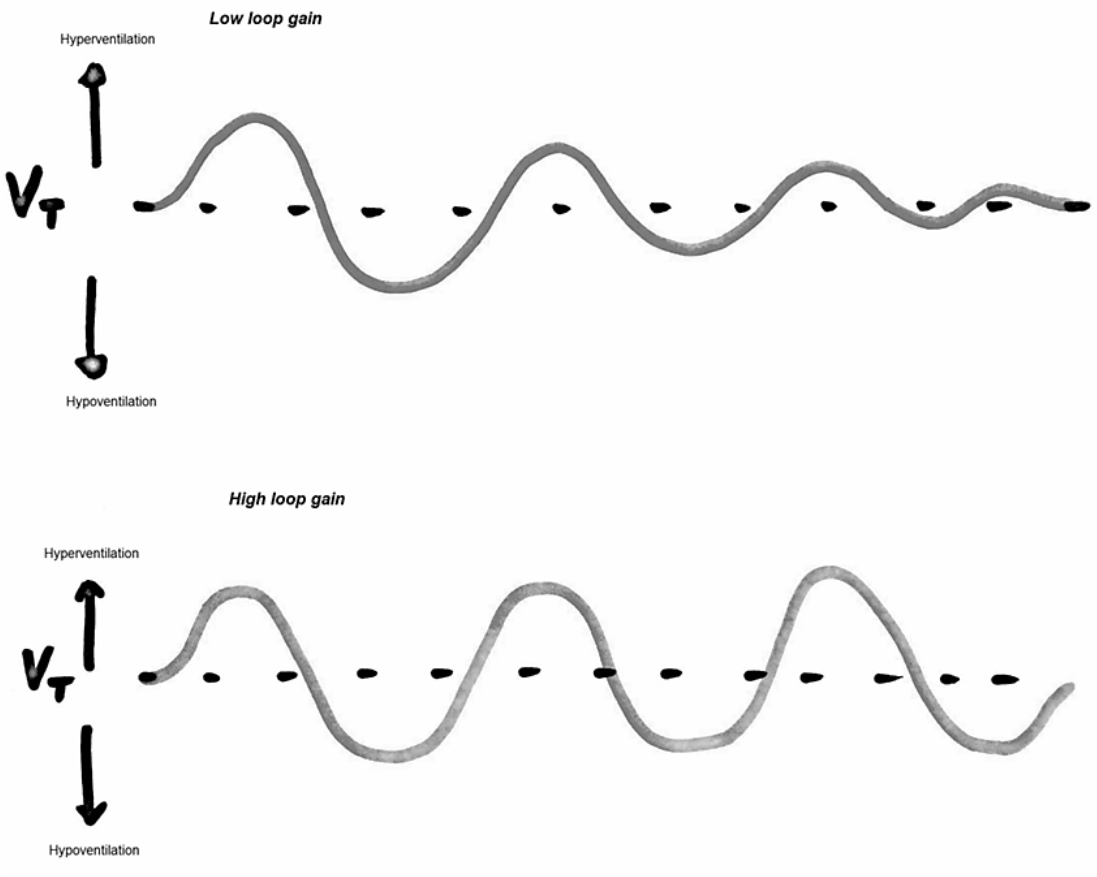


Figure 4.4: Schematic of an individual with low/normal loop gain (top) and an individual with high loop gain (bottom). In the individual with low/normal loop gain there is initial hyperventilation following termination of the hypopnoea, this is then followed by a few cycles of hypo- and hyperventilation, but ventilation quickly settles. In the individual with high loop gain the cycles of hypo- and hyperventilation persist, and could theoretically predispose to ongoing obstructive events as, during periods of hypoventilation, ventilatory drive to the upper airway dilator muscles will be reduced.

Loop gain has been shown to be an important factor in central sleep apnoea at altitude and oxygen therapy (reducing the hypoxic drive and reducing loop gain) is beneficial in improving altitude induced central sleep apnoea (221). In patients with OSA the Boston group showed

that hypoxia increased loop gain, and hyperoxia (via supplemental oxygen) reduced loop gain (220). Furthermore, using patients from a sample of convenience, including patients who have undergone their experiments before, they showed that the combination of eszopiclone and oxygen therapy reduced the mean AHI (219), with the AHI on active combination therapy being 26.5 ± 4.9/h versus 49.3 ± 6.2/h with placebo. Oxygen has been shown to reduce loop gain and stabilises ventilation which might reduce the AHI. Eszopiclone is a non-benzodiazepine hypnotic medication that is used in the treatment of insomnia. It increases the arousal threshold and might stop arousals from trivial obstructive events and stabilise ventilation, thus reducing the AHI. In this experiment loop gain was not a significant predictor of response to therapy, whilst the distance between active ventilation and arousal threshold ventilation did predict response. This suggests that the improvement in arousal threshold from eszopiclone was the reason for the reductions in AHI, and not improvements in loop gain from oxygen therapy. Lower therapeutic CPAP settings (implying less compressive forces on the pharynx) also predict response to this combination of therapies, indicating that this may be a useful surrogate of P_{CRIT}/upper airway collapsibility, and that response is more likely in those without overwhelming anatomic susceptibility to upper airway collapse (24). It is worth noting that these experiments tend to only expose patients to each intervention for relatively short periods of time, usually one or two nights. Also many of these patients were on CPAP at baseline. CPAP is known to normalise the increased hypoxic ventilatory responses seen in OSA (236), so the applicability of this work in the longer-term is not known. Therefore, whilst this work is interesting, further work needs to be done to show that oxygen and eszopiclone combination therapy can reduce the AHI in the longer-term, and that this translates to improved patient related outcome measures. In the longer-term, in patients not selected for particular physiological traits, oxygen has not

been shown to significantly reduce the AHI which is therefore similar to the findings of the supplemental oxygen trial.

4.5.3. Apnoea length and supplemental oxygen

The findings of my study showing increased apnoea length with supplemental oxygen are consistent with most observational studies, which have consistently shown that oxygen therapy increases the length of apnoeas (186). Gleeson's group showed that an individual's arousal threshold was determined by the level of respiratory effort/ventilatory drive (237). Regardless of whether the stimulus for increasing respiratory effort/ventilatory drive was hypoxia, hypercapnia or an upper airway resistive load, individuals had arousals from sleep at similar negative intra-thoracic pressures. During an apnoea all three of these stimuli would usually be present. It is likely that supplemental oxygen reduces the increased ventilatory drive from hypoxia, and therefore ventilatory drive is determined only by hypercapnia and upper airway obstruction. This is likely to increase the time to arousal and therefore apnoea length, as seen in the supplemental oxygen trial, and as reported by others (186). My study also showed a tendency towards increased interapnoea periods and so the apnoea: interapnoea length ratio was not altered. This is important as in patients with very severe OSA, who predominately had apnoeas, increased apnoea: interapnoea length has been shown to correlate with hypercapnia (187).

4.5.4. Changes in base excess and supplemental oxygen

Early descriptions of patients with OSA by Remmers *et al.* described patients with severe OSA, and often daytime hypercapnia (155). In these patients ventilatory responses to hypoxia and hypercapnia are likely to be vital in ending obstructive events to restore normal ventilation and to attempt to normalise blood gases. However, as others have described (157), most patients now treated for OSA do not match these early descriptions, and ventilatory responses leading to arousal may actually perpetuate OSA as discussed above. In my study, there were increases in carbon dioxide levels and increases in venous base excess, when supplemental oxygen was compared to air. This significant effect between arms was made up of both increases in carbon dioxide levels and base excess with oxygen, and decreases in carbon dioxide levels and base excess with air. It has previously been shown that CPAP reduces hypoxic ventilatory responses but not hypercapnic ventilatory responses (236). Therefore, it is likely that the converse occurs during CPAP withdrawal in the air arm, with the decreases in carbon dioxide levels and base excess being due to increased ventilatory responses to hypoxia. This is important as it suggests that the patients in my study are not like those described by Remmers *et al.* who often had daytime hypercapnia, as in my study median carbon dioxide levels went down with CPAP withdrawal onto air. During return of OSA, in the air arm, it seems there were compensatory increases in ventilation leading to reductions in carbon dioxide levels and base excess. Base excess and carbon dioxide levels have not previously been assessed in CPAP withdrawal. It is likely that this represents a redevelopment of heightened hypoxic ventilatory responses observed in patients with untreated OSA (236).

During CPAP withdrawal, supplemental oxygen increased carbon dioxide levels and base excess levels. This suggests that oxygen administration, by attenuating intermittent hypoxia, stops the increase in hypoxic ventilatory drive that was probably developing in the air arm. New algorithms are being developed to assess ventilatory traits/responses from sleep study parameters but these have not yet been developed for home sleep studies using nasal pressure transducers (238). These changes in ventilatory responses probably correspond to the changes in loop gain observed by Edwards and colleagues (220)(239). However, unlike the Edwards group (219), in my trial supplemental oxygen had little or no impact on the AHI. Change in ventilatory responses may not have led to greater and significant changes in the AHI for two reasons. First, patients were selected with rapidly recurring moderate to severe OSA, which is more commonly seen in patients with more severe OSA (181). Thus the patients with more severe OSA included in my study may have had predominant anatomical reasons for their OSA, making them less likely to respond to non-anatomical therapies such as supplemental oxygen (182)(24). Second, it may be that the combination of oxygen and eszopiclone is required to significantly alter the AHI (219).

Experiments by Gleeson showed that arousal occurs at a threshold of respiratory effort, regardless of whether the stimulus was hypoxia, hypercapnia or upper airway obstruction (237). In OSA it is therefore a combination of these factors that leads to increasing respiratory effort and results in an arousal once a certain threshold of respiratory effort has been reached. Therefore, having removed the hypoxic stimulus it is not surprising that apnoeas were extended, as respiratory effort was only dependent on hypercapnic and upper airway obstruction. Longer events are likely to be related to greater retention of carbon

dioxide. I observed increases in apnoea length and carbon dioxide levels without significant changes in the apnoea: interapnoea length ratio, and this differs from a previous report (187). In future trials the longer-term safety of oxygen therapy in OSA needs careful consideration as, although hypercapnia was not particularly marked in this study, oxygen has the potential to worsen/lead to hypercapnic respiratory failure in some individuals (240)(241), particularly those with OSA/obesity hypoventilation overlap (242)(243). Transcutaneous carbon dioxide monitoring or early morning capillary/arterial blood gases could be used in future studies to assess this, along with adverse event reporting focusing on symptoms of hypercapnia.

4.5.5. Sympathetic activation

Sympathetic activation was examined in two ways in the supplemental oxygen trial. First, overnight urinary normetadrenaline and metadrenaline levels were measured. I observed increases in urinary normetadrenaline with CPAP withdrawal, but these were only partially attenuated by supplemental oxygen, without significant changes in metadrenaline. In OSA, increased normetadrenaline levels have been more commonly reported (148)(222)(223)(224), than increased metadrenaline levels (225)(226). Differential sympathetic activation, with mainly increases in noradrenaline released from muscle and renal nerve endings, has been proposed in OSA (122). However, animal experiments show that both the adrenal medulla and sympathetic nerves are important factors in rises in blood pressure caused by intermittent hypoxia (106).

In the Mills study, examining the effects of supplemental oxygen in OSA, whilst they did not show a significant reduction in blood pressure, there was a reduction in daytime noradrenaline excretion (122). Mills *et al.* showed that CPAP reduced both night-time and daytime noradrenaline excretion, but supplemental oxygen only reduced daytime noradrenaline secretion. My study was not powered for the secondary outcome of change in urinary normetadrenaline, and the difference in changes in sympathetic activation, oxygen versus air, was only significant when looking at medians with urinary rate correction. This limited significance is likely to relate both to supplemental oxygen only partially attenuating sympathetic activation, and the variability in measurements of normetadrenaline levels. Normetadrenaline levels are affected by diet and medications, amongst other factors (194). Normetadrenaline levels were not significantly different when corrected by creatinine concentration, although there were trends towards reductions in levels with supplemental oxygen. Correcting the actual rate of urine production rather than a surrogate marker of urinary creatinine concentration seems reasonable. Endocrinology guidelines recommend this approach for 24-hour urine collections and use creatinine only as a check that collection was complete (195). I only measured normetadrenaline overnight, and perhaps the more marked attenuation of intermittent hypoxia in my study explains the reduction in overnight normetadrenaline, which is in contrast to changes in noradrenaline overnight in the Mills study. It is not surprising that sympathetic activation was not abolished by supplemental oxygen, as it did not have much impact on the AHI. As there was little change in the AHI it is likely that there was little impact on arousals from sleep, as evidenced by the minimal change in heart rate rises. Arousals are known to be associated with autonomic activation, independent of the effects of hypoxia (116)(118).

Heart rate rises overnight are a complex marker of arousal, sympathetic activation and parasympathetic inactivation. A threshold of >6bpm was set arbitrarily to ensure that small rises in heart rate that were likely to represent spontaneous variation were not included (11). I observed a small reduction in the heart rate rises index with oxygen therapy compared to air. I explored whether there were differences in the magnitude of heart rate rises between oxygen and air but did not observe significant differences. The small reduction in the number of heart rate rises >6bpm could have occurred due to reductions in arousals secondary to the small reduction in the number of obstructive events, as observed by others with combination oxygen and eszopiclone (219). Alternatively, this could have arisen due to changes in sympathetic activation as observed with oxygen therapy by Mills *et al* (122). In addition, heart rate rises are determined by reductions in parasympathetic activity (158). I observed both a partial attenuation of increases in sympathetic activity, measured by overnight urinary normetadrenaline, and a slight reduction in the AHI. The small changes in heart rate rises are difficult to interpret but were of similar magnitude to changes in the AHI suggesting that they are likely to be related. Supplemental oxygen caused marked attenuation of intermittent hypoxia along with a large effect on blood pressure, and a smaller effect on sympathetic activity. It is thus not clear, following CPAP withdrawal, that the limited reductions in sympathetic activation from reductions in intermittent hypoxia can fully explain the large attenuation in blood pressure rise seen in my study. Other effects of removing the intermittent hypoxia may also be important.

The relationship between hypoxia, sympathetic activity and daytime blood pressure is complex. After approximately 50 days at altitude, with associated chronic hypoxia, whilst the initial increases in blood pressure partially normalise, marked sympathetic activation remains (210). This is thought to be in part explained by baroreflex compensation (208). However, with two weeks of intermittent hypoxia in the air arm, there appear to be increases in both blood pressure and sympathetic activation, when compared to the oxygen arm, and there was no evidence of the blood pressure effect decreasing over this time course. Whether compensatory mechanisms similar to those seen at altitude occur in the longer-term in patients with OSA remains speculative.

4.5.6. Overnight urinary dilution

The mechanism(s) underlying urinary dilution in OSA is/are not fully understood. CPAP reduces the production of urinary sodium and water (229), and reduces the frequency and volume of nocturia (227)(228). OSA is thought to lead to changes in atrial natriuretic peptide (230)(231), and potentially to anti-diuretic hormone levels (231), although changes in anti-diuretic hormone have not been found by all (230). Intermittent hypoxia has been hypothesised to increase atrial natriuretic peptide by altering haemodynamics through peripheral vasoconstriction. Supplemental oxygen, whilst effectively attenuating intermittent hypoxia did not change the volume of urine produced overnight, and therefore another mechanism, other than intermittent hypoxia, must be involved in overnight urinary dilution in OSA. Intra-thoracic pressure swings leading to changes in haemodynamics and increased atrial natriuretic peptide production (230), is a potential mechanism along with sleep fragmentation blocking the normal reductions in urinary volumes overnight (244).

4.5.7. Supplemental oxygen in OSA and inflammation

There was no significant effect of oxygen treatment versus air, on hsCRP, which is a systemic marker of inflammation. Similarly, changes in hsCRP levels have not been shown either with CPAP treatment (153), of CPAP withdrawal (148). This lack of an effect of treatment with either oxygen or CPAP on hsCRP suggests that OSA, and OSA induced intermittent hypoxia, may not induce systemic inflammation in the short-term. However, hsCRP levels are elevated in patients with OSA in longer-term cohort studies (152), and animal models of intermittent hypoxia have highlighted the potential roles of inflammation in leading to hypertension and cardiovascular disease (151). There are alternative explanations for a lack of effect of supplemental oxygen on hsCRP levels. First, inflammation may be occurring in specific sites, such as the carotid body, without systemic increases in inflammation (218). Second, inflammatory changes induced by intermittent hypoxia may not be fully reversible (154). Third, hsCRP is not a very specific marker and may not reflect changes in individual inflammatory pathways. Therefore, whilst supplemental oxygen does not seem to change hsCRP, this does not mean that inflammation may not be important at a tissue level.

4.5.8. Correlations between physiological changes and blood pressure changes

The correlations between changes in measurements of intermittent hypoxia, AHI, heart rate, urinary normetadrenaline, base excess, hsCRP and blood pressure changes were explored to try to determine whether a single mechanism could explain the blood pressure changes observed. Many of the physiological parameters were co-correlated (ODI, AHI, HRR index) as they are all dependent on cyclical apnoeas/arousals. The correlations observed were modest

and it is difficult to interpret these results due to the co-correlations of variables. The sample size and degree of co-correlation did not allow further meaningful exploration as to the relative importance of cyclical deoxygenation/re-oxygenation, the degree of hypoxia, sympathetic activation or inflammation in causing elevated daytime blood pressure in OSA.

4.5.9. Conclusions (Chapter Four)

Supplemental oxygen therapy causes marked attenuation of intermittent hypoxia during CPAP withdrawal, compared to air. This contrasts with the small magnitude of changes in the AHI, heart rate rises, and thus presumably arousals. Supplemental oxygen appeared to only partially reduce the rise in overnight urinary normetadrenaline, a metabolite or noradrenaline, thus it is not clear if reductions in sympathetic activation explain the attenuation of awake morning blood pressure. There were small reductions in the heart rate rises index, and these may reflect reductions in sympathetic activation or arousals. The extent of attenuation of intermittent hypoxia in my study was more marked than in previous longer-term studies of supplemental oxygen therapy in OSA. This is likely to reflect the inclusion of patients with initially more marked hypoxia from their OSA, increased usage of the overnight oxygen therapy, and higher oxygen flow rates. The increases in carbon dioxide levels and base excess with oxygen therapy highlight potential changes in ventilatory responses following both CPAP withdrawal and with supplemental oxygen. In addition, the increased base excess levels illustrate the potential for oxygen precipitating daytime hypercapnia particularly in those with OHS overlap, which needs further careful monitoring in future trials.

5. Chapter Five: The effects of supplemental oxygen therapy on both subjective and objective sleepiness during CPAP withdrawal

5.1. Abstract (Chapter Five)

Daytime sleepiness is one of the most important symptoms of OSAS. Daytime sleepiness impacts upon quality of life, worsens performance at work, and increases risk of road traffic collisions. Intermittent hypoxia is thought to contribute to daytime sleepiness, with animal models showing increased total sleep time and reduced sleep latency with intermittent hypoxia. Both subjective sleepiness, measured by the ESS, and objective sleepiness, measured by the OSLER test, were assessed in the SOX trial. There were no significant differences in the changes in either the ESS or the original OSLER test in the supplemental oxygen trial, oxygen versus air. I sought to test if modifications to the original OSLER, proposed by others, improved its performance in detecting changes in sleepiness. These modifications marginally improved the OSLER test's performance both in minimally symptomatic patients from a different trial (the MOSAIC trial), and when comparing baseline and follow-up results from the combined arms in the SOX trial. Using these modifications, there were still no significant differences in the changes to the OSLER test, oxygen versus air. This suggests that supplemental oxygen does not improve daytime sleepiness, over a relatively short time period of two weeks of CPAP withdrawal, and conversely that intermittent hypoxia is unlikely to be the cause of daytime sleepiness in the short-term in OSA. There is little longer-term data on the direct effects of intermittent hypoxia in humans, and thus its longer-term effects are unknown. Given its lack of effect on sleepiness, any future trials of oxygen therapy in OSA probably should be restricted to non-sleepy patients.

5.2. Introduction

Excessive daytime sleepiness is one of the most important consequences of OSAS. OSAS patients have increased subjective (40)(42), and objective sleepiness (41). OSAS is also associated with reduced quality of life (189). It is likely that excessive daytime sleepiness is the cause of increased road traffic collisions in patients with OSA. It is worth noting that this has not been established, probably due to the difficulty and thus lack of studies looking at both sleepiness and actual road traffic collisions (7). In addition, excessive daytime sleepiness is associated with increased risk of work-based accidents (245), missed work days (246), and with reduced productivity in the workplace (247). CPAP has been shown to be effective in improving both subjective and objective sleepiness (4)(5)(188). Other treatments for OSA such as mandibular advancement devices are also effective in improving subjective sleepiness, but there is a lack of head-to-head data comparing alternative therapies with CPAP (4).

Arousals from sleep/sleep fragmentation are likely to be important in the development of daytime sleepiness. In addition, the degree of intermittent hypoxia may also contribute to daytime sleepiness. However, in one cross sectional study, respiratory sleep study parameters only explained 10-12% of the variance in objective sleepiness (41). Other factors such as total sleep time, shift work, alcohol consumption, and other factors are likely to influence daytime sleepiness.

5.2.1. Intermittent hypoxia and sleepiness

Evidence from rodent models suggests that intermittent hypoxia may be important in the development of sleepiness, independent of apnoea-induced arousals. Mice exposed to eight weeks of intermittent hypoxia had increased total sleep time and reduced sleep latency (248). In addition, oxidative injury to wake-promoting centres in the frontal cortex and brainstem persisted after two weeks of recovery from intermittent hypoxia. Therefore, intermittent hypoxia could contribute to sleepiness in OSA. It has been proposed that these changes persisting after recovery from intermittent hypoxia could explain "post-CPAP sleepiness" seen in some patients (249). However, others have argued that sleepiness persisting after CPAP therapy merely reflects the natural variation in sleepiness also seen in individuals without OSA (250).

5.2.2. Subjective sleepiness

The Epworth Sleepiness Scale (ESS) was developed as a method to assess subjective sleepiness in a range of sleep conditions including OSAS (39)(40). CPAP leads to marked improvements in the ESS in patients with symptomatic OSA (5), and smaller improvements in patients with minimally symptomatic OSA (46). Changes in the ESS seem to be the most sensitive questionnaire-based measures of improved symptoms following CPAP therapy (4). Changes in the ESS occur even in the context of a relatively short period of two weeks of CPAP withdrawal (148). In the supplemental oxygen trial, I assessed the ESS at each trial visit to assess the effect of supplemental oxygen versus air on subjective sleepiness. This would test the hypothesis that intermittent hypoxia is responsible for increases in subjective sleepiness in OSA.

5.2.3. Objective sleepiness

Objective sleepiness is commonly measured by either multiple sleep latency tests (MSLT) or maintenance of wakefulness tests (MWT). These are laborious and time consuming tests. Bennett *et al.* developed the Oxford Sleep Resistance (OSLER) test as a measure of objective sleepiness (188). The OSLER test requires an individual to lie in a darkened room and to press a sensor in response to a dim flashing light (red LED) appearing at regular 3 second intervals. The test ends when 7 consecutive lights (21s) are missed and the patient is deemed to have fallen asleep, or when 40 minutes (2400 seconds) have elapsed. The OSLER test's performance is comparable to the MWT in discriminating objective sleepiness in OSAS patients. Similarly it shows marked improvements after CPAP treatment (46)(62)(188)(189), and marked deteriorations following CPAP withdrawal (148).

5.2.4. Modifications to the OSLER test

Modifications have been proposed to the OSLER test (191)(192)(193). First, reducing the number of missed lights required for it to be deemed that the patient has fallen asleep to 6, 5, and 4 respectively improved its sensitivity to detect micro-sleeps (191). Second, looking at the total number of missed lights has been proposed to assess vigilance in addition to sleepiness (192)(193).

5.2.5. Aims

In the supplemental oxygen trial, I aimed to assess whether supplemental oxygen improved the time to 7 missed lights on the OSLER test ("original OSLER"), compared to air. Second, I aimed to see if improvements could be made to the OSLER test by reducing the required of number of missed lights to determine sleep latency and by introducing an error index as a measure of vigilance. I tested this aim in two study populations; in minimally symptomatic patients from the MOSAIC study, and in the current supplemental oxygen trial. Finally, I aimed to see if any of these modifications to the OSLER test better identified any differences between supplemental oxygen and air in the SOX trial.

5.3. Methods

5.3.1. Subjective sleepiness (5.4.1)

Subjective sleepiness was measured by the ESS test which patients completed during each of the four trial weeks as described in section 2.1.8.5. An example of the ESS questionnaire can be found in Appendix 8.2.

The differences in the ESS from baseline to follow-up were assessed for each arm separately and between supplemental oxygen and air. Wilcoxon rank test and paired t-tests were used as appropriate.

5.3.2. Objective sleepiness: Original OSLER test (5.4.2)

The OSLER test was measured at each trial visit as described in section 2.2.8.6. OSLER test data is generally non-normally distributed, due to patients completing the test without falling asleep being allocated scores of 2400. Often, changes in the OSLER test are also non-normally distributed due to a large number of values not changing. Differences in the change in the original OSLER test from baseline to follow-up were compared for each arm separately and between supplemental oxygen and air. Wilcoxon rank tests and paired t-tests were used as appropriate.

In addition, Kaplan-Meier survival curves were constructed to compare the original OSLER tests between supplemental oxygen and air, at baseline and follow-up. A patient who completed 2400 seconds of the test was deemed to have "survived". Alternatively, a patient's data was censored when they missed seven lights in a row. Cox proportional hazard ratios were also calculated for these comparisons using standard Cox proportional hazard models and using a mixed effect Cox proportional hazard model to account for the paired nature of the data (R software).

5.3.3. Development of modified OSLER test derivatives in the MOSAIC study (5.4.3 & 5.4.4)

Methodology for the development of a template to extract modified OSLER data from the original OSLER test and the MOSAIC study patients used to develop this can be found in section 2.2.8.7 and 2.6. Due to the large sample size in the MOSAIC trial, central limits

theorem can be applied and so unpaired t-tests were used to compare the difference in means in the CPAP arm. Cohen's d effect size was calculated by the following equation:

$$Cohen's\ d = \frac{Mean(follow\ up) - Mean(baseline)}{\sqrt{(SD(follow\ up)^2 + SD(baseline)^2)}}$$

Arbitrarily, Cohen's d effect sizes are considered small when 0.3≤d<0.5, moderate when 0.5≤d<0.8, and large when d≥0.8. Cohen's d effect sizes were calculated for the time to 7 missed lights ("original OSLER"), time to 6 missed lights ("sleep latency 6" or "SL6"), time to 5 missed lights ("sleep latency 5" or "SL5"), time to 4 missed lights ("sleep latency 4" or "SL4"), number of times 3-6 lights in a row were missed, and the total number of missed lights per hour of test time ("error index"). My hypothesis was that the SL6, SL5, SL4, number of times 3-6 lights in a row were missed, and the error index would all show greater improvements with CPAP versus standard care in the minimally symptomatic patients in the MOSAIC trial, than the original OSLER.

5.3.4. Modifications to the OSLER test in the SOX trial (5.4.5, 5.4.6, & 5.4.7)

The modifications to the OSLER test were then measured in the SOX trial. First, differences between baseline and follow-up in the modified OSLER test parameters were assessed for all observations (oxygen and air arms combined). Second, the difference in the changes in the modifications to the OSLER test were assessed, oxygen versus air. Differences were compared using Wilcoxon rank tests and Cox proportional hazard models. Kaplan-Meier

survival curves were also plotted. Cohen's d effect sizes could not be calculated as the data was non-normally distributed and the sample size was small.

5.3.5. Correlations between sleepiness, sleep study and blood pressure parameters (5.4.8)

Correlations between the changes in the ODI, the heart rate rises index, the systolic and diastolic blood pressure, and both subjective (ESS), and objective sleepiness were assessed. Changes in the ODI and heart rate rises index were calculated by subtracting screening on CPAP values from the ODI during nights 8-13 of either oxygen or air treatment.

5.4. Results

5.4.1. Subjective sleepiness

There were significant increases in the ESS from baseline to follow-up for both oxygen treatment (median difference +1.0, p=0.03, IQR -1.0, +4.0) and air treatment (median difference +2.0, p=0.003, IQR 0.0, +6.0). However, the difference in the change in the ESS from baseline to follow-up between treatment arms was not significantly different, oxygen versus air (mean difference -0.6, p=0.56, 95% CI -2.5 to +1.4 using a paired t-test). Baseline and follow-up ESS scores by treatment are displayed in *Figure 5.1* and in *Table 5.1*.

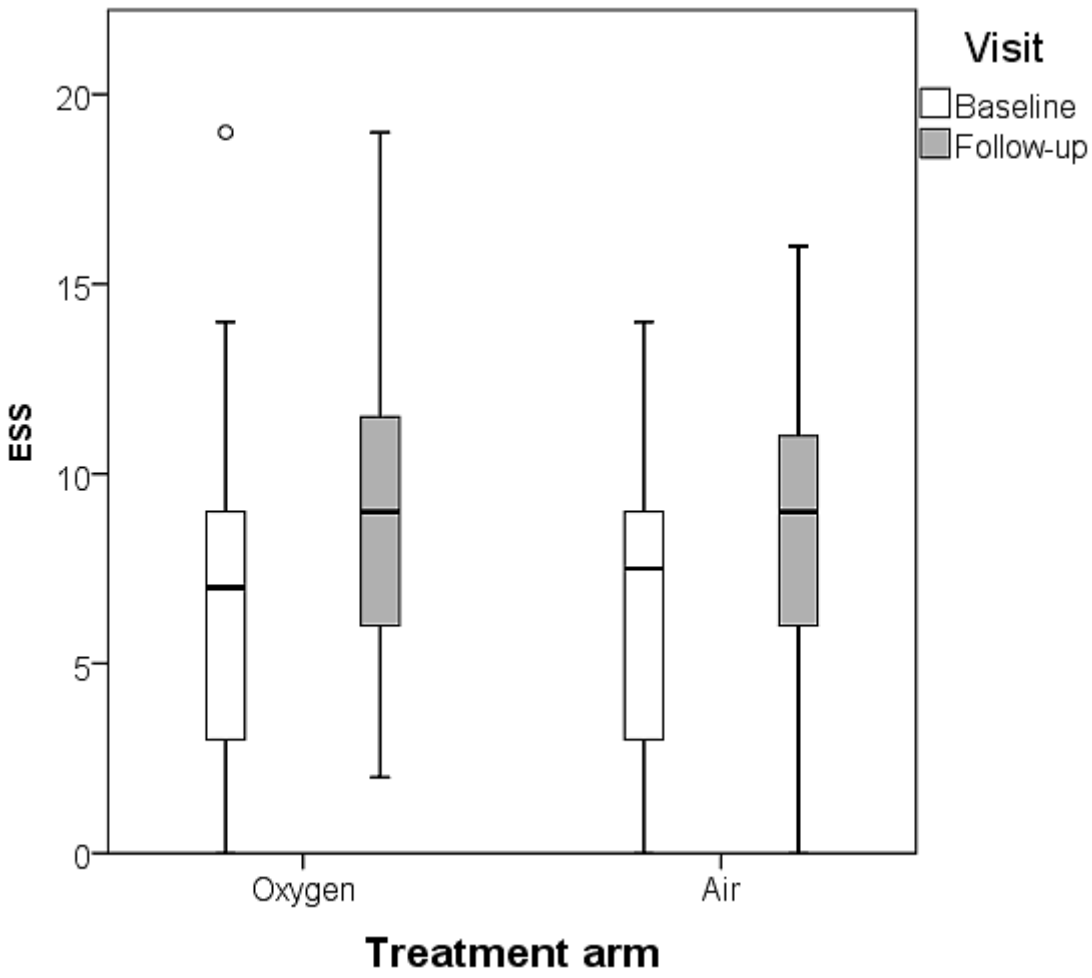


Figure 5.1: Box and whisker plots showing ESS scores at baseline and follow-up for oxygen and air treatment arms. Solid black lines represent the median, box edges represent the 1st and 3rd quartiles, the whiskers extend to 1.5 times the IQR from the 1st and 3rd quartiles, and outliers within 1.5 times the IQR of the whiskers are represented by open circles.

5.4.2. Objective sleepiness: Original OSLER test

Baseline/run-in values for the original OSLER test were comparable, with median values of 2400 seconds (1285, 2400) in the oxygen arm, and 2400 seconds (1286, 2400) in the air arm. At follow-up, there were trends toward reductions in the OSLER test for both oxygen (median reduction -3 seconds, p=0.11, IQR -796, 0.0) and air (median reduction 0 seconds,

p=0.11, IQR -437, 0), compared to baseline. There was no significant difference in the change in median times between treatment arms from baseline to follow-up, oxygen versus air (median difference 0 seconds, p=0.50, IQR -425, +14). Data are displayed in *Table 5.1*.

	Oxygen		*Air*		Between groups difference, oxygen versus air	p
	Baseline/ run-in	Follow-up	Baseline/ run-in	Follow-up		
ESS	7·0 ± 4·8	9·0 ± 4·2	6·3 ± 3·8	8·8 ± 4·5	-0·6 (-2·5 to +1·4)	0·56
Original OSLER (s)	2400 (1285, 2400)	1746 (770, 2400)	2400 (1286, 2400)	2025 (956, 2400)	0 (-425, +14)	0·50

Table 5.1: Objective and subjective sleepiness in both treatment arms. Baseline and follow-up data are displayed mean ± SD for ESS and median (IQR) for the original OSLER. Data for between group differences are displayed as mean (95% CI) for ESS and median (IQR) for the original OSLER.

Many individuals did not miss 7 lights and so were deemed to have "survived" the OSLER test. Kaplan-Meier survival curves were plotted comparing oxygen and air at follow-up (*Figure 5.2*). Both standard and mixed effect Cox proportional hazard models were constructed to assess if there was a difference in "survival" in the OSLER test between oxygen and air. In the standard Cox proportional hazard model there was no significant difference with oxygen compared to air (Hazard ratio or HR 1.40, p=0.57, 95% CI 0.44 to

4.46). In the mixed effect Cox proportional hazard model there was no significant difference with oxygen compared to air (HR 1.40, p=0.60, 95% CI 0.40, 4.97).

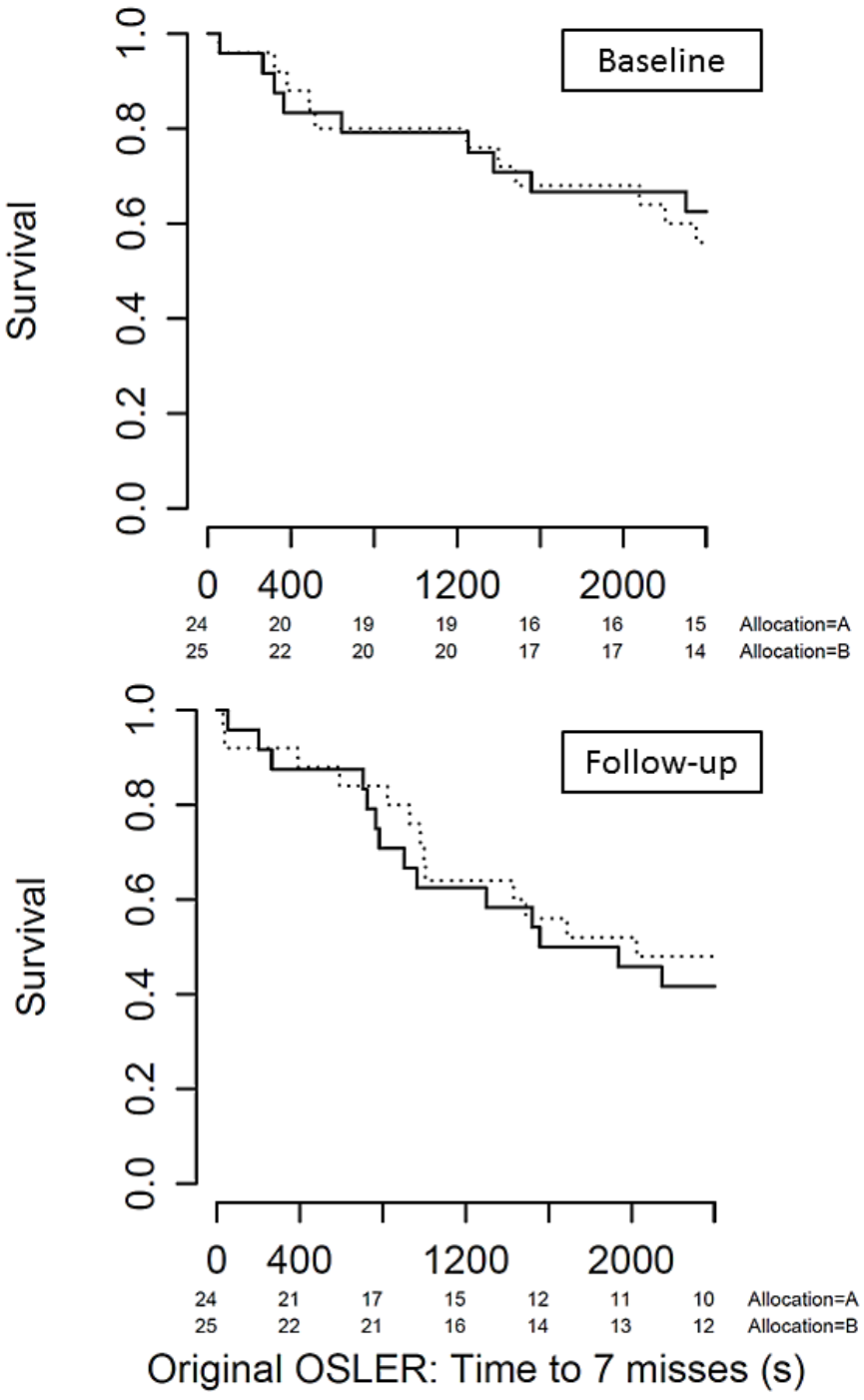


Figure 5.2: Kaplan-Meier Plot comparing the proportion of patients "surviving/awake" the OSLER test without missing 7 consecutive lights, oxygen versus air at baseline and follow-up. Solid line represents the oxygen arm (A), and the dotted line represents the air arm (B).

Numbers still at risk ("awake") shown below the x-axes for every 400 seconds. Log rank tests (unpaired) were not significant for differences between oxygen and air at baseline (p=0.73) or follow-up (p=0.66).

5.4.3. Development of modified OSLER test derivatives in the MOSAIC study: Cohen's d effect sizes

The MOSAIC trial randomised 391 patients to either CPAP or standard care. OSLER tests were available for re-analysis from 118 patients in the standard care arm and 116 in the CPAP group. The change in OSLER test scores were calculated from baseline to follow-up for; the original OSLER (time to 7 misses); time to 6, 5 and 4 misses; the error rate (total number of misses/h); and the number of 3-6 misses. The difference in these scores was compared between the CPAP group and the standard group using unpaired t-tests. The Cohen's d effect size was calculated for each variable. Results are shown in *Table 5.2*.

Change over 6 months	Standard care (Mean ± SD)	CPAP (Mean ± SD)	Mean difference in change (95% CI)	p	Cohen's d effect size
Time to 7 misses(s) **(Original OSLER)**	-80 ± 680	+165 ± 784	+244 (+55 to +433)	**0.01**	0.33
Time to 6 misses(s)	-31 ± 759	+213 ± 800	+244 (+43 to +445)	**0.02**	0.31
Time to 5 misses(s)	+3 ± 802	+246 ± 845	+244 (+31 to +456)	**0.03**	0.30
Time to 4 misses(s)	+44 ± 748	+329 ± 814	+285 (+84 to +487)	**0.006**	0.36
Error rate (events/hour)	-14 ± 142	-47 ± 130	-33 (-68 to +2)	0.07	-0.24
Number of 3-6 misses	-1 ± 4	-1 ± 4	-0 (-1 to +1)	0.96	-0.00

Table 5.2: Changes in OSLER scores from baseline to 6-month follow-up, comparison of the difference in the change of means CPAP versus standard care and Cohen's d effect sizes. A Cohen's d effect was considered small when 0.3≤d<0.5, moderate when 0.5≤d<0.8, and large when d≥0.8.

Therefore, modifications in the OSLER test made only slight improvements to its performance when assessed using Cohen's d effect sizes. The time to 4 misses performed the best with a Cohen's d effect size of 0.36.

5.4.4. Development of modified OSLER test derivative in the MOSAIC study: Cox proportional hazard ratio models

As many patients were deemed to have been "awake" at the end of the OSLER test in the MOSAIC trial there may have been minimal change in overall mean scores. Cox proportional

hazard models were constructed comparing CPAP with standard care in the MOSAIC trial patients. The time taken until the patient was deemed to have fallen asleep (either 7, 6, 5, or 4 lights missed in a row) was the dependent variable, with those staying awake to 40 minutes being deemed to have "survived". Patient visit (baseline=0, 6-month follow-up=1), treatment (standard care=0, CPAP=1) and the interaction between visit × treatment (the treatment effect) were modelled as fixed factors. In addition to these parameters the patient trial number was added as a random effect in the mixed-effects Cox proportional hazard model. Results are shown in *Table 5.3*.

	Standard Cox model			Mixed effects Cox model		
Time to (s)	HR	95% CI	p	HR	95% CI	p
7 misses (Original OSLER)	0.66	0.31 to 2.43	0.18	0.49	0.26 to 0.93	0.03
6 misses	0.70	0.39 to 1.24	0.22	0.53	0.29 to 0.98	0.04
5 misses	0.69	0.40 to 1.19	0.18	0.56	0.31 to 1.01	0.05
4 misses	0.66	0.39 to 1.11	0.12	0.46	0.26 to 0.81	0.008

Table 5.3: Results of standard (unpaired) and mixed effect (paired) Cox proportional hazard models for relative risk of not surviving the OSLER test dependent on the number of misses in the MOSAIC trial. Hazard ratios are quoted for follow-up on CPAP relative to a reference of 1.0 for standard care.

There were again, only marginal improvements in the OSLER test's performance with these modifications, when assessed with Cox proportional hazard models. As with Cohen's d effect size assessments, the time to 4 misses seemed to best discriminate the two groups.

5.4.5. Modifications to the OSLER test in the SOX trial, baseline versus follow-up in both oxygen and air arms combined

In the SOX trial, changes from baseline to follow-up for the oxygen and air arms combined were first assessed, to explore the effects of the modifications to the OSLER test. Changes in modified OSLER parameters were compared using Wilcoxon rank tests and results are shown in *Table 5.4*.

Combined (n=49)	Baseline Median (IQR)	Follow-up Median (IQR)	Median difference in change (IQR)	p	Z score
Time to 7 misses(s) (Original OSLER)	2400 (1316, 2400)	1935 (863, 2400)	0 (-584, 0)	0.02	-2.3
Time to 6 misses(s)	2400 (1313, 2400)	1551 (743, 2400)	0 (-644, 0)	0.009	-2.6
Time to 5 misses(s)	2400 (1245, 2400)	1425 (708, 2400)	-14 (-782, 0)	0.002	-3.0
Time to 4 misses(s)	2295 (1224, 2400)	1143 (657, 2400)	-80 (-733, 0)	0.001	-3.4
Error rate (/h)	18.0 (1.5, 73.4)	61.3 (14.3, 151.5)	+10.5 (0, +78.8)	0.008	-2.7
Number of 3-6 misses	0.0 (0.0, 2.0)	1.0 (0.0, 3.5)	0 (-1, +2)	0.13	-1.5

Table 5.4: Comparison of changes in OSLER scores from baseline to follow-up for both arms combined (oxygen and air).

There were significant changes in the median time to 7, 6, 5, and 4 misses, and a significant change in the error rate. The Z score for time to 4 misses was the largest suggesting that this

modification was a marginal improvement on the original OSLER time to 7 misses, as found above in the MOSAIC data.

5.4.6. Modifications to the OSLER test in the SOX trial Cox proportional hazard ratio, baseline versus follow-up in both oxygen and air arms combined

In the SOX trial, as there were no significant differences in Cox proportional hazard models in the "Original" OSLER test between oxygen and air treatment, both the oxygen and air arms were combined to see if reducing the number of lights missed increased the performance of the OSLER test in discriminating between baseline and follow-up. *Table 5.5* shows the results of standard and mixed effect Cox proportional hazard ratios using the original OSLER (time to 7 missed lights) and modifications to the OSLER of; time to 6, 5, and 4 missed lights. An example of the R script used to calculate these variables is shown in *Figure 5.3.*

Time to (s)	Standard Cox model			Mixed effects Cox model		
	HR	95% CI	p	HR	95% CI	p
7 misses (Original OSLER)	1.52	0.85 to 2.71	0.16	2.45	1.29 to 4.65	0.006
6 misses	1.54	0.87 to 2.71	0.14	2.21	1.20 to 4.08	0.01
5 misses	1.67	0.98 to 2.82	0.06	2.45	1.38 to 4.36	0.002
4 misses	1.79	1.07 to 2.98	0.03	2.60	1.49 to 4.53	<0.001

Table 5.5: Results of standard (unpaired) and mixed effect (paired) Cox proportional hazard models for relative risk of not surviving the OSLER test dependent on the number of misses. Hazard ratios are quoted for follow-up relative to a reference of 1.0 for baseline visit. Data are oxygen and air arms combined.

```
> ##Apply a standard Cox just for baseline to follow-up
> fit1 <- coxph(Surv(SL4, Survival4) ~ Visit_bin, data=mydata)
> print(fit1)
Call:
coxph(formula = Surv(SL4, Survival4) ~ Visit_bin, data = mydata)

          coef exp(coef) se(coef)    z    p
Visit_bin 0.582     1.789    0.261 2.23 0.026

Likelihood ratio test=5.06  on 1 df, p=0.0245
n= 98, number of events= 61
> ##Change to exp(coef + c(-1,1)*1.96*se(coef))
> exp(0.582 + c(-1,1)*1.96*0.261)
[1] 1.072980 2.984881
> ##Apply a mixed effects Cox just for baseline to follow-up
> fit2 <- coxme(Surv(SL4, Survival4) ~  Visit_bin + (1|Triano),
+             data=mydata)
> print(fit2)
Cox mixed-effects model fit by maximum likelihood
  Data: mydata
  events, n = 61, 98
  Iterations= 18 79
                NULL Integrated    Fitted
Log-likelihood -255.2085  -220.7415 -182.7128

                Chisq    df        p    AIC   BIC
Integrated loglik  68.93  2.00 1.1102e-15  64.93 60.71
 Penalized loglik 144.99 21.52 0.0000e+00 101.94 56.51

Model:  Surv(SL4, Survival4) ~ Visit_bin + (1 | Triano)
Fixed coefficients
            coef exp(coef)  se(coef)    z      p
Visit_bin 0.9549482  2.598536 0.2839042 3.36 0.00077

Random effects
 Group  Variable  Std Dev  Variance
 Triano Intercept 1.934207 3.741156
> ##Change to exp(coef + c(-1,1)*1.96*se(coef))
> exp(0.9549482 + c(-1,1)*1.96 *0.2839042)
[1] 1.489583 4.533075
```

Figure 5.3: An example R script for both standard Cox proportional Hazard models and for mixed effect Cox proportional hazard models. In this example baseline visits are compared to follow-up. "Fit1" is the model for a standard Cox proportional hazards model comparing baseline (x=0) and follow-up (x=1). "Fit2" is the model for a mixed effect Cox proportional hazard model comparing baseline (x=0) and follow-up (x=1) but also including a random effects term for patient identifier ("Triano"). Therefore, the exponential coefficient is the hazard ratio of the missing 4 lights ("Sleep latency 4" or "SL4") at the follow-up compared to baseline.

There were again minor improvements in performance of the OSLER test using the time to 4 misses compared to the original OSLER time to 7 misses. The Kaplan-Meier curve for the time to 4 misses for both arms combined is shown in *Figure 5.4.*

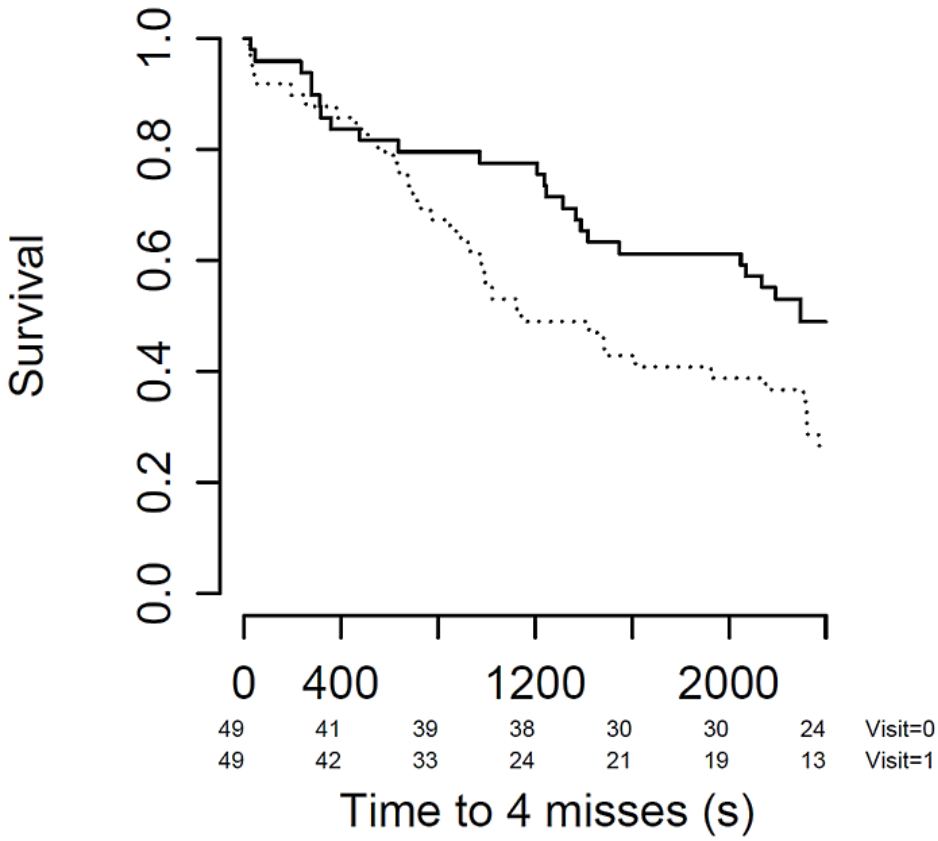


Figure 5.4: Kaplan-Meier survival curve showing time to 4 missed lights, for both arms combined, at baseline (solid line and Visit=0) and follow-up (dotted line and Visit=1). Number at risk/awake are shown below the x-axis. Log-rank p=0.02 (unpaired).

5.4.7. Modifications to the OSLER test in the SOX trial, oxygen versus air

The modifications to the OSLER test were assessed to see if they could distinguish differences in the change in parameters from baseline to follow-up, oxygen versus air. The results are shown in *Table 5.6*.

	Change in oxygen arm Median (IQR)	Change in air arm Median (IQR)	Median difference in change (IQR)	Z score	p
Time to 7 misses(s) (Original OSLER)	-3 (-796, 0)	0 (-437, 0)	0 (-504, +18)	-0.67	0.50
Time to 6 misses(s)	-60 (-965, 0)	0 (-357, 0)	0 (-639, +39)	-0.92	0.36
Time to 5 misses(s)	-278 (-1054, 0)	-2 (-508, 0)	0 (-792, +279)	-0.76	0.45
Time to 4 misses(s)	-279 (-1276, 0)	-51 (-398, 0)	0 (-741, +99)	-1.11	0.27
Error rate (/h)	+12.9 (-24.2, +100.1)	+10.5 (0, +72.6)	-3.0 (-77.4, +100.4)	-0.17	0.86
Number of 3-6 misses	+0.5 (-1.0, +3.8)	0 (-1.8, +1.0)	0.0 (0.0, +3.0)	-1.35	0.18

Table 5.6: Comparison of the median difference in the change in OSLER parameters baseline to follow-up, oxygen versus air. Comparisons using Wilcoxon rank tests.

There were no significant differences in median changes in any of these modifications to the OSLER test, oxygen versus air.

Cox proportional hazard models were constructed to compare these modifications to the original OSLER test, oxygen versus air. Results are shown in *Table 5.7* with an example R script in *Figure 5.5.* There were no significant differences identified using Cox proportional models in any modifications to the OSLER test, oxygen versus air.

Time to (s)	Standard Cox model			Mixed effects Cox model		
	HR	95% CI	p	HR	95% CI	p
7 misses (Original OSLER)	1.40	0.44 to 4.46	0.57	1.40	0.40 to 4.97	0.60
6 misses	1.40	0.45 to 4.35	0.56	1.48	0.43 to 5.15	0.53
5 misses	1.26	0.44 to 3.62	0.67	1.18	0.37 to 3.71	0.78
4 misses	1.15	0.41 to 3.18	0.80	1.15	0.38 to 3.51	0.81

Table 5.7: Results of standard (unpaired) and mixed effect (paired) Cox proportional hazard models for relative risk of not "surviving" the OSLER test dependent on the number of misses. Hazard ratios are quoted for oxygen relative to a reference of 1.0 for air.

```
> ##Apply a standard Cox just for fully adjusted treatment effect
> fit3 <- coxph(Surv(SL7, Survival7) ~ Visit_bin + Treatment_bin1 + Sequence + Order_bin
+ (Visit_bin * Treatment_bin1) + data=mydata)
> print(fit3)

                      coef exp(coef) se(coef)    z    p
Visit_bin             0.2648    1.3031   0.4100  0.65 0.518
Treatment_bin1        -0.0483    0.9529   0.4821 -0.10 0.920
Sequence              0.1781    1.1950   0.3385  0.53 0.599
Order_bin             -0.8280    0.4369   0.3390 -2.44 0.015
Visit_bin:Treatment_bin1  0.3338    1.3963   0.5922  0.56 0.573

Likelihood ratio test=9.24  on 5 df, p=0.0997
n= 98, number of events= 47
> ##Change to exp(coef + c(-1,1)*1.96*se(coef))
> exp(0.3338 + c(-1,1)*1.96 *0.5922)
[1] 0.4373979 4.4571609
> ##Apply a mixed effect Cox just for treatment effect - drop non-significant terms
> fit4 <- coxme(Surv(SL7, Survival7) ~ Visit_bin + Treatment_bin1 + Sequence + Order_bin
+ (Visit_bin * Treatment_bin1)  + (1|Triano), data=mydata)
> print(fit4)
Cox mixed-effects model fit by maximum likelihood
 Data: mydata
 events, n = 47, 98
 Iterations= 15 83
               NULL Integrated    Fitted
Log-likelihood -202.1295  -169.7083 -134.4703

               Chisq   df        p   AIC  BIC
Integrated loglik  64.84  6.00 4.6457e-12 52.84 41.74
 Penalized loglik 135.32 24.49 0.0000e+00 86.35 41.05

Fixed coefficients
                      coef exp(coef)  se(coef)    z    p
Visit_bin             0.74120034 2.0984529 0.4502301  1.65 0.10
Treatment_bin1        -0.06793838 0.9343180 0.5071395 -0.13 0.89
Sequence              0.20039396 1.2218840 0.3569678  0.56 0.57
Order_bin             -1.82960762 0.1604765 1.2123610 -1.51 0.13
Visit_bin:Treatment_bin1  0.33840796 1.4027126 0.6453871  0.52 0.60

Random effects
 Group  Variable  Std Dev  Variance
 Triano Intercept 2.624080 6.885795
> ##Change to exp(coef + c(-1,1)*1.96*se(coef))
> exp(0.33840796 + c(-1,1)*1.96 *0.6453871)
[1] 0.395917 4.969736
```

Figure 5.5: An example R script for both standard Cox proportional Hazard models and for mixed effect Cox proportional hazard models. In this example the treatment effect via an interaction term ("Visit_bin × Treatment_bin1") is assessed. "Fit3" is the model for a standard Cox proportional hazards model. "Fit4" is the model for a mixed effect Cox proportional hazard model including a random effects term for patient identifier ("Triano"). Therefore, the exponential coefficient is the hazard ratio of the missing 7 lights ("Sleep latency 7" or "SL7") with oxygen treatment versus air.

5.4.8. Correlations between sleepiness, sleep study and blood pressure parameters

The correlations between the change in sleepiness (ESS and time to 4 misses) and; the change in pulse oximetry parameters (change in ODI, change in heart rate rises, mean oxygen saturations); the AHI; the change in systolic and diastolic blood pressure were assessed using Spearman's rank correlations. The results are shown in *Table 5.8*.

	Δ Time to 4 misses (s)	Δ ODI (/hour)	Δ HRR (/hour)	Δ Systolic BP (mmHg)	Δ Diastolic BP (mmHg)
Δ ESS	R=0.03 p=0.83	R=0.05 p=0.71	R=0.12 p=0.42	R=0.20 p=0.18	R=0.08 p=0.58
Δ Time to 4 misses (s)	-	R=-0.08 p=0.57	R=0.00 p=0.99	R=0.02 p=0.91	R=-0.16 p=0.29

Table 5.8: Spearman's rank correlations between the changes in measures of sleepiness (both objective and subjective) and physiological parameters (overnight oximetry, heart rate rises, AHI and blood pressure) for both treatment arms combined. Δ = change.

There were no statistically significant correlations between measures of either objective or subjective sleepiness and any other parameters.

5.5. Discussion

There were no differences in the changes in sleepiness during CPAP withdrawal between the supplemental oxygen and air arms. Both objective and subjective measures of sleepiness deteriorated during CPAP withdrawal with both supplemental oxygen and air. Modifications to the OSLER test marginally improved its performance at detecting small changes in sleepiness in minimally symptomatic patients in the MOSAIC trial, and in identifying sleepiness during CPAP withdrawal. However, even after these modifications to the OSLER test, there were still no differences in objective sleepiness or vigilance scores with oxygen compared to air, during CPAP withdrawal.

The estimates of the effect of supplemental oxygen on returning daytime sleepiness were broad, both for subjective (ESS change mean difference -0.6; 95%CI -2.5 to +1.4) and objective measures (time to four misses change median 0s; IQR -741, +99s). However, supplemental oxygen tended to improve subjective sleepiness and worsen objective measures of sleepiness, making it unlikely that a small effect of supplemental oxygen on daytime sleepiness was missed.

5.5.1. Short-term effects of supplemental oxygen on sleepiness

These results are in keeping with previous findings. Loredo *et al.* (who studied the same patients as Mills *et al.* (122)), showed no difference between supplemental oxygen and placebo-CPAP in changes in the ESS over two weeks of treatment (251). However, it is worth noting that in their trial CPAP did not significantly reduce the ESS, compared to placebo-

CPAP, so it perhaps not surprising that supplemental oxygen did not reduce sleepiness either. A small study looked at the effect of oxygen on objective sleepiness in OSA as a secondary outcome (206); it showed significant reductions in the MSLT with CPAP but no change in the MSLT with supplemental oxygen compared to air, although it only contained eight patients and was probably underpowered to detect a small effect size of oxygen therapy. In addition, the design of this trial makes its results hard to interpret. It was a cross-over trial with randomisation for the order in which oxygen and air order were delivered (1st and 2nd treatment months), but CPAP was always given as the third and final treatment. Therefore, improvements with CPAP in the MSLT, compared to scores at trial entry, could represent a learning effect or regression to the mean. The largest RCT of oxygen therapy did not report sleepiness outcomes (121) thus data on the effect of oxygen therapy on sleepiness in OSA is limited. However, in the SOX trial there were no improvements in either objective or subjective sleepiness with supplemental oxygen. Thus, it is likely that supplemental oxygen has little effect on sleepiness and that other mechanisms, such as sleep fragmentation, led to daytime sleepiness in OSA, at least in the short-term.

5.5.2. Short-term effects of intermittent hypoxia on sleepiness

Experimentally inducing intermittent hypoxia is an alternative way to assess the effects of intermittent hypoxia on sleepiness, rather than the approach of abolishing intermittent hypoxia with supplemental oxygen. A small experiment, which included seven patients in a cross-over design, did not show any short-term effects of induced intermittent hypoxia on daytime sleepiness (252). Seven patients with OSA underwent PSG over six nights; two nights as baseline studies to confirm OSA diagnosis, then for two nights on CPAP and for two

nights on CPAP with induced intermittent hypoxia. The order of CPAP and CPAP with induced intermittent hypoxia was randomised, and there were three days of washout with no therapy between experimental conditions. Intermittent hypoxia was induced whilst patients were on CPAP by the intermittent addition of 100% nitrogen to the inspired gas mixture. There were improvements in objective daytime sleepiness, as measured by the MSLT, with both CPAP and CPAP with induced intermittent hypoxia. There were no significant differences between CPAP and CPAP with induced intermittent hypoxia in improvements in sleep latency. There was a marked increase in central apnoeic events and desaturations with induced intermittent hypoxia, but no change in sleepiness despite central events often being associated with arousal. Therefore, intermittent hypoxia does not seem to lead to increases in daytime sleepiness in the short-term of up to two weeks. The finding of central events that caused arousal in the induced intermittent hypoxia group without sleepiness demonstrates that other mechanisms are likely to be involved other than arousals.

5.5.3. Potential longer-term effects of intermittent hypoxia on sleepiness

However, others have argued that longer-term intermittent hypoxia may have very different effects (253), and that these effects may even be irreversible (249). The presence of "post-CPAP sleepiness" may be a consequence of irreversible damage to the wake-sleep centres of the brain (249), although some remaining abnormal sleepiness in a CPAP population may simply reflect a high tail within the natural variation in daytime sleepiness found amongst the normal population (250). Animal intermittent hypoxia experiments have shown: oxidative injury to wake-sleep promoting brain centres that do not fully reverse after

restoration of normoxia (248), oxidative injury linked to neurodegenerative conditions (254), and increases in sleep promoting prostaglandins (255).

An MRI study of patients with OSA showed widespread non-Gaussian diffusion changes, when compared with control subjects (256). Wake-sleep centres were affected by MRI changes and the authors suggest that changes represent an acute tissue injury, which could relate to intermittent hypoxia. This study did not perform sleep studies on control subjects, and OSA subjects had higher mean BMI, systolic and diastolic blood pressure. Therefore, it is possible that these effects are related to elevations in blood pressure or BMI, and sleep disordered breathing may not be responsible for their observations.

Whilst, it is clear that intermittent hypoxia does not seem to have effects on daytime sleepiness in the short-term, it may play a role in the longer-term via oxidative injury in wake-sleep centres of the brain but at present the data are weak.

5.5.4. Modifications to the OSLER test

Modifications to the OSLER test showed only marginal improvements in its performance to detect change. Introduction of an error index, or an analysis of the distribution in the errors, has been suggested to measure vigilance as well as sleepiness (191)(192)(193). Alakuijala *et al.* (192), carried out three OSLER tests and one multiple unprepared reaction time (MURT) test, in 192 patients referred for investigation of possible OSA. The MURT test involves responding to an auditory or visual stimulus as quickly as possible, with approximately 100

stimuli occurring over 10 minutes, at 1 to 10 second intervals. The OSLER test was repeated three times at approximately 9am, 11am and 1pm. There was a moderate correlation between MURT and an OSLER test derived error index ($r=0.512$, $p<0.01$) and between MURT and OSLER sleep latency ($r=0.436$, $p<0.01$). These correlations were only moderate, and there was only a slight increase in the correlation when using the error index compared to the original OSLER sleep latency. Mazza *et al.* (193), carried out OSLER tests at 9am, 11am and 1:30pm in 27 patients with OSAS and 20 age matched non-snoring control subjects. Patients with OSAS had shorter sleep latency and an increased percentage of errors when compared to control subjects. Interestingly, whilst sleep latency was significantly different at all three time points, the percentage of errors was only significantly different at 9am and 11am. At 1:30pm the patients with OSAS had a similar error rate percentage to the control subjects. Whilst introduction of an error index may help to identify impaired vigilance, improvements are marginal over the original OSLER test, and still do not closely match the MURT.

Priest *et al.* showed in ten healthy volunteers that whilst single errors were common, four or more missed lights on the OSLER test almost always corresponded to an EEG-defined micro-sleep (191). In the supplemental oxygen trial, when comparing baseline to follow-up in both arms combined, and when comparing CPAP to control in the MOSAIC trial, there were marginal improvements in the OSLER test's ability to detect change when looking at the time to four missed lights, but not when looking at the error index. However, even when considering the best performing measure of change, the time to four missed lights, there were no differences in the change from baseline to follow-up, oxygen versus air, in the

supplemental oxygen trial. Whilst, considering the time to four missed lights in addition to the original OSLER test sleep latency may marginally improve its performance, I was still unable to demonstrate the possibility that short-term intermittent hypoxia might be important in causing sleepiness. Future research trial should consider using the time to four missed lights as a more sensitive discriminant of objective sleepiness than the original OSLER test.

5.5.5. Implications for future trials

When considering future trials looking at the effects of supplemental oxygen on blood pressure it is important to consider its lack of effect on sleepiness. As there seems to be no effect of supplemental oxygen on sleepiness, the most sleepy patients would be likely to be exposed to significant risk (7)(245)(246)(247). Similarly to the entry criteria for the SAVE trial, which assessed the role of CPAP in secondary prevention of cardiovascular disease in OSA, the most sleepy patients (those with an ESS >15) probably should not be considered (49). A concern with this approach would be that this might exclude those most likely to benefit from therapy (9). However, the SAVE trial also excluded the most hypoxic patients (those with oxygen saturations <80% for >10% of the night), and showed no improvement in blood pressure with CPAP treatment. Hypoxia is thought to be a key determinant of cardiovascular risk in OSA (10), and so those with the most severe hypoxia potentially may benefit the most from intervention. Patients with resistant hypertension show a more marked blood pressure benefit from CPAP therapy (70). It would therefore seem sensible to consider patients with resistant hypertension, with severe hypoxia, but without sleepiness, for any future trials of supplemental oxygen therapy in OSA (see section 7.6).

5.5.6. Conclusions (Chapter Five)

Supplemental oxygen therapy during CPAP withdrawal does not affect the return of either objective or subjective sleepiness, compared to air. Whilst modifications to the OSLER test marginally improved its performance at measuring change in sleepiness, there remained no significant differences in objective sleepiness in the supplemental oxygen trial, oxygen versus air. This probably suggests that other mechanisms, such as sleep fragmentation from recurrent arousals, and not intermittent hypoxia are responsible for daytime sleepiness in OSA, at least in the short-term. However, there is little data on the longer-term effects of intermittent hypoxia on sleepiness. Given its lack of effect on sleepiness, future trials of oxygen therapy in OSA probably should probably be restricted to non-sleepy patients with OSA.

6. Chapter Six: Biomarkers in other CPAP withdrawal trials

6.1. Abstract (Chapter Six)

The supplemental oxygen trial shows the importance of intermittent hypoxia in determining morning blood pressure rises in OSA. However, the underlying molecular mediators of this are not clear but might involve pathways mediated by hypoxia-inducible factor. Furthermore, other mechanisms, including endothelial dysfunction, adipose tissue/hypoxia mediated inflammation, and oxidative stress, are thought to contribute to the pathogenesis of cardiovascular disease in OSA. Utilising blood samples from three previous trials and overnight urine samples from one previous trial I explored blood and urine markers of these processes. These included proteins regulated by the hypoxia-inducible factor pathway (adrenomedullin, endothelin-1 and vascular endothelial growth factor), endothelial derived proteins (adrenomedullin, endocan, endothelin-1), a marker of adipose tissue/hypoxia mediated inflammation (resistin), and a marker of systemic oxidative stress (overnight urinary F2-isoprostanes). Patients in these trials were randomised to either two weeks of sham CPAP (CPAP withdrawal) or two weeks of continued CPAP (acting as a control). All markers were analysed at baseline and after two weeks of either sham CPAP or continued CPAP. Unexpectedly, CPAP withdrawal led to a small but significant reduction in the vasodilator adrenomedullin. The reduction in adrenomedullin was unexpected as previous observational studies have shown increased adrenomedullin levels in patients with OSA. This small reduction in adrenomedullin may contribute to increased daytime blood pressure during CPAP withdrawal. There were no other significant changes in any other markers suggesting that OSA does not cause systemic increases in these other pathways, at least over a period of two weeks.

6.2. Introduction

OSA is associated with significant comorbidities, particularly arterial hypertension (68)(69), and cardiovascular disease (9). The underlying mechanisms for the association of cardiovascular disease with OSA are not fully understood. There is a clear association between OSA and endothelial dysfunction (148)(149), and I have presented data supporting the importance of chronic intermittent hypoxia in elevating daytime blood pressure (Chapters 3 and 4). The relative importance of oxidative stress, inflammation, and the molecular pathways involved in the development of cardiovascular disease remain unclear (167)(257).

6.2.1. Hypoxia-inducible factor pathway proteins

Blood markers of cardiovascular disease have previously been explored in OSA, but much of this data is observational. Cohort studies have explored markers induced by hypoxia, via the hypoxia-inducible factor (HIF) pathway, and have identified differences between patients with OSA and control subjects. Adrenomedullin (ADM) is a short peptide that is found almost ubiquitously in human tissues. Hypoxia, amongst other signals, increases ADM levels. Endothelial cells produce ADM at high levels and therefore its potent vasodilatory action is thought to be its most important function (258). Cross-sectional cohort studies have shown elevations of ADM in patients with OSA compared to matched control subjects (259)(260). The role of increased ADM levels found in OSA is controversial. Increases in ADM levels are proposed to represent a compensatory mechanism protective against cardiovascular disease in OSA (259), as it is a potent vasodilator and increases should reduce blood pressure.

Alternatively, ADM might induce cell surface expression of adhesion molecules on endothelial cells, increasing the risk of cardiovascular disease (260).

Endothelin-1 (ET-1) is a peptide hormone and was first discovered as an endothelium-derived vasoconstrictor, although other functions exist. The HIF pathway activates ET-1 production (261). Increased ET-1 levels have been found in patients with OSA compared to matched control subjects (262). Increases in ET-1, triggered by intermittent hypoxia and causing vasoconstriction, may promote hypertension. In addition ET-1 levels are inversely related with flow-mediated dilatation which is a standard way of assessing endothelial function (263), so increases in ET-1 may be a useful biomarker of endothelial dysfunction in OSA, as well as potentially being a marker of HIF activation.

Vascular endothelial growth factors (VEGF) are a family of glycoproteins that act as angiogenesis stimulators. There are a number of mechanisms by which OSA could affect VEGF expression including the HIF pathway, circadian rhythm genes, and inflammation (264). Reports of the effect of OSA on VEGF are varied. In case control studies OSA has been reported to increase VEGF levels (265), whereas other authors report no differences in VEGF levels between patients with OSA and control subjects (266)(267). In patients with OSA no correlation could be found between the severity of OSA and VEGF levels (268).

6.2.2. Endothelial function and endocan

Endothelial function describes the role of the vascular endothelium in regulating blood flow. Impaired endothelial function occurs in both hypertension (139), and in patients with coronary artery atherosclerosis (140), therefore it is thought to represent an early stage in the development of cardiovascular disease. Endothelial function is commonly assessed by measuring flow-mediated dilatation at the brachial artery (141), and this non-invasive measurement is used as a surrogate marker of endothelial function elsewhere, such as in the coronary arteries. OSA is associated with impaired endothelial function (148)(149). Blood biomarkers of endothelial dysfunction would be helpful, as measuring flow mediated dilatation is time consuming and requires on a skilled operator. Endocan, which is an endothelial derived protein, is a potential biomarker of endothelial dysfunction. Increased levels of endocan have been reported in OSA (269), and are inversely correlated with endothelial function as measured by flow mediated dilation in patients with OSA (270).

6.2.3. Adipose tissue mediated inflammation and resistin

Adipose tissue exposed to intermittent hypoxia has been proposed to have a "pro-inflammatory" role in OSA (151). However, a randomised control trial of CPAP did not show reductions in either hsCRP or adiponectin, which is a hormone linked to the "pro-inflammatory" adipose tissue hypothesis (153). Resistin, an adipose derived cytokine, has also been proposed as an important marker in adipose tissue mediated inflammation. An observational study showed that patients with OSA have increased resistin levels, when compared with matched control subjects (271). The authors propose that resistin possibly

mediates systemic inflammatory processes in OSA, which might be of importance in the development of cardiovascular disease in OSA.

6.2.4. Oxidative stress, urinary isoprostanes and urinary dilution

Oxidative stress is thought to be of importance in the development of cardiovascular disease in OSA (257). Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and antioxidant mechanisms. Intermittent hypoxia is thought to lead to oxidative stress by decreasing antioxidant mechanisms in periods of hypoxia and increasing reactive oxygen species production during periods of reoxygenation; termed an ischaemia-reperfusion injury (123). Animal experiments have shown tissue specific increases in oxidative stress following intermittent hypoxia (128)(129)(130). Prabhakar and colleagues suggest a central role of oxidative stress in the carotid chemoreceptors, leading to hypertension (136). However, a previous CPAP withdrawal experiment using data from two centres (including the Oxford Sleep Unit), showed that systemic markers of oxidative stress were not increased by the return of OSA (135). Unexpectedly, there were decreased levels of urinary isoprostanes in early morning urine specimens and this, combined with increased levels of blood superoxide dismutase (which reduces oxidative stress), raised the possibility that compensatory changes might actually decrease oxidative stress in OSA. However, concerns over this paper's methodology were raised by Monneret and Bonnefont-Rousselot (272). First, spot morning urine samples were taken later in the morning and might not reflect any overnight changes in urinary isoprostanes. Although isoprostanes are a relatively stable biomarker of oxidative stress (273), their half-life is short in blood (minutes), but

longer in urine (hours) (274). Second, as OSA is known to cause overnight diuresis, using uncorrected urinary isoprostane levels may have influenced the results.

6.2.5. Aims

I hypothesised that return of OSA during CPAP withdrawal would adversely change blood markers of cardiovascular disease. Therefore, I aimed to explore the effects of OSA in a CPAP withdrawal model on the following blood markers of cardiovascular disease; hypoxic regulated proteins (ADM, ET-1, and VEGF), on endothelium-derived proteins (ADM, endocan and ET-1), and on an adipose tissue derived protein (resistin). Second, I hypothesised that that urinary isoprostanes might have been increased overnight, and the reductions observed in early morning spot urines reflecting a secondary compensatory fall. I also explored the possibility that urinary dilution might indeed have influenced the change in urinary isoprostanes previously observed by reanalysing following corrections for urinary creatinine and urinary volume.

6.3. Methods

6.3.1. Protein biomarkers of cardiovascular disease in CPAP withdrawal (6.4.1)

This analysis used samples and data from three trials and detailed methodology is described in section 2.4. All three trials randomised patients with known OSA, established on and compliant with CPAP therapy, to either sham CPAP (CPAP withdrawal) or continued CPAP (acting as a control) for 14 days.

The main outcomes are the change in blood cardiovascular marker levels (ADM, endocan, ET-1, resistin, and VEGF) and EPO levels (as a control that is known to be hypoxically regulated). Changes from baseline to follow-up were compared, sham CPAP versus continued CPAP. Blood samples were collected from participants at baseline and at 14-day follow-up appointments. Blood samples were collected before 10am, spun, and stored at -80^{O}C for later analysis. Samples were then analysed using commercially available enzyme-linked immunosorbent assay (ELISA) kits: ADM (Caltag Medisystems #E-EL-H0275), ET-1 (R&D-Systems #QET00B), endocan (Aviscera #SK00318-01), EPO (R&D Systems #DEP00), resistin (BioVendor #RD191016100) and VEGF (R&D Systems #DVE00). EDTA plasma was used for all tests except for VEGF, which was performed on serum. Samples were measured in duplicate, except for VEGF which was measured in triplicate.

Treatment effects (sham CPAP versus continued CPAP) on follow-up blood protein levels were modelled using linear regression, controlling for baseline blood protein level, age, gender, BMI, smoking status, recruiting centre and comorbidities.

6.3.2. Urinary isoprostanes in CPAP withdrawal (6.4.2)

Detailed methodology can be found in section 2.5. Overnight urinary samples were available from patients from one of the two centres involved in the original oxidative stress biomarker study (135). Overnight urine samples were analysed from all 14 patients included in the original study from this site, and from 9 further patients from this site. Patients were

randomised to either sham CPAP (CPAP-withdrawal) for two weeks or continued CPAP (acting as a control) for two weeks.

Patients completed overnight urine collections on the night prior to their baseline visit and on the final night of their treatment period, prior to their two week follow up. Non-acidified urine was collected. The start and end times for collection, along with the total urine volume was recorded by the patients in a study diary. Aliquots of urine were then stored at -80^{O}C for later use. Urinary F2-isoprostanes were measured by ELISA technique (Abcam, Cambridge, UK, Cat# ab175819) and urine creatinine was measured from the same samples.

Overnight urinary F2-isoprostane levels are reported as uncorrected concentrations (ng/ml) or corrected values. Corrected values were either: creatinine corrected (ng/µmol) - the F2-isoprostanes concentration (ng/ml) divided by the urinary creatinine concentration (mmol/l); or the rate corrected (ng/h) - urinary F2-isoprostanes concentration (ng/ml) multiplied by the overnight volume of urine produced (ml) divided by the hours of urine collection (h).

The treatment effect of CPAP withdrawal versus control on two week urinary isoprostanes levels was modelled using multivariable linear regression, adjusting for the baseline urinary isoprostanes levels, age, gender, BMI, smoking status, antihypertensive usage, and statin usage. Data are expressed as mean ± standard deviation, median (1st quartile, 3rd quartile) or number (%).

6.4. Results

6.4.1. Protein biomarkers of cardiovascular disease in CPAP withdrawal

One-hundred and nine patients were included in this study, 86 from Zurich and 23 from Oxford. Fifty-five patients were randomised to sham CPAP (CPAP withdrawal) and 54 patients to continued CPAP. The sham CPAP group had slightly more severe OSA (both at the time of original diagnosis and during the pre-trial screening), were more commonly on treatment for pre-existing hypertension, and included more ex-smokers (see *Table 6.1*). Analyses were adjusted for these baseline differences.

	Sham CPAP (n=55)	Continued CPAP (n=54)
Mean ± SD Age (years)	63.5 ± 7.9	62.2 ± 7.9
Number male gender (%)	49 (89%)	48 (89%)
Mean ± SD BMI (kg/m^2)	34.2 ± 6.0	33.8 ± 6.2
Median (IQR) ODI at diagnosis (/h)	46.2 ± 22.2	41.9 ± 19.1
Median (IQR) ODI during pre-trial screening (/h)	31.5 (20.4, 45.4)	26.7 (20.9, 34.2)
Number with coronary artery disease (%)	5 (9%)	7 (13%)
Number with hypertension (%)	39 (71%)	30 (56%)
Number with diabetes (%)	10 (18%)	11 (20%)
Number (%): *Current smokers* *Ex-smokers* *Never smokers*	 8 (15%) 24 (44%) 23 (42%)	 8 (15%) 17 (32%) 29 (54%)

Table 6.1: Baseline characteristics for patients in the blood markers of cardiovascular disease study.

As shown previously, there was a significant effect of CPAP withdrawal on ODI of +36.0 (p<0.001; 95%CI +29.9 to +42.2) and on ESS of +3.9 (p<0.001; 95%CI +2.7 to +5.0) at 14 days, versus continued CPAP.

There was a significant effect of CPAP withdrawal on ADM levels of -26.0 pg/ml (p=0.02, 95%CI -4.3 to -47.8) at 14 days, versus continued CPAP. No other protein marker significantly changed at 14 days in the sham CPAP group versus continued CPAP (*Table 6.2*).

	Adjusted treatment effect	95% CI	p
ADM (pg/ml)	-26.0	-47.8 to -4.3	**0.02**
Endocan (ng/ml)	0.1	-0.7 to +0.8	0.87
EPO (mIU/ml)	-1.3	-2.8 to +0.3	0.12
ET-1 (pg/ml)	-0.2	-0.5 to +0.0	0.09
Resistin (ng/ml)	2.1	-2.8 to +7.1	0.39
VEGF (pg/ml)	26.0	-64.1 to +116.2	0.57

Table 6.2: Treatment effect of sham CPAP versus continued CPAP on levels of blood markers of cardiovascular disease.

The treatment effect for ADM levels comprised of both a decrease in the sham CPAP group (mean difference -14.2 pg.ml, 95%CI -31.2 to +2.9, p=0.10) and an increase in the continued

CPAP group (mean difference +18.3 pg/ml, 95%CI +0.65 to +35.9, p=0.04) (*Figure 6.1 & Table 6.3).*

	Sham				*CPAP*			
	Median baseline (IQR)	Median follow-up (IQR)	Mean difference (95% CI)	p	Median baseline (IQR)	Median follow-up (IQR)	Mean difference (95% CI)	p
ADM (pg/ml)	146.0 (99.5, 189.6)	134.2 (79.0, 206.8)	-14.2 (-31.2 to +2.9)	0.10	154.7 (82.4, 237.7)	173.1 (105.2, 247.0)	+18.3 (+0.65 to +35.9)	**0.04**
Endocan (ng/ml)	0.0 (0.0, 0.2)	0.0 (0.0, 0.2)	+0.02 (-0.09 to +0.14)	0.70	0.0 (0.0, 1.2)	0.0 (0.0, 1.7)	-0.43 (-0.15 to +0.28)	0.23
EPO (mIU/ml)	10.9 (8.9, 16.2)	10.9 (8.4, 15.5)	+0.21 (-1.1 to +1.5)	0.74	11.6 (9.3, 14.2)	12.5 (10.3, 16.2)	+1.47 (+0.54 to +2.39)	0.003
ET-1 (pg/ml)	2.1 (1.7, 2.5)	2.0 (1.6, 2.4)	+0.00 (-0.18 to +0.19)	0.98	1.8 (1.4, 2.5)	2.0 (1.7, 2.6)	+0.19 (+0.04 to +0.35)	**0.01**
Resistin (ng/ml)	4.4 (3.8, 5.4)	4.4 (3.5, 5.1)	+2.2 (-2.7 to +7.0)	0.38	4.6 (3.9, 5.3)	4.7 (3.7, 5.4)	-0.03 (-0.27 to +0.20	0.78
VEGF (pg/ml)	304.0 (155.9, 462.5)	299.1 (192.9, 407.0)	-28.8 (-125.3 to +67.7)	0.55	259.2 (171.1, 444.8)	205.7 (128.2, 384.8)	-55.4 (-99.4 to -11.4)	**0.02**

Table 6.3: Baseline, follow-up and mean differences in blood marker levels in sham CPAP and continued CPAP groups. Paired t-tests were used to compare mean changes in each arm from baseline to follow-up.

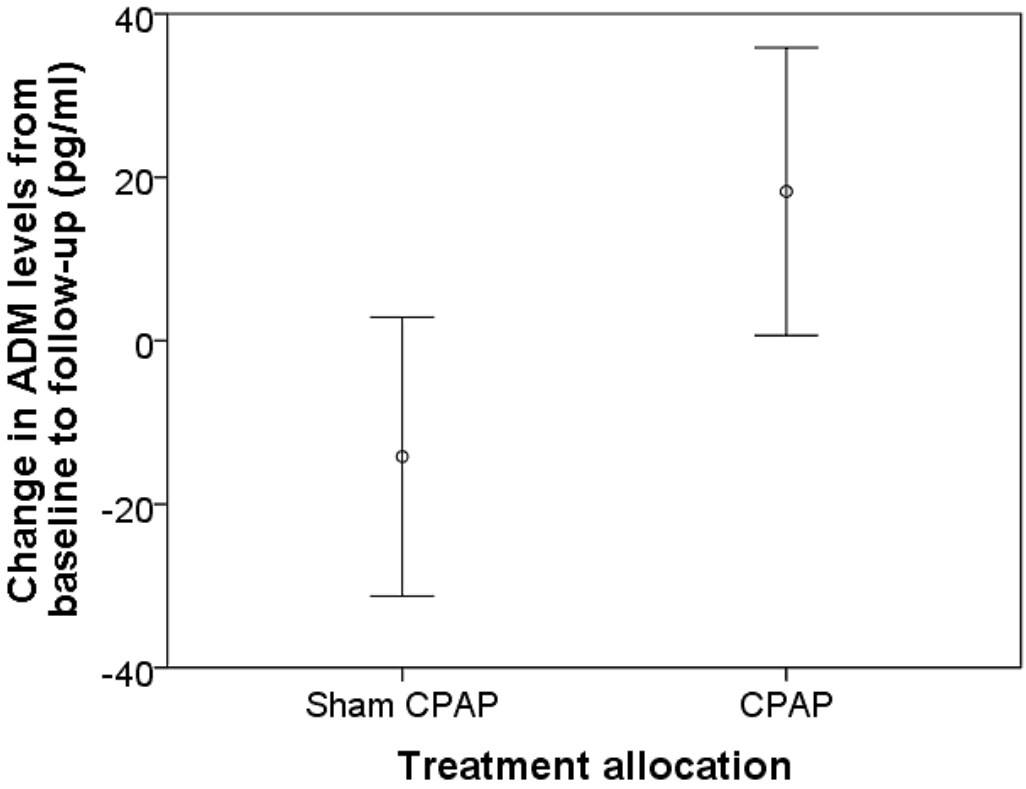


Figure 6.1: Change in adrenomedullin (ADM) levels from baseline to follow-up by treatment allocation. Circles represent the mean change and bars represent the 95% confidence intervals.

Over half of all patients' endocan levels were undetectable. As a sensitivity analysis the treatment effect of CPAP withdrawal was assessed only in those patients with detectable endocan levels at baseline. 14 patients randomised to sham CPAP with median (IQR) levels of 2.1ng/ml (0.2, 5.4) and 19 patients randomised to continued CPAP with median (IQR) levels of 3.3ng/ml (0.5, 33.5) had detectable endocan levels at baseline. There remained no significant effect of CPAP treatment on endocan levels (treatment effect +0.8ng/ml, 95%CI -2.2, +3.7, p=0.60).

6.4.2. Urinary isoprostanes in CPAP withdrawal

Twenty-three patients from Oxford completed the trial with 11 randomised to continued CPAP and 12 randomised to sham-CPAP (CPAP-withdrawal). Baseline characteristics are shown in *Table 6.4*. Baseline characteristic in both groups were comparable.

	Sham-CPAP (n=12)	Continued CPAP (n=11)
Mean ± SD age (years)	61.1 ± 8.9	59.9 ± 6.7
Number male gender (%)	10 (83%)	10 (91%)
Mean ± SD BMI (kg/m^2)	36.9 ± 7.5	36.8 ± 7.4
Median (IQR) ODI during pre-trial screening (/h)	34.4 (25.8, 64.6)	35.2 (23.7, 53.3)
Number on anti-hypertensives (%)	7 (58%)	5 (46%)
Number on statins (%)	7 (58%)	6 (55%)
Number current smokers (%)	1 (8%)	1 (9%)

Table 6.4: Baseline characteristics for patients in the overnight analysis of urinary isoprostanes study.

Whilst there was a significant effect of CPAP-withdrawal on uncorrected overnight urinary F2-isoprostanes, there was however, no significant effect of CPAP withdrawal on corrected F2-isoprostanes, either when correcting by creatinine, or by rate of urine production (*Table 6.5*). There were non-significant increases in overnight urine production and reductions in urinary creatinine, as has previously been described in Chapter 4 from the SOX trial.

	CPAP (n=11)		CPAP-withdrawal (n=12)			
	Baseline mean ± SD	Follow-up mean ± SD	Baseline mean ± SD	Follow-up mean ± SD	Treatment effect (95% CI)	p
Uncorrected F2-isoprostanes (ng/ml)	3.1 ± 1.3	3.4 ± 1.4	3.2 ± 1.4	2.2 ± 0.8	-1.3 (-2.4 to -0.1)	**0.03**
Creatinine (mmol/l)	13.8 ± 10.5	13.8 ± 10.6	11.3 ± 4.3	8.4 ± 2.9	-3.0 (-8.0 to +1.9)	0.21
Urine volume (ml)	814 ± 497	889 ± 569	699 ± 312	988 ± 335	+205 (-88 to +499)	0.16
Urinary excretion rate (ml/h)	78.1 ± 43.1	83.9 ± 53.2	57.0 ± 31.1	86.7 ± 28.5	+18.9 (-12.3 to +50.1)	0.22
Corrected F2-isoprostanes/ creatinine ratio (ng/µmol)	0.31 ± 0.18	0.37 ± 0.19	0.29 ± 0.11	0.27 ± 0.07	-0.10 (-0.23 to +0.03)	0.11
F2-isopostanes production(ng/h) corrected by urine excretion rate	216.6 ± 120.7	251.7 ± 145.8	168.2 ± 116.3	182.8 ± 78.7	-56.9 (-161.0 to +47.2)	0.26

Table 6.5: Overnight urinary F2-isoprostanes, uncorrected and corrected.

6.5. Discussion

The return of OSA during CPAP withdrawal for 14 days was not associated with changes in several hypoxically regulated proteins, protein markers of endothelial function, or in resistin, an adipocyte derived protein. There was an unexpected reduction in adrenomedullin levels, an endothelial-derived vasodilator, associated with CPAP withdrawal. These results differ from models of sustained hypoxia, other models of intermittent hypoxia, and previous cohort studies of patients with OSA. In contrast to a previous study showing a reduction in morning urinary isoprostane levels, there was no change in corrected overnight isoprostane

levels with CPAP withdrawal, suggesting that isoprostane levels, in this situation of changing urine production, should be corrected for either urine volume or urinary creatinine concentration.

6.5.1. Proteins induced by intermittent hypoxia

ADM, ET-1, and VEGF are activated via HIF during hypoxic conditions and have been proposed to play a role in cardiovascular disease in OSA. ADM levels have previously been reported to be increased in untreated patients with OSA compared with patients without OSA (259)(260). This increase in ADM has been proposed to either have a protective role through compensatory vasodilation (259), or a pathological role through inducing endothelial cell surface adherence markers (260). CPAP treatment is associated with reductions in ADM levels in patients with OSA (259)(260). On the contrary, I found a reduction in ADM levels in patients following CPAP withdrawal. This suggests that the extent of intermittent hypoxia seen in OSA may not lead to increases in ADM and that other mechanisms lead to a reduction in ADM levels. Reductions in levels of the vasodilator ADM could have a deleterious effect by contributing to hypertension.

It is important to mention differences in study design between previous cohort studies and my observations. Previous cross-sectional studies found differences in ADM levels between patients with OSA and matched control subjects, and went on to show improvements in ADM levels in the patients with OSA treated with CPAP (259)(260). Whilst there were modest reductions in ADM at two nights, there were more marked reductions following

long-term CPAP. However, reductions with long-term CPAP were only reported in a subgroup of patients, presumably those compliant with CPAP therapy. This introduces a significant bias into these findings. Blood tests were taken earlier in the morning in the studies by Schulz *et al.* and Teramoto *et al.*, between 730-830 AM (259)(260), whereas I measured levels before 10 AM. However, in these previous studies ADM levels were measured after laboratory polysomnography at baseline, but while sleeping at home after two nights of CPAP (259), which potentially introduces bias into these findings. Therefore, given the risk of bias in previous potential studies, my findings question previous conclusions that ADM levels are increased by intermittent hypoxia in OSA.

Increased VEGF levels have been proposed to promote vascular injury in OSA in a previous study (265). In this previous study, patients with OSA and age matched control subjects all underwent sleep studies. It is not clear whether control subjects were recruited from the sleep clinic or from the wider population. Increases in VEGF levels and hsCRP levels were found in patients with OSA, and when endothelial cells were cultured in serum from these patients with OSA, endothelial healing was impaired.

However, the effect of OSA on VEGF levels is not clear with VEGF levels being reported to be: increased in OSA (265), increased only in patients with both OSA and hypertension (266), increased with CPAP therapy (275), comparable in patients with OSA and control subjects (267), and unrelated to the severity of OSA (268). My results showing no differences in VEGF levels are comparable to a previous CPAP withdrawal trial (276). My study differs from this

previous CPAP withdrawal trial by including a control arm of continued CPAP and withdrawing CPAP for 14 days instead of 7 days. Therefore, it seems that OSA does not cause increases in VEGF levels, at least in the short-term.

ET-1 levels have been reported to be increased in patients with OSA and these increases were linked with hypertension in a previous study (262). This previous study carried out split night sleep studies, measuring ET-1 at baseline, at 2 AM after 4 hours of sleep without CPAP, and at 7am after 4 hours of sleep with CPAP. Control subjects were younger, less obese, included a greater percentage of females, and had not undergone sleep studies to exclude OSA. These factors may well have influenced their findings. Other studies have found only the precursor to endothelin-1 to be elevated in OSA (277), and obesity rather than OSA was found to be the cause for elevated ET-1 levels in patients with predominately moderate OSA (278). My results suggest that short-term return of OSA is not associated with changes in ET-1 levels in patients with predominately severe OSA.

EPO is upregulated by sustained hypoxia (279)(280). My results suggest that the intermittent hypoxia seen in OSA during CPAP withdrawal is insufficient to lead to changes in EPO levels seen in sustained hypoxia. This is comparable to some previous reports which have not found differences in EPO or haemoglobin levels in patients with OSA compared with control subjects (281)(282). However, increased EPO levels have been reported in a small cohort of patients with very severe OSA compared to control subjects who were not matched for BMI or comorbidities (283). Reductions in EPO levels have been reported in a

small cohort of severe patients with OSA treated with CPAP (284), although such patients are not generally polycythaemic (282). These findings were not replicated in my results, in patients with predominately severe OSA. This suggests that more sustained hypoxic periods are needed to induce EPO, even in patients with severe OSA.

6.5.2. Endocan as marker of endothelial dysfunction

Endothelial dysfunction is thought to be an early and important step in the development of cardiovascular disease (285). Reduced endothelial function has been shown to be associated with OSA; endothelial function improves with CPAP therapy (149), and worsens with withdrawal of CPAP (148). Endocan is an endothelial-derived protein and increased levels have been proposed as markers of endothelial dysfunction and cardiovascular disease (286). In observational studies, endocan levels have been reported to be increased in patients with OSA compared to healthy control subjects (269)(270), and endocan levels were inversely correlated with endothelial function and positively correlated carotid intimal thickness. Altintas *et al.* carried out a case-control study with 40 patients with OSA and 40 matched control subjects (270). Control subjects were recruited from referrals to the sleep clinic but all had an AHI < 5/h. Control subjects were slightly younger and slightly less obese than patients with OSA but were reasonably well matched. Patients with OSA had elevated endocan levels at baseline and these were correlated with reduced flow-mediated dilatation. Whilst CPAP reduced endocan levels, endocan levels were not repeated in control subjects, meaning this comparison is uncontrolled. Changes in endocan levels could represent other factors such as changes in diet/lifestyle following patients with OSA's initial clinic assessment. I did not find differences in endocan levels during CPAP withdrawal, despite

Kohler *et al.* showing significant endothelial dysfunction following CPAP withdrawal (148). Although a significant proportion of patients had undetectable endocan levels, a sensitivity analysis looking at just those with detectable baseline levels did not change this finding. The technique I used was a similar sandwich based ELISA technique to that used by others (270). My results suggest that endocan is not necessarily useful as a marker of endothelial dysfunction in OSA. However, more sensitive endocan assays are now becoming available and further work is needed to determine if differences in endocan levels can be detected with these newer assays in patients with OSA.

6.5.3. Resistin as a marker of adipose and hypoxia mediated inflammation

Resistin, an adipose derived cytokine, has been suggested as an important link between OSA, obesity and inflammation (271). Yamamoto *et al.* carried out an observational study with 31 male patients with OSA and 10 matched male control subjects. Patients with OSA had increased resistin levels, and resistin levels were more markedly increased in those with severe OSA. The degree of hypoxaemia and IL-6 levels were independent predictors of resistin levels. However, this link is not certain, and others reported no difference in resistin levels in patients with OSA (287), or found a reduction in resistin levels in patients with OSA (288). Reductions in resistin levels have been reported with CPAP treatment (271), but others have reported no change in resistin levels with CPAP therapy (289)(290). My results show that resistin levels are not changed during return of OSA seen following 14 days of CPAP withdrawal. It is possible that a longer time period is required for changes in resistin levels to occur but the short-term return of intermittent hypoxia in CPAP withdrawal does not increase resistin levels.

6.5.4. Overnight urinary isoprostanes as a marker of oxidative stress

The return of OSA with CPAP-withdrawal does not lead to an increase in oxidative stress as measured by corrected levels of overnight urinary isoprostanes. Using overnight urine samples corrected for urine production/dilution, in this smaller subgroup of patients, the previous finding of decreased urinary F2-isoprostanes levels in early morning spot urine samples following CPAP withdrawal was not reproduced (135). This previous CPAP withdrawal study specifically did not correct for urinary creatinine, as it used early morning spot urine samples, when correction for urinary creatinine could have introduced other sources of variability. Uncorrected isoprostane levels are commonly reported for this reason and no consensus for reporting has been established (291).

Whilst urinary volumes were increased, and urinary creatinine levels were decreased, there was significant variability in these values and this result was not significant. OSA causes increased atrial natriuretic peptide (230)(231), and decreased anti-diuretic hormone production leading to increased urinary production, and urinary dilution have been shown by some (231). Therefore in future OSA trials, to allow for any effect of urinary dilution, it would seem sensible to collect overnight urine samples and correct for urine creatinine or rate of urine production.

The previous CPAP withdrawal trial assessing key biomarkers of oxidative stress found no changes (malondialdehyde, lipid hydroperoxides, total antioxidant capacity) (135), and my

results show no changes in overnight corrected isoprostanes. A recent study similarly found no changes in overnight creatinine-corrected urinary F2-isoprostanes during CPAP treatment, compared with sham CPAP (134). The previous CPAP withdrawal trial did find increased superoxide dismutase levels (135). Any increase in tissue oxygen generation appears to be eliminated by the increased superoxide dismutase via a "pre-conditioning-type effect", which may explain why systemic markers of oxidative stress do not change. Hypoxic preconditioning has been proposed to explain the lower peak troponin levels observed in acute coronary syndrome in patients with OSA, suggesting a potential protective effective of OSA (292)(293). Therefore, other mechanisms may be responsible for the development of cardiovascular disease in OSA. However, a novel breath analysis technique using mass-spectrometry showed changes in a family of aldehydes during CPAP withdrawal (133). Authors report that this family of aldehydes could relate to increased oxidative stress. This suggests that there may be increases in oxidative stress, but traditional biomarkers of systemic oxidative stress are not sensitive to these increases. Indeed, systemic biomarkers have been shown to be poorly correlated with oxidative stress in coronary artery bypass graft samples (138). These results suggest that either oxidative stress is not increased overnight by OSA or that increases in superoxide dismutase more than compensates for any increased tissue oxygen generation.

6.5.5. Limitations

These studies have some important limitations to consider. The blood protein marker data are from three trials with primary outcomes published elsewhere, and the overnight urinary isoprostanes are from a previously published trial. As none of these data were nominated as

primary outcomes, all results are therefore exploratory and hypothesis-generating. Whilst patients included were from different trials, they had similar inclusion criteria and the treatment effect was adjusted for recruitment site. Patients prepared to undergo CPAP withdrawal are a selected population, as during pre-trial screening a significant proportion either did not have sufficient return of OSA, or were unable to continue due to being unable to tolerate the return of symptoms following being off CPAP. The inclusion of patients with presumably less symptomatic and mild-to-moderate OSA may mean that treatment effects observable only in severe OSA may have been missed in the blood biomarkers study. However, almost half of the patients (52% in the sham CPAP group and 43% in the continued CPAP group) had severe OSA by conventional definitions (ODI>30/h).

6.6. Conclusions (Chapter Six)

There were unexpected small reductions in adrenomedullin during CPAP withdrawal. Reductions in adrenomedullin, which is a potent vasodilator, could contribute to the increased morning blood pressure seen during CPAP withdrawal. No other blood protein markers significantly changed, suggesting little or no acute effects on the cardiovascular system from OSA. Alternatively, these may not be the right assays or biomarkers to detect any effect that might exist. The assays used were reliable and repeatable. There were concerns over the endocan assay with low detection levels and newer assays for this biomarker are reportedly more sensitive. Overnight corrected urinary F2-isoprostanes were unchanged by CPAP withdrawal suggesting that systemic oxidative stress is not changed. End-organ oxidative stress could still occur in OSA, but it seems that this does not cause increases in markers of oxidative stress. Previous studies have shown increases in the

anti-oxidant superoxide dismutase, which protect against oxidative stress. Much of the previous evidence to support the markers reviewed in this chapter comes from observational cohort studies. CPAP withdrawal provides a more robust and powerful model to examine the underlying molecular pathways involved in cardiovascular disease in OSA.

7. Discussion

7.1. Abstract

The results of this thesis highlight the importance of intermittent hypoxia in elevated morning blood pressure in OSA. Supplemental oxygen during CPAP withdrawal had minimal effect on the AHI or arousals, and no effect on the return of daytime sleepiness, suggesting that the supplemental oxygen had the relatively pure effect of reducing intermittent hypoxia. It is not clear which exact mechanisms underlie intermittent hypoxia-mediated elevations in morning blood pressure in OSA. Whilst changes in markers of sympathetic activation were inconclusive in the SOX trial, previous studies have highlighted the potential importance of intermittent hypoxia-mediated sympathetic activation in diurnal increases in blood pressure. The lack of changes in markers of oxidative stress, inflammation, or hypoxia-induced protein markers in blood during CPAP withdrawal suggest that these mechanisms are less important in determining the cardiovascular effects of OSA. Alternatively, changes occurring at the tissue/end-organ level may not be reflected by these systemic biomarkers. The supplemental oxygen trial was a physiological mechanistic study and was not designed to assess the potential therapeutic benefits of supplemental oxygen in OSA, although I will discuss potential therapeutic applications. In this chapter I will discuss the potential mechanisms involved in cardiovascular disease in OSA in more detail. Future directions including assessing the potential therapeutic effects of supplemental oxygen on blood pressure in OSA will be discussed, along with an outline of other ongoing aspects of my research. In any future trials of supplemental oxygen, safety considerations will be important, taking note of both the lack of effect of supplemental oxygen on reducing sleepiness in OSA and the possible effect of supplemental oxygen raising carbon dioxide levels.

7.2. OSA, intermittent hypoxia and blood pressure

Cross sectional studies have shown increased diagnoses of hypertension (67), and increased blood pressure in OSA patients (68). OSA is also associated with increased diagnoses of hypertension in prospective studies (66). Hypertension is an important cause of both ischaemic heart disease and stroke, and it leads to significant cardiovascular disease (103). Elevated blood pressure in OSA is a possible cause of the observed increases in cardiovascular morbidity and mortality in OSA patients (9)(76).

Although CPAP has not yet been shown to reduce cardiovascular risk in OSA (49), CPAP has been shown to be effective in lowering blood pressure in OSA (69)(70) and in improving endothelial function (150). Improvements in blood pressure with CPAP tend to be more marked in those with more severe OSA/intermittent hypoxia, in those who use CPAP therapy for longer (69), and in patients already on at least one anti-hypertensive medication (70).

However, it may be that the extent of sleepiness is an important factor. One study including only non-sleepy patients with resistant hypertension and OSA did not show an effect of CPAP on blood pressure (72). This trial included patients with an ESS<10 and all severities of OSA (ODI>10). There are several possible reasons for this negative result. First, the inclusion of non-sleepy patients may have excluded those likely to benefit from CPAP. Second, the inclusion of patients with mild OSA may have affected the result. Third, this study was powered to find a large difference in mean blood pressure of 5mmHg, which is much larger than previous reports in more symptomatic patients (69). The HIPARCO study was a multi-

centre RCT assessing the effect of CPAP on blood pressure in patients with resistant hypertension and moderate to severe OSA (74). It included 194 patients, with approximately 60% being non-sleepy, and found a significant effect of CPAP on blood pressure. It seems that there is therefore an effect of CPAP on blood pressure in non-sleepy patients with resistant hypertension, at least in those individuals with moderate to severe OSA.

The SAVE trial, which was the first large randomised control to assess the effects of CPAP on cardiovascular events in a secondary prevention population, showed no additional benefit of CPAP over and above standard care (49). There are limitations to the SAVE trial. These include the low average usage of CPAP at 3.3 h/night, the exclusion of the most sleepy patients (ESS>15), and the exclusion of the most hypoxic patients (>10% of the nights with oxygen saturations <80%). In addition, the inclusion of only patients already receiving secondary cardiovascular prevention may have diminished any potential benefit from CPAP. Finally, CPAP had no effect on blood pressure in the SAVE trial, which has been shown elsewhere (70), and could the mechanisms via which CPAP has an effect on cardiovascular risk.

Punjabi *et al.* looked at cross-sectional patients from the Sleep Heart Health Study and showed that the extent of hypoxia associated with hypopnoeas was a predictor of prevalent cardiovascular disease (10). Hypopnoeas with oxygen desaturations above a 4% threshold were associated with increased risk of cardiovascular disease, whereas hypopnoeas with desaturations of less than 4% were not associated with increased cardiovascular risk. This

suggests that the extent of intermittent hypoxia is important in determining cardiovascular risk and patients excluded on the basis of severe hypoxia in the SAVE trial may be those who stood to benefit most.

Grimaldi *et al.* argue that lower CPAP therapy hours are likely to leave patients exposed to significant OSA, and perhaps specifically REM OSA (205). REM sleep tends to occur in the latter part of the night and the average usage of CPAP of 3.3 h/night in the SAVE trial, if all in the first part of the night, would potentially leave patients particularly vulnerable to REM OSA. Mokhlesi and Varga argue that the low usage in the SAVE trial would have meant patients were still exposed to significant cardiovascular risk via REM-related OSA (294). Alternatively, if patients used CPAP for the entire night on nights they used CPAP and slept for 6-7 h/night, which is the UK average (295), the 3.3 h/night usage in the SAVE trial would equate to using CPAP on only approximately 50% of nights. However, in the SAVE trial, even a highly compliant sub-group did not have reduced cardiovascular events.

The usage of CPAP in the SAVE trial is comparable to most other trials which have trialled CPAP in OSA patients recruited with few or no symptoms (46)(48)(296). However, the Spanish sleep network has had success in achieving greater hours of CPAP usage in minimally symptomatic OSA patients, with a median CPAP usage of 5h/night (297). It is not entirely clear why the Spanish sleep network was able to achieve better adherence with CPAP than others, but in general CPAP adherence is modest in patients without excessive sleepiness.

Therefore, the usage of CPAP in SAVE is probably a reflection of what is realistically achievable with CPAP in minimally symptomatic patients in the "real world".

The lack of an effect on blood pressure in the SAVE trial is difficult to interpret. This may reflect the efficacy of secondary preventative medications in controlling blood pressure. In patients with OSA and previously untreated hypertension, CPAP made little difference over and above the effect of losartan on blood pressure (71). Approximately 80% of patients in the SAVE trial were on anti-hypertensive medications and it may be that for most, CPAP had no further benefit in addition to anti-hypertensive medications on blood pressure. In contrast, patients with resistant hypertension, in whom blood pressure is not adequately controlled despite three medications or more, there is a more marked reduction in blood pressure with CPAP (70), as has already been discussed.

Previous trials of supplemental oxygen, whilst showing attenuation of intermittent hypoxia (121)(122)(186), have not shown a reduction in blood pressure (121)(122). This is in contrast to my finding of a substantial effect of supplemental oxygen on blood pressure during CPAP withdrawal. Importantly my trial was able to include patients with more severe hypoxaemia, and delivered oxygen at higher flow rates, more likely to attenuate intermittent hypoxia. In addition, the use of ambulatory blood pressure in previous trials is known to cause arousals from sleep (112), and this could have altered sleep architecture which is known to cause elevations in blood pressure (201)(204). When compared to the study by Gottlieb *et al.* (121), the SOX trial showed markedly increased hours of usage of supplemental oxygen (7:12

± 1:01 h:min/night versus 4.8 ± 2.4 h/night). Similarly to the SAVE trial, lower hours of usage of supplemental oxygen in the Gottlieb study could have led to significant intermittent hypoxia during REM sleep, which has been particularly linked to cardiovascular disease (294). Another important difference is in the duration of intervention with supplemental oxygen. Gottlieb *et al.* gave supplemental oxygen for a much longer period of 12 weeks (121). Blood pressure increases with exposure to chronic hypoxia on travel to altitude within the first few days, but after 50 days this blood pressure rise is attenuated (210). Whilst I did not observe any attenuation of the effect of supplemental oxygen on blood pressure during two weeks of CPAP withdrawal, the longer-term effects are not known. It is possible that compensatory mechanisms may attenuate the effect of intermittent hypoxia in the longer-term, but this is speculative.

7.3. Mechanisms of intermittent hypoxia causing elevated morning blood pressure in OSA

7.3.1. Sympathetic nervous system activation

The experiments of Fletcher and colleagues suggest a dominant role of the sympathetic nervous system in determining the rise in blood pressure following exposure to intermittent hypoxia (106). Rodents exposed to intermittent hypoxia during sleep have elevations in diurnal blood pressure (104). Removal of the carotid body, and therefore the carotid chemoreceptors, which sense hypoxia, removed this effect. In addition, removal of either the adrenal medulla or chemical blockade of sympathetic nerve endings abolished this rise in blood pressure. In healthy human subjects intermittent hypoxia has been shown to increase blood daytime blood pressure and daytime muscle sympathetic nerve activation (111). OSA

increases urinary metabolites of sympathetic activation (148)(276), and Mills *et al.* showed that supplemental oxygen reduced the daytime excretion of noradrenaline in OSA (122). Mills *et al.* observed a reduction only in daytime noradrenaline excretion with supplemental oxygen; night-time excretion was unchanged. The authors speculate that the night-time noradrenaline levels were unchanged as night-time levels were determined by arousal induced sympathetic activation and not intermittent hypoxia. In contrast, they argue that the daytime changes were determined by intermittent hypoxia. There was a suggestion of a partial reduction in normetadrenaline in the SOX trial and it is possible that measuring daytime rather than overnight sympathetic activation in the supplemental oxygen trial may have shown more marked reductions in the oxygen arm.

7.3.2. Inflammation and oxidative stress

In addition to sympathetic activation, inflammation and oxidative stress have been proposed to underlie the development of hypertension and cardiovascular disease in OSA (257). The lack of change in hsCRP in the supplemental oxygen trial could either mean that inflammation is not triggered by intermittent hypoxia in OSA, or that inflammatory changes occur at a tissue/end-organ level which might not be reflected by systemic changes in hsCRP.

Prabhakar and colleagues suggest a central role for an oxidative injury induced by intermittent hypoxia in the carotid chemoreceptors (136). They argue that oxidative stress causes increased sympathetic output from the carotid body in response to hypoxia. Traditional biomarkers of oxidative stress were assessed in a previous CPAP withdrawal trial

(135). Biomarkers of oxidative stress were not increased, and the authors argued that a paradoxical decrease in morning urinary uncorrected isoprostanes and a decrease in superoxide dismutase suggested that OSA may actually reduce oxidative stress. Concerns were raised by others that the urinary isoprostanes might not reflect overnight changes as they were measured in the morning and that urinary isoprostanes were not corrected for diuresis (272). I have subsequently shown that overnight urinary isoprostanes, corrected for diuresis, do not change with CPAP withdrawal. This is in keeping with the findings of a randomised control trial which found that CPAP did not change corrected urinary isoprostane levels over two months (134). Systemic biomarkers of oxidative stress show little correlation with oxidative stress measures in coronary artery bypass graft tissue (138). Therefore, the lack of a change in systemic biomarkers in OSA does not exclude the possibility that oxidative stress may occur at a tissue level. Increases in superoxide dismutase seen in CPAP withdrawal suggest a compensatory increase in the mechanisms to counter oxidative stress in OSA. A separate withdrawal trial used a mass spectrometry based technique to assess the breath signature of patients undergoing CPAP withdrawal (133); this showed increases in a family of aldehydes that are linked to oxidative stress. Therefore, whilst evidence from intermittent hypoxia experiments in animals shows oxidative stress, the role of oxidative stress following intermittent hypoxia in patients with OSA is not yet established.

Adipose tissue/hypoxia mediated inflammation has also been suggested to lead to cardiovascular disease in OSA (298). However, in patients with OSA, changes in adipose tissue derived inflammatory proteins have not been shown in a traditional RCT, assessing

adiponectin (153), or during CPAP withdrawal, where resistin levels were unchanged. Whilst the negative findings with these systemic markers do not support the role of adipose tissue/hypoxia mediated inflammation in OSA they do not exclude it being of importance. Assessing tissue-specific changes may be more helpful in determining the importance/non-importance of adipose tissue hypoxia in OSA (299).

7.3.3. Urinary dilution in OSA

OSA causes increased overnight urine production and commonly causes nocturia (227) (228), with OSA causing increased losses of sodium and water (229). Increases in urinary sodium losses are correlated with overnight increases in diastolic blood pressure in OSA (300). Hyperaldosteronism and OSA are both common conditions in patients with resistant hypertension, and increases in aldosterone levels have been suggested to cause hypertension in OSA (301). A cross-over trial in 31 OSA patients with elevated blood pressures showed that a β-blocker was more effective in lowering blood pressure than a diuretic (302). OSA-related diuresis might make diuretic medications less effective, however, it is difficult to know if the doses used in this study would be expected to be equipotent and have similar effects on blood pressure. Potential stimuli for increased aldosterone secretion in OSA include renin-angiotensin activation from either sympathetic activation or diuresis. Therefore, diuresis in OSA could lead to hypertension in OSA by increasing aldosterone, offsetting any potential benefit from the diuresis itself. It is important to understand the mechanisms underlying diuresis in OSA to allow an understanding of the overall effect of OSA on BP.

Intermittent hypoxia or the thoracic pressure swings occurring during OSA have been hypothesised to underlie changes in atrial natriuretic peptide thought to be responsible for this urinary diuresis (230)(231). Overnight urinary diuresis was unaffected by supplemental oxygen during CPAP withdrawal. As supplemental oxygen did not affect overnight urinary diuresis, it seems that another mechanism, such as intrathoracic pressure swings (230), or sleep fragmentation blocking the normal physiological overnight reduction in urinary volumes (244), are more likely to determine urinary diuresis in OSA than intermittent hypoxia.

7.3.4. Blood hypoxically-regulated protein markers

Several proteins increased by hypoxia-inducible factor have been proposed to be of importance in the development of cardiovascular disease. Increased blood adrenomedullin (259)(260), endothelin-1 (262), and vascular endothelial growth factor levels (265), have been found in OSA patients. In CPAP withdrawal none of these hypoxically-regulated proteins were increased, questioning previous conclusions of their importance in the development of cardiovascular disease in OSA. Unexpectedly, there was a slight decrease in adrenomedullin levels. Adrenomedullin is a potent vasodilator (258), and reductions in its levels could contribute to increased blood pressure in OSA. However, the reductions in adrenomedullin are likely to be caused by another mechanism and not hypoxia, as this would be expected to increase levels.

7.3.5. Endothelial dysfunction

Endothelial dysfunction is thought to be of importance in the development of cardiovascular disease (285), with both arterial stiffness and the endothelium playing an important role in hypertension (303). Oxidative stress, triggered by intermittent hypoxia, is thought to be of importance in the development of endothelial dysfunction in OSA (257). OSA is associated with endothelial dysfunction; both improving with CPAP treatment (149), and deteriorating with CPAP withdrawal (148). Endocan has been proposed as useful biomarker for identifying endothelial dysfunction in OSA, with elevated levels compared to controls, and increased endocan levels are inversely correlated with endothelial dysfunction (270). Whilst CPAP withdrawal worsens endothelial function (148), it did not alter endocan levels, suggesting that endocan may not be useful as a biomarker of endothelial dysfunction in OSA.

The potential mechanisms leading to elevated daytime blood pressure from intermittent hypoxia are shown in *Figure 7.1*.

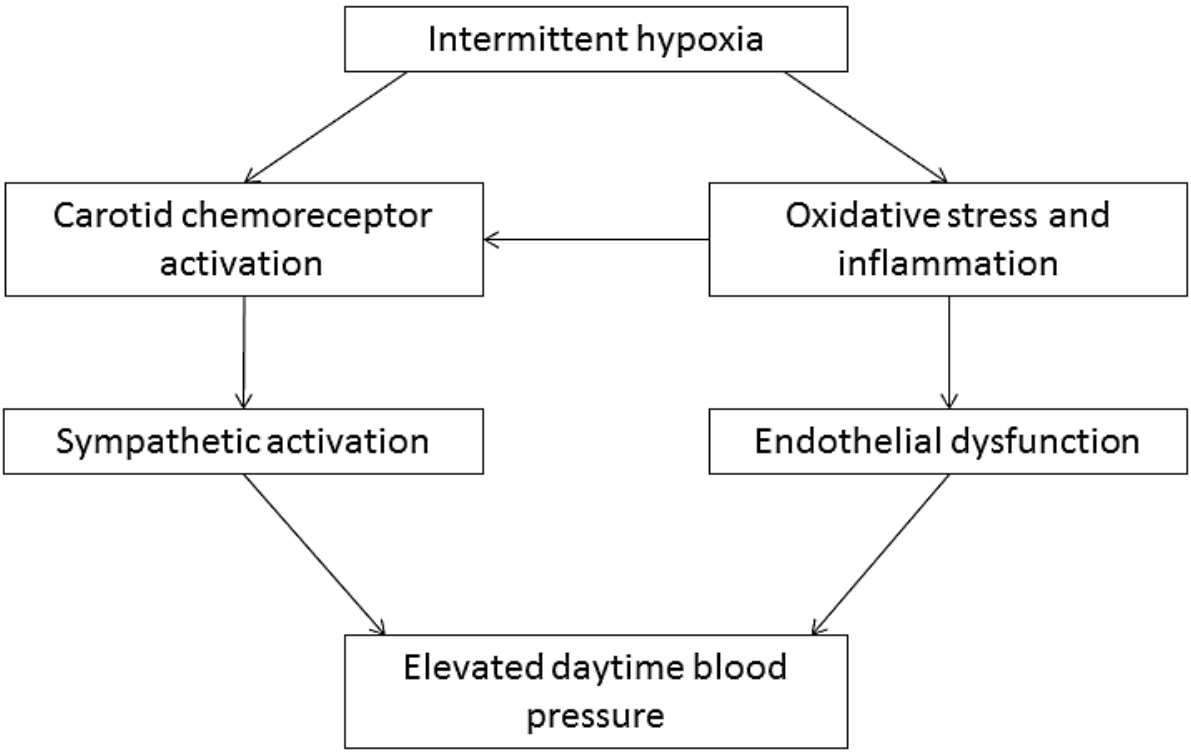


Figure 7.1: The potential effects of intermittent hypoxia in relation to daytime blood pressure.

7.4. Effects of supplemental oxygen on the AHI

Supplemental oxygen reduces loop gain (220), and has been hypothesised to reduce the AHI in OSA patients. Supplemental oxygen is certainly a helpful therapy in treating central sleep apnoea at altitude (221). Short-term reductions in the AHI have been shown with supplemental oxygen therapy, both in OSA cohort studies (186), and in one randomised control trial in selected patients, and in combination with eszopiclone (219). In the longer-term, supplemental oxygen has not been shown to greatly reduce the AHI (121)(122). In the supplemental oxygen trial the small reductions in the AHI were not significant. This suggests that reductions in blood pressure were not due to reductions in arousal mediated

sympathetic activation but a separate effect of supplemental oxygen on intermittent hypoxia. Supplemental oxygen did increase the apnoea length, along with the venous base excess, and carbon dioxide levels. This suggests that supplemental oxygen therapy had an effect on ventilatory drive, perhaps blocking increases in hypoxic ventilatory drive that are seen in patients with OSA (236). However, this did not reduce the overall AHI, as would be predicted by the work by Edwards *et al.* (219)(220). This could either reflect predominantly underlying anatomical susceptibility to OSA in my patients (rather than loop gain issues), or that the combination of both eszopiclone and supplemental oxygen is needed to reduce the AHI in a sub-group of patients with OSA.

There was a small reduction in the heart rate rises index following supplemental oxygen. Heart rate rises are a complex marker of arousal, sympathetic activation at arousal, and reduced parasympathetic activity at arousal (159)(304); thus the small decrease in heart rate rises may not only be due to a reduced number of arousals. Therefore, it is unlikely that this small reduction in heart rate rises explains the marked attenuation of the morning blood pressure rise in the supplemental oxygen trial. It seems more likely that attenuation of intermittent hypoxia is directly responsible for blood pressure changes.

7.5. Effect of intermittent hypoxia on sleepiness

Supplemental oxygen did not affect the return of sleepiness during CPAP withdrawal. Experimentally-induced intermittent hypoxia, in a small cross-over trial where patients with OSA were given CPAP, with or without the intermittent addition of 100% nitrogen, also

showed no difference between the two arms as regards the improvement in objective sleepiness (252). Roehrs *et al.* showed that respiratory-related arousals were a better predictor of multiple sleep latency scores than measures of intermittent hypoxia (41). However, the respiratory arousal index only predicted approximately 13% of the variance in objective sleepiness, and other factors such as total sleep time and the quality of sleep were also important.

In the short-term intermittent hypoxia does not seem to be important in determining daytime sleepiness. However, this does not mean that longer-term intermittent hypoxia may not cause sleepiness. Indeed animal intermittent hypoxia, and MRI studies in OSA patients, suggest oxidative injuries to wake-sleep promoting centres (248)(256). This has been proposed as a possible basis for post-CPAP sleepiness (249). There is a lack of longer-term data on the effects of supplemental oxygen in OSA on daytime sleepiness as the largest trial in this area did not report sleepiness outcomes (121). Further work is needed to clarify if intermittent hypoxia increases daytime sleepiness in the longer-term.

7.6. Future directions

The supplemental oxygen trial was designed as a physiological mechanistic study to determine if intermittent hypoxia causes elevated daytime blood pressure in OSA. Animal and human intermittent hypoxia experiments suggested the importance of intermittent hypoxia in daytime blood pressure (106)(111). Previous RCTs assessing the effect of supplemental oxygen on 24-hour blood pressure profiles in OSA suggested that hypoxia may

not be important but had significant methodological limitations and were thus not definitive (121)(122). The supplemental oxygen trial, whilst not designed to determine the therapeutic benefit of oxygen in OSA, does suggest that there may be a role for supplemental oxygen as a treatment in patients with both OSA and hypertension.

Blood pressure is decreased by CPAP (69)(70), and in longitudinal cohort studies has been shown to reduce the risk of cardiovascular events (9)(76). However, CPAP is not always indicated/tolerated, particularly in minimally symptomatic patients (46)(48)(49)(296). Therefore, the effect of supplemental oxygen on blood pressure could be assessed in OSA patients, when CPAP is not indicated or tolerated. CPAP has little additional beneficial effect when given in combination with losartan in patients not previously treated with anti-hypertensives (71), whereas its effects are more marked in patients who have resistant hypertension (70). Future trials of supplemental oxygen focussing on patients with OSA and resistant hypertension, when CPAP is not indicated or tolerated, are therefore justifiable.

Supplemental oxygen had no effect on the return of either subjective or objective sleepiness during CPAP withdrawal. Sleepiness is an important consequence of OSAS and is probably the cause of increased road traffic incidents (7), work place accidents (245), and work days missed in OSA (246). In any future trials of supplemental oxygen therapy, patients with excessive sleepiness, or those presenting to the sleep clinic with symptoms should not be included due to concerns over safety. These symptomatic patients should, in any case, be offered CPAP (305). A concern about this approach is whether there would be any benefit in

terms of blood pressure in treating non-sleepy patients. Robinson *et al.* showed no benefit of CPAP in reducing blood pressure in non-sleepy patients with hypertension (72), this study was relatively small with only 32 patients completing both arms in a cross-over design. In a meta-analysis the effects of CPAP on blood pressure were more marked in patients with resistant hypertension, and this meta-analysis included many patients with an ESS <10 (70). The mean ESS ranged from 5.3 (in the Robinson *et al.* study) to 15.0, so including patients from hypertension clinic with an ESS <15, and without concerns about safe driving, seems a reasonable approach.

A further potential safety concern was the finding of increased venous base excess and carbon dioxide levels in the supplemental oxygen trial. Whilst this did not lead to any adverse events in this trial it may be more of a concern in patients with obesity hypoventilation (242). These patients were excluded here and probably also should be in future research. In future trials careful monitoring for adverse events relating to hypercapnia (such as early morning headaches) and checks of early morning blood gasses, or overnight transcutaneous carbon dioxide levels, would be necessary.

Resistant hypertension is commonly defined as an office blood pressure of >140/90mmHg on three or more optimal anti-hypertensive medications. Hypertension clinics screen for secondary causes of hypertension, ensure compliance with medications (which can be checked by direction observation or urine toxicology), and exclude “white-coat” hypertension (306).

Next I propose conducting a trial to assess the therapeutic benefit of supplemental oxygen in reducing blood pressure in OSA, I would propose screening patients with resistant essential hypertension (defined as a blood pressure of >140/90mmHg despite taking three or more optimal anti-hypertensive medications without a secondary cause) for OSA. Those with an ODI >20/hour, an ESS of <15 and no concerns about safe driving, and an early morning arterialised capillary blood gas showing PCO_2 <6kPa or base excess <2 mmol/l, would then be randomised to either one month of supplemental oxygen or air. After one month patients would then undergo two weeks of washout before receiving the alternate treatment. A cross-over design would increase the power of this study design and a longer intervention would enable monitoring of the blood pressure effect of oxygen in the medium term. Blood pressure could be monitored daily at home in the morning, and 24-hour blood pressure profiles could be assessed prior to each treatment and in the last week of each treatment using a portable non-invasive Finapres device (211). Weekly visits to check safety outcomes including an early morning capillary blood gas to check carbon dioxide and base excess levels could also be included. It may be necessary for an unblinded member of staff to carry out safety capillary blood gas checks.

7.6.1. Messenger ribonucleic acid (mRNA) work

There are limitations to the targeted approach I have taken in trying to understanding the molecular mechanisms underlying the development of cardiovascular disease in OSA. Next generation sequencing allows a hypothesis free approach to explore the potential molecular

pathways that are altered in OSA. Samples for transcriptomics analyses were collected both in a previous CPAP withdrawal trial (135), and from the SOX trial. Blood samples were taken in both trials to perform mRNA sequencing on isolated peripheral blood leucocytes. By comparing paired samples of patients when they are on and off treatment, relative differences in the transcriptome may be detected and crucial gene networks may be identified.

In OSA most previous studies have focused on targeted microRNA and exosome profiling. MicroRNAs in exosomes have been linked to endothelial dysfunction in childhood OSA (307), and microRNAs and mRNAs in exosomes that are linked to endothelial dysfunction are upregulated in a model of intermittent hypoxia in healthy adult subjects (146). Exploratory work, based on a RCT assessing the effects of CPAP in resistant hypertension, linked a specific microRNA profile to blood pressure "responders" to CPAP therapy (308), although this work was a post-hoc analysis and needs further validation. Whilst most studies focused on microRNA profiling, few studies have assessed RNA analysis in peripheral blood leucocytes. One study used a hypothesis free approach, using micro-array technology, and found upregulation of toll-like-receptor-2 mRNA in healthy volunteers exposed to intermittent hypoxia (309). The authors conclude that pro-inflammatory toll-like-receptor-2, induced by intermittent hypoxia, might mediate inflammatory changes in OSA patients leading to cardiovascular disease. Another study investigated a single hypothesis and found increases in a potassium channel involved in vasomotor control in OSA patients (310).

There have been no attempts to date to use RNA sequencing to gain a comprehensive understanding of the molecular pathways involved in OSA. By utilising samples from a previous withdrawal trial and the SOX trial I aim to assess the effects of OSA with and without intermittent hypoxia on peripheral blood leucocyte profiles. Samples have been extracted and sequenced by the Wellcome Trust Centre for Human Genetics core laboratory. At present the sequencing results are being analysed.

7.6.2. Retinal vasculature reactivity following CPAP withdrawal study

In addition to the SOX trial, I have been running another CPAP-withdrawal trial assessing the retinal vasculature. OSA is associated with type 2 diabetes mellitus, diabetic retinopathy, and particularly with diabetic macular oedema (94)(311). The cause of worsened diabetic macular oedema in OSA is not clear. Patients with diabetes have impaired retinal vascular responses representing reduced endothelial function (312)(313)(314), and impaired retinal vascular autoregulation (315). I am investigating the effects of OSA, independent of the effects of diabetes, on retinal vascular endothelial function and autoregulation. I am testing the hypothesis that OSA causes impaired retinal endothelial function and autoregulation accelerating diabetic macular oedema (*Figure 7.2*).

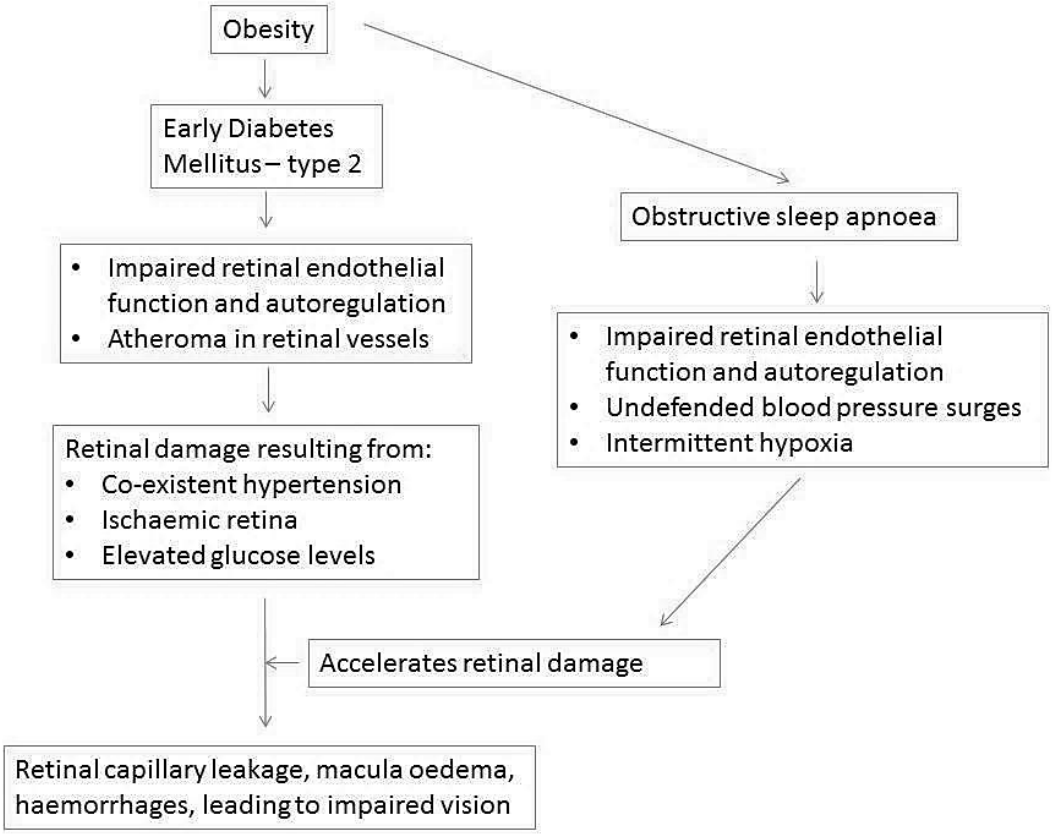


Figure 7.2: Proposed mechanisms by which OSA leads to accelerated diabetic maculopathy

Retinal dynamic vessel analysis cameras are able to measure the diameter of retinal arterioles and venules in real time, and record changes in response to challenges such as a "flickering-light" (316). Alternating light and dark in the "flickering-light" protocol maximally stimulates photoreceptors and acts as a metabolic stimulus. This requires increases in blood flow, and endothelial mediated processes are thought to underlie increases in the diameters of retinal arterioles and venules that facilitate increased blood flow. *Figure 7.3* shows a typical response to a flickering light and illustrates the standard way in which this response is measured (the dilation amplitude as a percentage of baseline). This response is reduced in

patients with type 2 diabetes, particularly in those with established retinopathy (312)(313)(314), and in patients with coronary artery disease (317).

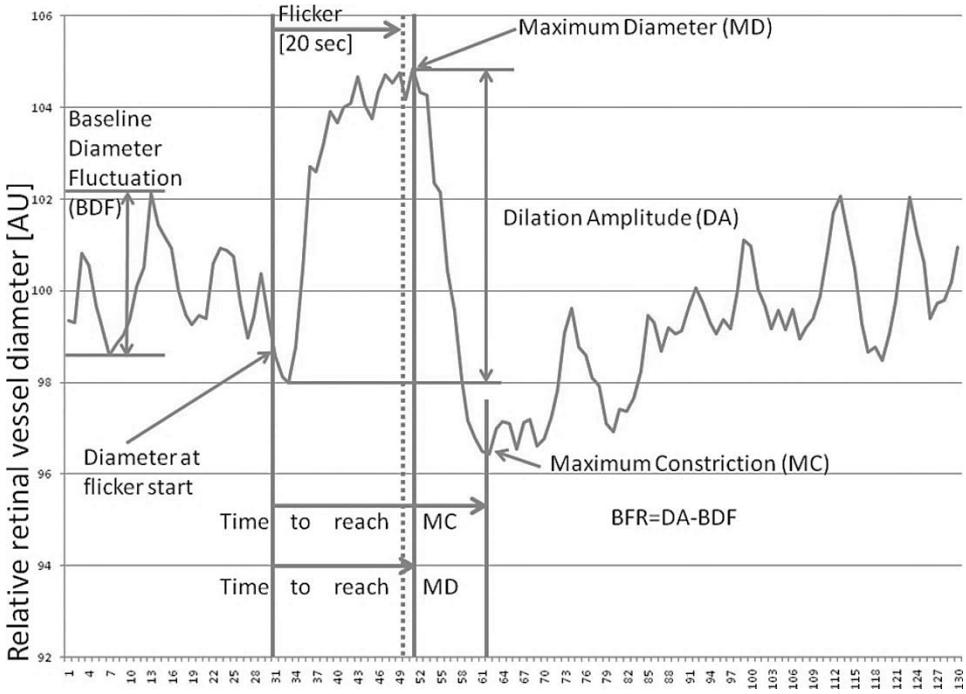


Figure 7.3: Relative retinal vessel diameter at baseline and during 'flickering-light' protocol illustrating my primary outcome; the percentage increase from 'diameter at flicker' start to 'maximal diameter' (increase in retinal vessel diameter following 'flickering-light'). Each patient's response to the 'flickering-light' is repeated 3 times with 80 seconds in between. Adapted with permission from Heitmar et al. (317).

The retinal vasculature reactivity CPAP withdrawal trial is a multi-centre prospective randomised controlled trial. Four sites are open and recruiting, including sites in Birmingham, Oxford, and Coventry. This trial has been registered (ISRCTN: 78082983) and approved by a research ethics committee (14/SC/1235). Patients are eligible if they have

been on CPAP for >6 months without known diabetes. Patients are randomised to receive 14 nights of sham CPAP or continued therapeutic CPAP. The primary outcome of the trial is the effect of CPAP withdrawal on retinal vasculature reactivity during a 'flickering-light' protocol as previously described. Currently 29 patients have completed the protocol, with a target recruitment of 40 patients per protocol.

Baseline results show that there are measurable responses to the "flickering-light" protocol in OSA patients on CPAP treatment (mean arteriole response 2.1 ± 0.7% and mean venular response 3.8 ± 1.7%). This is important as coronary artery disease patients have reduced "flickering-light" responses (317). Although unstable coronary artery disease was an exclusion criterion, many OSA patients have cardiovascular disease and OSA patients might have had very low baseline reactivity making an assessment of change in reactivity difficult.

Patients with known diabetes were excluded and patients underwent recording of HbA1C. Mean HbA1C was 5.6 ± 0.5%, with two patients having values above 6.5% suggestive of a new diagnosis of diabetes (6.6% and 7.4%). These two individuals did not have evidence of diabetic retinopathy and will be included in the per protocol analysis. Following 14 days of either sham CPAP of continued CPAP, those randomised to sham CPAP had severe OSA on average, with a mean $ODI_{\geq 4\%}$ of 31.7 ± 20.1/h, whilst those who received continued CPAP had well controlled OSA with a mean $ODI_{\geq 4\%}$ of 6.0 ± 4.3/h. Therefore, the patients identified have measurable retinal vasculature responses that may be affected by CPAP withdrawal in my RCT, are free from diabetic retinopathy, and have measurable return of OSA during CPAP withdrawal.

Recruitment to date has been slow for the retinal reactivity trial. This was designed as a single centre trial, recruiting from University Hospitals Birmingham sleep clinic. Study visits have to be performed at Aston University as this is the only UK site with both the dynamic vessel analyser and expertise to perform this protocol. Unfortunately recruitment to date has been slow in Birmingham. Even after opening recruitment sites at Heartland's Hospital and Coventry Hospital, only 12 patients have been recruited from Midlands's sites. Due to slow recruitment Oxford University Hospitals has opened as a site and this, in combination with an increase in nursing time dedicated to the trial from June 2017, has led to accelerated recruitment. Initially Oxford was not a recruiting site as patients attending study visits at Aston University require dilating eye drops and therefore cannot drive to their Aston visits. I have driven patients up from Oxford to their study visits at Aston University. Seventeen patients have now completed the trial from Oxford, significantly more than Oxford's target of 5 patients. Recruitment for this study is due to end in 2018 (see *Figure 7.4*) with projections based on average recruitment over the last two years and not on the accelerated recruitment since June 2017.

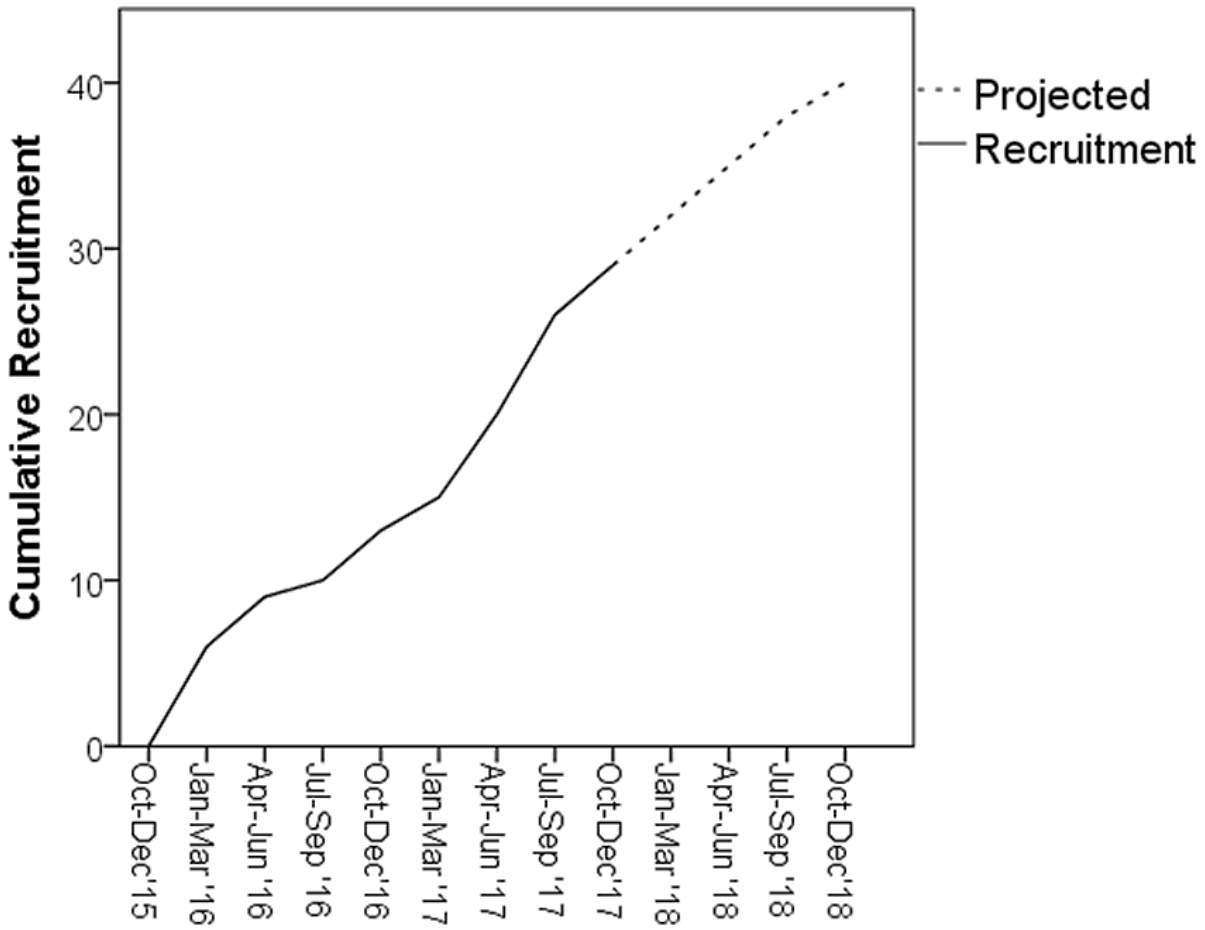


Figure 7.4: Retinal reactivity trial recruitment. Projected recruitment is based on the overall recruitment rate and does not take into account accelerations since June 2017 at the Oxford site.

7.7. Conclusions

The supplemental oxygen trial shows the importance of the intermittent hypoxia seen in OSA in morning elevations in blood pressure. Intermittent hypoxia is likely to increase sympathetic activation over and above the effect of arousal mediated sympathetic activation overnight. Whilst there was a small effect of supplemental oxygen on the AHI and heart rate rises, as a surrogate marker of arousal, this was small and unlikely to explain the marked attenuation of the rise in blood pressure seen following CPAP withdrawal. The exact

mechanisms for intermittent hypoxia causing elevations in morning blood pressure in OSA are unclear. The carotid chemoreceptors and sympathetic activation may be involved, although this not certain. The supplemental oxygen trial was a physiological mechanistic study highlighting the importance of intermittent hypoxia in elevated morning blood pressure in OSA. Future trials are needed to assess whether supplemental oxygen is a viable treatment option in certain situations for blood pressure reduction in OSA. Supplemental oxygen does not affect daytime sleepiness in OSA, at least in the short-term, and so sleepy patients should be excluded from future trials as they may be at increased risk of road traffic incidents, and would be better treated by CPAP. In those who do not to present to the sleep clinic, usage of CPAP has been disappointing (49). Therefore, recruiting patients from the resistant hypertension clinic who do not present with typical symptoms of OSA could identify a group of patients who would be unlikely to tolerate CPAP and therefore may derive more benefit from oxygen than CPAP in terms of blood pressure. Future trials of supplemental oxygen should also exclude patients with daytime hypercapnia and closely monitor early morning blood gasses as hypercapnia is a potential safety concern with supplemental oxygen.

8. Appendix

8.1. Oxygen delivery interface in healthy volunteers

The primary aim of the SOX trial was to assess the effects of intermittent hypoxia on blood pressure as a marker of cardiovascular function. In order to assess this hypothesis it was important that oxygen delivery was optimised.

There were two major concerns about oxygen delivery. First, I wanted to ensure that our oxygen concentrator delivers a high percentage of oxygen at all flow rates. Second, there were concerns about the efficacy of the patient interface on the oxygen delivery. A minority of patients will be habitual mouth breathers, although the incidence of mouth breathing is not known in adults, it can be as high as 25% in 6 year olds (318). Anecdotally, even in those who are not habitual mouth breathers, breathing frequently occurs via the mouth after the cessation of an apnoea. Oxygen delivery is probably reduced when using a nasal-only device during these periods of mouth breathing.

Face masks however, contain dead space and will mix exhaled air with gas from the concentrator and normal room air. There is a theoretical possibility therefore with these systems of CO_2 build-up in the dead space leading to rebreathing of CO_2.

From the first two patients who completed the SOX trials some concerns arose. One patient still had significant oxygen desaturations, which could have been due to mouth breathing whilst using nasal cannulae (*Figure 8.1*).

	Patient1		Patient2	
Concentrator given	***Air***	***Oxygen***	***Air***	***Oxygen***
ODI (events/ hr)	31.4	13.2	43.1	1.4
Mean O_2 saturations (%)	90.7	93.8	93.0	97
Time O_2 saturations <90% (%)	30.6	6.8	9.5	0
Mean nadir O_2 saturations (%)	83.6	87.0	88.9	89.7

Figure 8.1: ***A-D*** *are approximately 20 minute segments from the overnight oximetry recordings from the first two patients showing the oxygen saturations (top line) from 70-100%, and pulse rate (bottom line) from 50-130bpm.* ***A*** *and* ***B*** *are from patient 1 with air and*

oxygen treatment respectively. ***C*** *and* ***D*** *are from patient 2 with air and oxygen treatment respectively. Displayed in table* ***E*** *are the results of the four oximetry tracings. Whilst patient 2 had a dramatic improvement in all oximetry indices, patient 1 had only a partial response, thought to be mainly due to mouth breathing after apnoeas.*

In patient 1 the $ODI_{\geq 4\%}$ was only reduced from 31.4/h on air treatment to 13.2/h on oxygen treatment. This contrasts with the dramatic reduction in from 43.1/h with air treatment to 1.4/h with oxygen treatment in patient 2.

The percentage oxygen delivered by the oxygen concentrators at increasing flow rates at increments of 1l/min to a maximum flow rate deliverable of 5.5 l/min was checked using an oxygen percent analyser. The inspiratory and end-tidal oxygen and carbon dioxide levels were checked using a nasal catheter for different mask interfaces: standard nasal cannulae, nasal cannulae with pendant reservoir (Oxymizer), standard-use non-rebreathe mask, high-flow non-rebreathe face mask with reservoir bag removed, and with a fitted face mask.

Table 8.1 shows the results of the oxygen percentages obtained at different flow rates via the concentrator.

Flow rate (l/min)	Concentrated oxygen percentage (%)
1	99.2
2	99.0
3	98.5
4	98.2
5	98.1
5.5	98.0

Table 8.1: Percentage oxygen delivery by study oxygen concentrator at different flow rates

Four healthy volunteers underwent experiments to assess inspired and end-tidal gas analyses. Results of end-tidal oxygen and carbon dioxide levels and inspired carbon dioxide levels are shown in *Table 8.2*, for each mask interface. *Figure 8.2* shows the end-tidal oxygen for each interface.

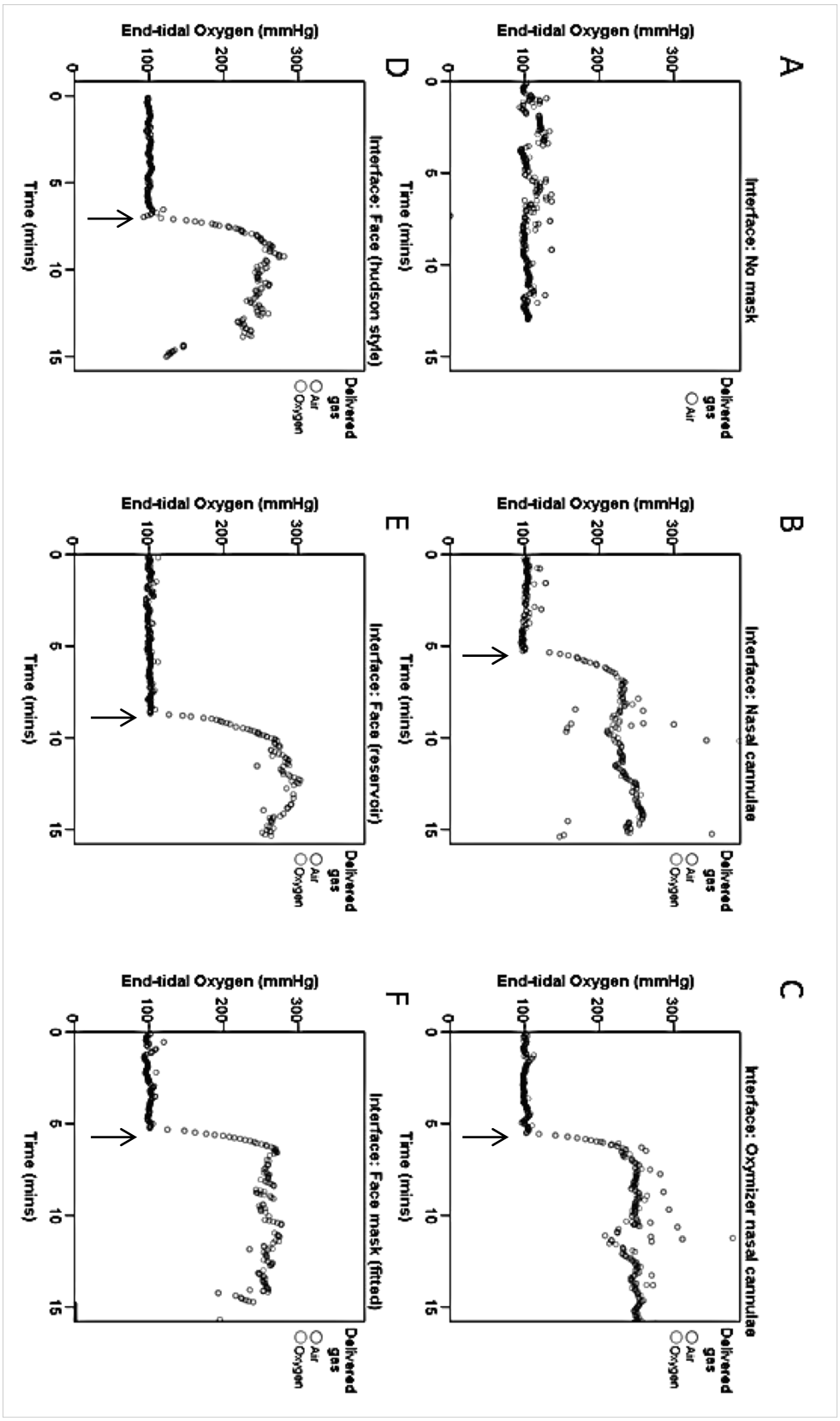


Figure 8.2: End-tidal oxygen for each interface breathing air for 5-10 minutes before breathing oxygen (arrow indicates the point when room air was switched to oxygen).

	End-tidal oxygen (mmHg)	End-tidal carbon dioxide (mmHg)	Inspired carbon dioxide (mmHg)
None	106.9 ± 3.6	35.0 ± 4.3	0.5 ± 0.3
Nasal cannulae	257.1 ± 27.3	35.9 ± 3.4	0.2 ± 0.2
Oxymizer	269.4 ± 21.8	37.0 ± 3.9	0.4 ± 0.3
Face mask (Hudson)	269.2 ± 37.7	37.5 ± 3.2	1.0 ± 1.0
Face mask (reservoir)	265.8 ± 24.0	37.2 ± 4.5	0.8 ± 0.4
Face mask (fitted)	258.1 ± 9.9	37.3 ± 3.7	1.0 ± 0.6

Table 8.2: Summary of inspired and end-tidal oxygen for each interface. Mean (SD) values from four subjects. All values are expressed as mean ± SD.

The results of these experiments show that the oxygen concentrator delivers ≥98% oxygen at all flow rates. All interfaces achieved a high end tidal oxygen level above 230mmHg and the highest value were seen for the non-rebreathe reservoir face mask, levels were also higher with the Oxymizer nasal cannulae and fitted face mask than with ordinary nasal cannulae, where end-tidal oxygen levels were lowest. With all of the face masks there was a small rise in the inspired CO_2 of 2-3mmHg, this was greatest in the non-rebreathe reservoir face mask. There was a small increase in the end tidal CO_2 with the face masks, but differences were small.

Therefore, at a flow rate of 5l/min the percentage oxygen delivered is high and achieves good end-tidal oxygen levels with only small increases in end-tidal and inspired carbon dioxide levels. Nasal cannulae and face masks achieve similar end-tidal oxygen levels. Face

masks do so with small increases in end-tidal and inspired carbon dioxide levels whilst nasal cannulae could possibly be affected by mouth breathing. Given the equivalence in end-tidal oxygen levels, and slight, but different, drawbacks with nasal cannulae and face masks, patients were given the choice between these interfaces.

8.2. Epworth Sleepiness Score questionnaire

Participant's number:							Participant's Initials:				Date of testing: D D/M O N/Y Y Y Y

SUPPLEMENTAL OXYGEN STUDY

EPWORTH SLEEPINESS SCALE

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

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

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

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

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

Situation	Chance of Dozing (0-3)
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. 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 the traffic	

THANK YOU FOR YOUR COOPERATION

8.3. SOX trial primary outcome statistical analysis plan

- Values downloaded at each trial visit will be used and patient's diary recordings will be used as a back-up
    - Machine records will be checked against the diary card
    - In the case of a discrepancy BP machine recording will be used
- Blood pressure and heart will be averaged for each day from the first three values that the patient recorded
- The daily mean of blood pressure and heart rate will be averaged over the three days in the run up to Visit 1 to give baseline values prior to first intervention (days -3, -2, and -1)
- The daily mean of blood pressure and heart rate will be averaged on days 11, 12 and 13 in the run up to Visit 2 to give values following intervention 1
    - Day 14 (the morning of the trial visit) will not be used as this is the night of the sleep study and this may have interfered with sleep
- The daily mean of blood pressure and heart rate will be averaged over the three days in the run up to Visit 3 to give run-in values prior to second intervention (days -3, -2, and -1)
- The daily mean of blood pressure and heart rate will be averaged on days 11, 12 and 13 in the run up to Visit 4 to give values following intervention 1
    - Day 14 (the morning of the trial visit) will not be used as this is the night of the sleep study and this may have interfered with sleep
- The difference in the change in mean blood pressure and heart rate from baseline to follow-up will be compared using a paired t-test, oxygen versus air
- Adjustments for potential modifiers of age, gender, BMI and treatment order will be made using mixed linear effects models.

8.4. Personal correspondence

Turnbull Christopher (RTH) OUH

From: Redline, Susan,M.D.,M.P.H. <SREDLINE@BWH.HARVARD.EDU>
Sent: 14 November 2017 16:23
To: Turnbull Christopher (RTH) OUH
Subject: RE: British Sleep Society Talk

Follow Up Flag: Flag for follow up
Flag Status: Completed

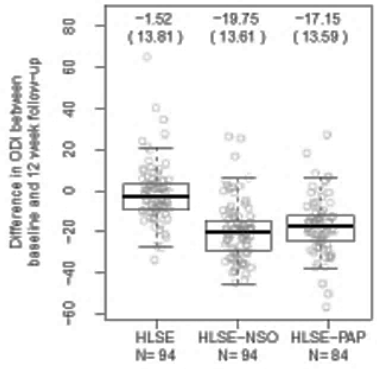
Hi Chris,

The group receiving CPAP and the group receiving supplemental oxygen had similar reductions in nocturnal hypoxemia, with each group having a reduction of at least 64% in the frequency of 3% desaturation events and a decline of at least 2.6% in the percentage of sleep time during which oxygen saturation was below 90% (P<0.001 for the comparison of each of these groups with the control group); there was no significant difference in the rates of either event between the CPAP group and the supplemental-oxygen group.

ODI

Sample size	Test if the effect of CPAP, NSO, and HLSE are the same	Geometric mean ratio in 12 week response (95% CI); p-value		
		CPAP versus HLSE	NSO versus HLSE	CPAP versus NSO
HLSE: 94 CPAP: 84 NSO: 94	P <.0001	0.37 (0.28, 0.47) P<.0001	0.29 (0.23, 0.38) P<.0001	1.24 (0.97, 1.60) P=0.090

- HLSE has higher ODI at 12 weeks than HLSE-PAP and HLSE-NSO.
- Effect of HLSE-PAP on ODI at 12 weeks is similar to HLSE-NSO.



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8.5 Analysis of apnoea length protocol

Creating the Wordpad file:

1. Open Visi-Download
2. Click review and open the file you wish to view
3. File cleaning:
    a. Place the markers over an approximately 20-minute period of stable sleep apnoea
    b. Resave the file and either create a new Wordpad file or write over the last file ensuring that all previous excel file is saved first
4. Run an analysis report inside these markers
5. Once analysis report is open close the report
6. Go to file and then save, this will create a WP1 file in the file directory

Analysing the data for apnoeas

7. Open folder with the file in; desktop, dummy trial files, choose the specific patient and sleep study and open the WP1 file
8. Open the Excel template: Template Interlength 01032016
9. Find all data about unclassified apnoeas:
    a. Copy the raw data from the WP1 file into the RawData tab
    b. Search for [iNumofEvents13] and highlight this row
    c. Copy from here to the row before [iNumofEvents14]
10. Copy this into column A on the RawApnoeas Tab and sort column A alphabetically
11. First find all the apnoea start time information
    a. Copy all data in the format [iEventStartxxx] from column A
    b. Paste in column C
12. Second find all the apnoea length information
    a. Copy all data in the format [iEventLengthxxx] from column A
    b. Paste in column K
13. This should then run several formulae on this tab
    a. The formulae should extend far enough down in terms of rows but check this
14. Copy the values into columns S-V *(paste as values)*
15. Make sure data is stored as a number (click tab to convert to numbers)
16. Check that all values in Row W are correct (y=True, FALSE=false)
    a. If this is the case then all of the event numbers for apnoea length and start times match
17. Copy columns Y-AB and paste this information into SummaryApnoeas Tab *(paste as values)*
18. Sort the apnoea by start time
    a. All except for the last cell should be correct in column F (y=True, FALSE=false)
19. Copy columns I-M and paste to SecondApnoeas Tab *(paste as values)*
20. Delete the last apnoea length value in both columns E
    a. There is no data for this event to say when the next apnoea starts
21. Values should be computed for apnoea length, apnoea interlength and apnoea length: interlength ratio
22. Save file in the file directory under a different name i.e. dummytrialnumber_sleepstudy1

8.6. The relationships between overnight pulse oximetry and sleep study parameters and the primary outcome

	Change HRR (/hour)	AHI (/hour)	Urinary normetadrenaline (nmol/hour)	Venous base excess (mmol/l)	hsCRP (mg/l)	Change in systolic BP (mmHg)	Change in diastolic BP (mmHg)
Change ODI (/hour)	**R=0.32 p=0.02**	**R=0.35 p=0.04**	**R=0.40 p=0.004**	**R=-0.48 p=0.02**	R=0.19 p=0.19	**R=0.38 p=0.007**	**R=0.33 p=0.02**
Mean % time<90% (%)	R=0.09 p=0.54	R=0.04 p=0.81	**R=0.32 p=0.02**	R=-0.37 p=0.08	**R=0.29 p=0.04**	**R=0.35 p=0.01**	**R=0.36 p=0.01**
Mean Sats (%)	R=-0.15 p=0.30	R=-0.08 p=0.65	**R=-0.28 p=0.05**	R=0.53 p=0.008	**R=-0.28 p=0.05**	**R=-0.33 p=0.02**	**R=-0.34 p=0.01**
Change HRR (/hour)	-	R=0.31 p=0.08	R=0.22 p=0.13	R=-0.28 p=0.19	R=0.12 p=0.40	**R=0.37 p=0.008**	**R=0.29 p=0.04**
AHI (/hour)	-	-	R=0.17 p=0.32	R=-0.43 p=0.08	R=0.08 p=0.66	**R=0.37, p=0.03**	R=0.25, p=0.15
Urinary normetadrenaline (nmol/hour)	-	-	-	R=-0.32 p=0.13	R=0.05 p=0.73	**R=0.31 p=0.03**	**R=0.29 p=0.04**
Venous base excess (mmol/l)	-	-	-	-	R=-0.40 p=0.06	R=-0.20 p=0.36	R=-0.16 p=0.46
hsCRP (mg/l)	-	-	-	-	-	**R=0.34 p=0.02**	R=0.26 p=0.07

The correlations between oximetry, heart rate, sleep study and blood pressure parameters for all observations. Results displayed are Spearman's rank correlation coefficients. The change in ODI is the average from nights 8-13 minus the screening on CPAP ODI. The change HRR is the average from nights 8-13 minus the screening on CPAP heart rate rises index. All other pulse oximetry parameters were averaged over nights 8-13. Means sats = the mean oxygen saturations. % time <90% = the percentage of time with oxygen saturations less than 90%. The AHI was taken from the single night sleep study. AHI = apnoea hypopnoea index. The change in blood pressure is the value at either visit 2 or 4 minus the value at either visit 1 or 3.

9. References

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