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REFERENCE
Physiological effects of treatments in obstructive sleep apnoea syndrome

James Moss

This thesis is submitted in partial fulfilment of the requirements of Sheffield Hallam University for award of the degree Doctor of Philosophy

June 2013

In collaboration with Sheffield Teaching Hospitals NHS Foundation Trust
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I would like to extend special thanks to those who have supported my research programme, including the administrative and technical staff at the Centre for Sport and Exercise Science and the physiology and medical staff at Sheffield Teaching Hospitals NHS Foundation Trust. Also to my students, teaching you has been a pleasure and a welcomed distraction.

And finally, to my fiancée Louise, who has endured this process along with me. You have provided daily the right dose of motivation, inspiration and encouragement to allow me to finish my doctoral studies. I dedicate this thesis to you.
Statement of originality

I hereby certify that I am solely responsible for the work contained in this thesis, unless otherwise acknowledged. Others work has been duly cited and the locations of source material can be found in the epilogue. I confirm that neither this thesis, nor the data included within it, has been submitted to Sheffield Hallam University or any other institution in partial or complete fulfilment for an undergraduate or postgraduate degree.

Study one was conceived by Mr Martin Stout and Dr Stephen Bianchi, who also secured funding. Ethics approval was obtained by Dr Garry Tew. The overwhelming majority (>99%) of study activities such as participant recruitment, familiarisation and assessment were conducted by myself (with occasional support from Centre for Sport and Exercise Science staff). Similarly, the delivery of the lifestyle intervention, and data collection were completed by myself. Data analyses were also conducted by myself with guidance from two independent external biostatisticians (based at University College London and University of Sheffield).

Study two was my own concept and design. I and Dr Stephen Bianchi secured funding for the study and I applied for research ethics approval. I conducted all recruitment (with support from Mrs Leslie Mattock), familiarisation and assessment activities with no exceptions. Data analyses were conducted by myself with support and verification of findings from Dr Garry Tew.
Original scientific material arising from this research

Original journal articles:

Presentations:
Sleep Apnoea Trust Association Annual General Meeting (October 2012): Results of SATA-funded research: Effects of a lifestyle intervention in obstructive sleep apnoea. Oxford, UK. (Oral communication; invited speaker)
21st Meeting of the European Sleep Research Society (September 2012): Effects of a lifestyle intervention in overweight OSA patients treated with CPAP. Paris, France. (Oral communication)
22nd European Respiratory Society Annual Congress (September 2012): Effects of a lifestyle intervention in overweight OSA patients treated with CPAP. Vienna, Austria. (Poster communication)
British Association of Sport and Exercise Sciences (September 2011): Effects of a lifestyle intervention in patients being treated for obstructive sleep apnoea. Essex, UK. (Oral communication)

Published abstracts:
### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
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<tr>
<td>ACh</td>
<td>acetylcholine</td>
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<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
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<tr>
<td>AF</td>
<td>atrial fibrillation</td>
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<tr>
<td>AHI</td>
<td>apnoea hypopnoea index</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>ANCOVA</td>
<td>analysis of co-variance</td>
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<td>APU</td>
<td>arbitrary perfusion units</td>
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<td>BL</td>
<td>baseline</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CAD</td>
<td>coronary artery disease</td>
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<tr>
<td>CBT</td>
<td>cognitive behavioural therapy</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CIH</td>
<td>chronic intermittent hypoxia</td>
</tr>
<tr>
<td>CONSORT</td>
<td>consolidated standards for reporting clinical trials</td>
</tr>
<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
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<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
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<td>CPET</td>
<td>cardiopulmonary exercise test</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSA</td>
<td>central sleep apnoea</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>CVC</td>
<td>cutaneous vascular conductance</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>DXA</td>
<td>dual-energy x-ray absorbiometry</td>
</tr>
<tr>
<td>EDS</td>
<td>excessive daytime somnolence</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<tr>
<td>EP</td>
<td>end-point</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>EPC</td>
<td>endothelial progenitor cell</td>
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<tr>
<td>EQVAS</td>
<td>EuroQol visual analogue scale</td>
</tr>
<tr>
<td>ESS</td>
<td>Epworth sleepiness scale</td>
</tr>
<tr>
<td>FITT</td>
<td>frequency, intensity, time, type</td>
</tr>
<tr>
<td>FMD</td>
<td>flow-mediated dilatation</td>
</tr>
<tr>
<td>FU</td>
<td>follow-up</td>
</tr>
<tr>
<td>GTN</td>
<td>glyceryl trinitrate</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>homeostatic model of assessment - insulin resistance</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>HRR</td>
<td>heart rate reserve</td>
</tr>
<tr>
<td>HRT</td>
<td>hormone replacement therapy</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>ICC</td>
<td>intra-class correlation coefficient</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>ISWD</td>
<td>incremental shuttle walk distance</td>
</tr>
<tr>
<td>ISWT</td>
<td>incremental shuttle walking test</td>
</tr>
<tr>
<td>LDF</td>
<td>laser Doppler flowmetry</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LED</td>
<td>low energy diet</td>
</tr>
<tr>
<td>LOCF</td>
<td>last observation carried forward</td>
</tr>
<tr>
<td>LTH</td>
<td>local thermal hyperaemia</td>
</tr>
<tr>
<td>MAD</td>
<td>mandibular advancement device</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>MCID</td>
<td>minimal clinically important difference</td>
</tr>
<tr>
<td>MID</td>
<td>minimal important difference</td>
</tr>
<tr>
<td>MPA</td>
<td>medroxyprogesterone acetate</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MSA</td>
<td>mixed sleep apnoea</td>
</tr>
<tr>
<td>MSNA</td>
<td>muscle sympathetic nerve activity</td>
</tr>
<tr>
<td>NHS</td>
<td>National Health Service</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>OCP</td>
<td>oral contraceptive pill</td>
</tr>
<tr>
<td>ODI</td>
<td>oxygen desaturation index</td>
</tr>
<tr>
<td>OSAS</td>
<td>obstructive sleep apnoea syndrome</td>
</tr>
<tr>
<td>OSA-LC</td>
<td>low-compliance OSAS patients</td>
</tr>
<tr>
<td>OSA-HC</td>
<td>high-compliance OSAS patients</td>
</tr>
<tr>
<td>OSA-UN</td>
<td>untreated OSAS patients</td>
</tr>
<tr>
<td>P-eNOS</td>
<td>phosphorylated endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>PBF</td>
<td>percentage body fat</td>
</tr>
<tr>
<td>PGI₂</td>
<td>prostacyclin</td>
</tr>
<tr>
<td>PIS</td>
<td>participant information sheet</td>
</tr>
<tr>
<td>PORH</td>
<td>post-occlusion reactive hyperaemia</td>
</tr>
<tr>
<td>PSG</td>
<td>polysomnogram</td>
</tr>
<tr>
<td>RCT</td>
<td>randomised controlled trial</td>
</tr>
<tr>
<td>RDI</td>
<td>respiratory disturbance index</td>
</tr>
<tr>
<td>REM</td>
<td>rapid-eye movement</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RPE</td>
<td>rating of perceived exertion</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDB</td>
<td>sleep disordered breathing</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SNP</td>
<td>sodium nitroprusside</td>
</tr>
<tr>
<td>TEM</td>
<td>technical error of measurement</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TSD</td>
<td>tongue stabling device</td>
</tr>
<tr>
<td>TTM</td>
<td>trans-theoretical model</td>
</tr>
<tr>
<td>UPPP</td>
<td>uvulopalatopharyngoplasty</td>
</tr>
<tr>
<td>VLED</td>
<td>very low energy diet</td>
</tr>
<tr>
<td>VSMC</td>
<td>vascular smooth muscle cell</td>
</tr>
</tbody>
</table>
Structure of the thesis

Purpose of the thesis
The increasing prevalence of obesity and its associated morbidities are well reported. Obesity is an independent risk factor for obstructive sleep apnoea syndrome (OSAS), which in turn has been identified as an independent cardiovascular risk factor. OSAS contributes to the development of vascular atheroma and clinical events such as myocardial infarction and stroke. The aetiology of OSAS is multifactorial; however the principal modifiable cause is widely regarded to be obesity. Consequently, the bidirectional relationship of OSAS and obesity has been exploited by inducing a state of negative energy balance using paradigms that address energy intake, energy expenditure or both to reduce adiposity and its associated obstructive events. Prevalence and public awareness of OSAS is likely to increase in coming years. Although continuous positive airway pressure (CPAP) is effective it is not a patient-preferred treatment and interventions to address the causes of airway obstruction (e.g. cervical obesity) as opposed to simple management are warranted. Furthermore, not all patients are fully compliant with CPAP for several reasons, including apathy towards its importance (especially in the absence of daytime symptoms), mask discomfort and intolerance to the air pressure. Consequently, many patients within the population have low compliance, yet rarely are such patients included (let alone allocated together in a low compliance group) in medical research - perhaps because of problems defining what exactly constitutes a low-compliance patient. Consequently, there is a paucity of evidence regarding what level of protection occasional CPAP use offers in terms of the management of daytime symptoms and the prevention of future comorbidity (e.g. cardiovascular disease). Therefore, the purpose of this thesis was to investigate the feasibility of a combined lifestyle intervention in the management of OSAS and to improve our understanding of how treatment of OSAS with CPAP (in particular when compliance with treatment is suboptimal) affects vascular endothelial function in the macro- and microvasculature.
SECTION 1
INTRODUCTION AND LITERATURE REVIEW

Chapter 1
Overview

Chapter 2
Literature review

SECTION 2
Lifestyle intervention in OSAS - a randomised controlled feasibility study

Chapter 3
Introduction
Research question

Chapter 4
Methods

Chapter 5
Results

Chapter 6
Discussion

SECTION 3
Effects of OSAS and its treatment with CPAP on macrovascular and microvascular function

Chapter 7
Introduction
Research question

Chapter 8
Methods

Chapter 9
Results

Chapter 10
Discussion

SECTION 4
Conclusions

Chapter 11
Summary of findings
Implications for practice
Future research
Chapter summary

Chapter one
This chapter provides an overview of this thesis. It includes a brief summary of the history of medicine, a description of the current obesity problem and an appreciation for the development of exercise medicine as a stand-alone field.

Chapter two
Chapter two is a detailed literature review of obstructive sleep apnoea syndrome. It broadly covers aspects of the condition such as epidemiology and economic burden, and in more detail topics such as cardiovascular risk in obstructive sleep apnoea and what kind of lifestyle interventions have already been investigated.

Chapter three
This chapter provides a specific introduction to the first study: Lifestyle intervention in obstructive sleep apnoea syndrome - a randomised controlled feasibility study obstructive sleep apnoea. It includes an outline of the aims and objectives of the study, the research question and original hypotheses.

Chapter four
Chapter four describes in detail the procedures utilised in study one including recruitment strategies, inclusion criteria and exclusion criteria. It also outlines the lifestyle intervention used and what were used as the primary and secondary outcome measures. The methodology is discussed and the procedures employed are justified. The methods of analysis that were to be used to explore the data are also described.

Chapter five
This chapter reports the results of study one. It describes the feasibility of the study design, explores the effects of the intervention on important health outcomes and investigates relationships between the key variables.
Chapter six
Chapter six discusses the primary and secondary findings of study one. It compares key findings with data published in the scientific literature and assesses the strengths, weaknesses and limitations of the study design and of the conclusions that were inferred.

Chapter seven
Chapter seven provides a specific introduction to the second study: effects of OSAS and its treatment with CPAP on macro- and microvascular function. It specifies the aims and objectives of the study, the research question and original hypotheses.

Chapter eight
This chapter describes in detail the equipment and procedures involved in study two. It includes an explanation and justification of the study design and details of the sample size calculation.

Chapter nine
Chapter nine describes the primary and secondary findings of study two.

Chapter ten
This chapter provides discussion of the results of study two. It outlines the interpretation of the research in the context of the original research question.

Chapter eleven
Chapter eleven summarises and discusses the main findings of this research, describes what implications these finds may have on practice and proposes future research.
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The original research in this thesis aimed to investigate physiological effects of different treatment approaches in obstructive sleep apnoea syndrome (OSAS). OSAS is a prevalent public health concern independently associated with increased cardiovascular risk. Specifically, study 1 examined the feasibility of conducting a pragmatic lifestyle intervention in patients reporting compliance with continuous positive airway pressure (CPAP) and collected provisional data about its efficacy, and study 2 investigated the physiological effects of low compliance to CPAP therapy in a four-arm observational study.

The intervention in study 1 involved supervised exercise, dietary advice and behaviour change counselling. Primary outcome measures were recruitment, retention and compliance data and secondary outcome measures assessed anthropometrics, cardiovascular risk, quality of life and exercise capacity. Study 2 investigated macro- and microvascular function, anthropometrics, quality of life, cardiovascular risk and exercise capacity.

The novel findings of this research were: 1) the lifestyle intervention was feasible to deliver; 2) the intervention improved key health outcomes such as exercise capacity (Δ +16%) and serum C-reactive protein (Δ -57%), which were maintained after 3 months of independence (Δ +22% and -57%, respectively); 3) self-reported CPAP compliance is an unreliable indicator of actual compliance; 4) it is difficult to recruit low-compliance patients onto research trials, and recruiting newly diagnosed patients is also difficult without interrupting the patient pathway; 5) vascular function seems impaired in low-compliance patients versus high-compliance patients, although further work is needed to confirm this.

These findings contribute to the growing evidence base for the role of lifestyle intervention in OSAS, and provide provisional data on the effects of low compliance to CPAP therapy on vascular endothelial function. In summary, future research investigating pragmatic lifestyle interventions in OSAS and the physiological effects of low-compliance to CPAP is certainly warranted.
Medicine is a relatively new word for a discipline that is at least 5 millennia old. Evidence of Ancient Egyptian practice from approximately 2000 B.C. describes several ailments and injuries, their prognoses and recommended treatments. Several other texts based on empiricism also date to the second millennium B.C. From the ancient world to recent history, different civilisations have developed their own methods according to their experiences and beliefs. Similar developments based partly on observation, religion and spiritualism have been identified in Ancient China, India, Greece and Rome (Porter, 1999). Hippocrates (b. ~480 BC) and Galen (b. ~ 129 AD) are often credited with the development of the academic teaching and practice of medicine, although until the last 200 years this was not widely accepted. Medicine in Medieval England was principally practiced by Barber Surgeons who trained through apprenticeships and learnt by trial and error.

It is important when considering the state-of-the-art to credit the important discoveries or pioneering procedures that have contributed to the progression of modern medicine. To name a few, the development of the antiseptic principles of surgery by Joseph Lister in 1867 had emphatic results on post-operative outcomes and quickly became ubiquitous (Lister, 1867), the discovery of penicillin by Alexander Fleming in 1928 revolutionised the treatment of bacterial infection (Fleming, 1929), and the first successful organ transplant (kidney) by Joseph Murray and colleagues in 1954 confirmed the feasibility of replacing a dysfunctional human organ with a healthy donor organ (Porter, 1999). On the cutting edge, after the existence of pluripotent stem cells was first purported in the early 1900s, research exploded during the late 1990s, which promises one day to yield laboratory techniques that will allow physicians to simply grow a patient a replacement organ using their own cells - abolishing the issues of organ-matching and organ rejection. Although such wonderful endeavours will help some branches of medicine, others still require alternatives.
Since the mid-twentieth century the prevalence of obesity has increased dramatically. This epidemic can be fundamentally attributed to a sustained period of positive energy balance within the population; however the precise factors responsible for this have not yet been unequivocally identified. Two common-cited culprits are the augmented energy intake (e.g. the availability and consumption of energy-dense foods) and decreased daily energy expenditure consequent of technological advances and modern conveniences. Changes in diet patterns from home-cooked meals prepared with local produce to store-bought pre-prepared meals have increased the amount of complex carbohydrates and saturated fats consumed. Similarly, urbanisation, improved transport (e.g. personal motor vehicles and public transport) and an increase in sedentary behaviour both in the workplace (e.g. working at a desk) and at home (e.g. watching television or playing computer games) has affected all developed countries, both sexes, all ages and all levels of socioeconomic class. Beyond these, more novel factors have been proposed including infections, maternal age, sleep debt and ambient temperature (McAllister et al., 2009). The majority of evidence investigating these factors is retrospective and correlative, and it is often impractical or unethical to conduct randomised controlled trials to properly test these hypotheses. Current treatments for obesity include interventions to promote positive changes in lifestyle (e.g. adoption of healthy eating and increased physical activity), surgery (e.g. gastric banding) and pharmacological therapy to reduce energy intake (e.g. Orlistat therapy).

The role of exercise training in the prevention, management and treatment of chronic disease has gained increasing interest from medical researchers in recent decades for several reasons. Firstly, regular exercise is associated with holistic personal benefits such as improved fitness, improved vitality, weight loss, improved self-image, improved mood and expanded social networks (Burnham, 1998; Penedo & Dahn, 2005). Secondly, compared with other treatments such as medication or surgery, the provision of exercise can be very cost-effective. Thirdly, the intensity, duration, frequency and type of exercise are reasonably within the control of the patient, which allows patients to reduce or withdraw the exercise stimulus at the first sign of any adverse reactions. Evidence of the public health burden of physical inactivity has
accumulated over recent decades and the recognition of Sport and Exercise Medicine as a stand-alone section of the Royal Society of Medicine is testament to this.

It is important to discern the difference between exercise and physical activity, which are often incorrectly used interchangeably. Definitions proposed by Caspersen et al. (1985) described physical activity as deliberate bodily movement resulting from coordinated contraction of skeletal muscle groups; and, exercise as a planned, structured and goal-directed subset of physical activity. However, such definitions have their limitations by including movement as a requisite feature, which excludes activities involving isometric muscular activation (Winter & Fowler, 2009). These authors proposed an alternative definition for exercise: "a potential disruption to homeostasis by muscle activity that is either exclusively, or in combination, concentric, eccentric or isometric". However, it is unclear using this definition how one could discern the difference between exercise and non-exercise physical activity. Hereafter, physical activity will refer to routine activities that require meaningful effort (e.g. climbing three flights of stairs instead of using an elevator) and exercise will refer to an allocated period of time used for purposeful activity of at least moderate intensity.
2.1 What is obstructive sleep apnoea syndrome?

2.1.1 Overview

Obstructive sleep apnoea syndrome (OSAS) is the most common form of sleep disordered breathing (SDB), and is characterised by episodes of obstructed ventilation during sleep. This leads to reduced arterial blood oxygen saturation, intrathoracic pressure fluctuations and subsequent centrally-stimulated microarousals to restore airway patency. The aetiology of these obstructions is often multifactorial, with excessive cervical adiposity, reduced nocturnal tonus of airway smooth muscle and enlarged soft structures of the oropharynx usually implicated. The degree of obstruction can vary from complete cessation of airflow (apnoea) to reduced (and inadequate) airflow (hypopnoea; defined as <20% air flow). The frequency of apnoeic events is referred to as the apnoea hypopnoea index (AHI), which constitutes the defining criteria for different severities of OSAS (National Institute for Care and Health Excellence, 2010); mild (5 to 15 events per hour), moderate (15 to 30 events per hour) and severe (more than 30 events per hour).

The nocturnal symptoms include habitual excessive snoring (caused by turbulent airflow through the upper airway), witnessed pauses in breathing and awakening with a choking sensation. However, the principal symptoms for OSAS are excessive daytime sleepiness (EDS) and fatigue which are often dismissed as trivial by patients and general practitioners alike - consequently it is likely that only a small proportion of people afflicted have actually received a diagnosis and are receiving treatment. In addition to EDS, OSAS can contribute to impaired cognitive function, reduced concentration, irritability and increased risk of falling asleep during the day (possibly when driving a motor vehicle, which could have potentially fatal consequences).

The most common treatment for OSAS involves positive airway pressure therapy delivered continuously via a full-face or nasal mask interface. This high-pressure air provides an internal pneumatic splint that increases airway patency and thereby reduces the collapsibility of the airway (see section 2.8.1). This mainstay treatment
provides management only and does not constitute a cure; patients must adhere to therapy while trying to address modifiable underlying causes (such as obesity). Although other treatment options (e.g. mandibular repositioning devices and surgery) are available they are not as widespread. Many are useful only in particular aetiologies or for lower severities of OSAS.

2.1.2 Pathogenesis

SDB can be described as irregular breathing patterns during sleep and classified as obstructive (i.e. the airway is mostly or completely occluded), central (i.e. impaired autonomic respiratory control) or mixed (both obstructive and central). When SDB is obstructive and completely occludes airflow it is termed apnoea, and airflow reduced to 20 per cent of normal is called hypopnoea. Obstructive events mostly occur in the upper airway in the retropalatal and retroglossal parts of the oropharynx and less often in the nasopharynx and laryngopharynx. Apnoea occur in healthy individuals and SDB is only present when the mean event frequency exceeds five per hour of sleep. If the apnoea frequency is normalised with the application of positive airway pressure then a diagnosis of OSAS can be made.

Patients with OSAS are generally considered to have an airway that has an anatomical predisposition to collapse, although there is no direct evidence to support this. This likely involves a narrower-than-normal airway, which itself could be hereditary or acquired (caused by inflammation or increased pharyngeal fat deposits). Fatty infiltrates in the glossus and soft palate increase the mass of these structures, which compounds congestion in the upper airway and reduces airway calibre from the anterior direction. Similarly, increased parapharyngeal fat stores reduce airway calibre bilaterally, which contributes to reduced cross-sectional area of the airway and an increased propensity for collapse. Apnoea are absent during wakefulness because of upregulated activation of the smooth muscle of the airway, which maintains the patency. In addition to ventilation, upper airway patency is important for deglutition and speech. Also, when the thorax is upright gravity pulls loose structures in a downward direction – when supine gravity pulls them in a transverse direction across the airway and occluding it. Once an individual begins sleeping, the upper airway musculature that is under voluntary control (e.g. which controls the complex
movements required for speech) is no longer activated. Similarly, activation of the involuntary dilator muscles is reduced (hypotonic) and the aforementioned gross structures can easily cause obstruction in response to the negative pressures caused by inspiration. Moreover, there is evidence to support a rostral fluid shift as a contributory factor to airway collapse in OSAS. This theory proposes that the gravity-induced fluid accumulation in the lower extremities during the daytime redistributes overnight when in a supine position, which induces mild oedema in tissue around the neck that contributes to airway narrowing. Experimental evidence has shown significant relationship between the frequency of apnoeic events and the volume of fluid displaced from the legs (Redolfi et al., 2009).

Obstructive events are much more common than central events and a discrete risk profile has been identified that includes, in likely order of importance, obesity (Dempsey et al., 2002), the male sex (Young et al., 1993) and particular craniofacial structures (Dempsey et al., 2002). There is evidence to suggest that age is a risk factor for OSAS during middle-age (40 to 60 years), however beyond 60 its effects are negligible (Young et al., 2002a). The contribution of obesity to the development of OSAS is discussed further in section 2.7. Briefly, increasing obesity is associated with an increased risk of developing OSAS – a 10% increase in body mass is associated with a six-fold increase in risk of developing OSAS over four year period (Peppard et al., 2000a). Observational studies have also identified relationships between neck girth, adiposity and visceral fat to the presence and/or severity of OSAS (Vgontzas et al., 2003). Tendency for Western populations becoming “fatter” over the last 40 years suggests that the incidence of obstructive SDB is probably increasing. Incidence of other obesity-related ailments such as hypertension, diabetes and arthritis has also increased over the same period (Whelton, 1994; Winer & Sowers, 2004; Reginster, 2002).

There could also be a familial component to the risk profile for OSAS, although evidence is unclear whether this is an environmental (familial eating habits, lifestyle behaviours or socioeconomic status) or genetic (inherited craniofacial structures or genes that promote obesity) link. Varvarigou et al. (2011) reviewed the field of OSAS genetics and highlighted that although several studies have provided evidence, these studies have potentially been underpowered. They are also relatively scarce; the
authors tried to meta-analyse current literature but acknowledged the small pool of suitable studies, and the absence of reported covariates (e.g. age, sex, apnoea frequency) as limitations.

2.1.3 Events of the apnoeic cycle

The apnoeic cycle starts with an obstruction of the upper airway that leads to a state of hypoxia and hypercapnia. This activates peripheral chemoreceptors causing a compensatory increase in pulmonary effort that is unable to restore airflow. Eventually, hypoxaemia activates central chemoreceptors which cause a microarousal (the patient rouses just enough to change body position and restore upper airway patency) and a subsequent period of hyperventilation to counter the hypoxia and hypercapnia (Wiegand & Zwillich, 1994). These arousals are associated with a brief rise in blood pressure (Davies et al., 1993). The frequency of apnoea per hour of sleep is presented as the apnoea hypopnoea index (AHI). The cyclic desaturation and reoxygenation that accompanies each apnoea can produce excess reactive oxygen species (ROS; Lavie et al., 2003), or free radicals, which play a key role in cell damage (Valko et al., 2007). This is discussed further in section 2.5.1.

2.2 Historical perspectives

2.2.1 Obstructive sleep apnoea syndrome

Although potential evidence of SDB has been identified as long ago as 300 BC (Michalopoulos et al., 2003), the physiological manifestations of OSAS were first described not by scientists or physicians, but by English novelist Charles Dickens (b. 1812 AD) in 1836 (Dickens, 1949) in a regular publication entitled “The Pickwick Papers”. Dickens described a fat boy with an enormous appetite, who had a tendency to fall asleep at inconvenient times. The obesity and daytime sleepiness to which he referred would in due course become hallmarks of OSAS.

Caton (1889) published a case study describing an obese patient who had gained 43 kg in weight over 2 years and had started suffering from chronic sleepiness, falling asleep whenever he sat down and occasionally when upright. Caton observed his patient asleep and noticed the upper airway closure, futile hypercontraction of respiratory
muscles and cyanotic skin. This was followed by an arousal which did not awaken the patient, but restored airway patency, reversed the cyanosis and stimulated a brief deeper-than-usual period of ventilation. In his article, Caton pleaded for assistance from readers as to how to manage this patient as he could find no prior evidence of the condition in medical literature. His provisional diagnosis was narcolepsy, which had been described with a similar presentation nine years prior by Jean-Édouard Gélineau (1881).

Later in 1889, Morison described how a patient would fall asleep while engaged in an enjoyable game of cards and begin snoring (Morison, 1889). Morison wrote “I have myself observed him asleep in bed with an intensely cyanotic countenance, a condition from which he was aroused after a snorting and choking sound issued from his respiratory passages, the cyanosis then gradually disappearing”. Resulting from Dickens’ description half a century prior, Morison coined the condition he observed as Pickwickian Syndrome. Burwell et al. (1956) published a case study describing a patient presenting with obesity, fatigue and somnolence. Their patient described a recent weight gain and a tendency to fall asleep during daily routine. Historically, obesity was regarded as fashionable and an indicator of esteem, wealth and power. Consequently, many prominent historical figures reported to have been obese may have suffered from OSAS. Researchers have identified historical reports of now-established symptoms being exhibited by prominent leaders such as included Queen Victoria of the British Empire (Brewer, 2000), Emperor Napoleon Bonaparte (Chouard et al., 1988) and President William H. Taft of the United States (Sotos, 2003). The potential impact of undiagnosed OSAS on their decision-making and leadership and how treatment may have changed the course of history is worth consideration (Polkey et al., 2004).

### 2.2.2 Lifestyle

The bidirectional relationship between lifestyle (diet and exercise) and health is not a novel idea. Prominent Ancient Greek philosopher Hippocrates and Galen of Ancient Rome advocated the role of exercise in health and wellbeing. Hippocrates is reported to have said that “Eating alone will not keep a man healthy; he must also take exercise. For food and exercise, while possessing opposite qualities, yet work together to
produce health.” (Jones & Withington, 1952). His statement promotes the importance of balanced sustenance and physical activity in the maintenance of good health. Similarly, Galen’s theory of health involved six non-natural (or what we would now refer to as modifiable) components that included diet and exercise independently (as well as air, sleep, excretion and thought) as factors of which too much or too little would contribute to disease (Berryman, 1989). This thesis was still maintained by physicians during the sixteenth (Méndez, 1960) and eighteenth (Fuller, 1718; Buchan et al., 1774) centuries. In the modern medical literature the appreciable role of exercise (or a lack thereof) resurfaced in the mid-twentieth century and the American College of Sports Medicine (ACSM) was formed (1953), which is now an influential organisation in the field of sports medicine. Similarly, books such as Kraus & Raab (1961) explored the impact of a sedentary lifestyle on the prevalence of disease. Throughout history, the importance of a balanced lifestyle has been appreciated and with the current economic downfall, therapies (adjunct or otherwise) utilising the relative low cost of lifestyle modification must continue to be investigated.

2.3 Diagnosis

A positive diagnosis for OSAS requires confirmation of frequent nocturnal apnoea. This can be evidenced by monitoring ventilation dynamics and/or oxyhaemoglobin saturation during sleep. Overnight polysomnography (PSG) is the measurement multiple variables during sleep to diagnose sleep disorders. Common inputs include airflow (using a nasal cannula and pressure transducer to detect interruptions in the sinus breathing pattern), chest expansion (using a thoracic pressure band; expansion in the absence of airflow indicates an apnoea), electroencephalography (to determine states of sleep using brain activity), electrooculography (to identify rapid eye movement sleep), pulse oximetry (to identify periodic blood oxygen desaturations as a consequence of interrupted breathing) and heart rate (Flemons et al., 2003). An example of what a typical apnoeic trace could look like is presented in Figure 2.1. Note the peri-apnoeic cessation of nasal airflow, reduction in oxygen saturation and increase in heart rate, and the post-apnoeic increase in respiratory muscle activation,
increased depth of inspiration that steadily normalises, a return to pre-apnoeic oxygen saturation, increased skeletal muscle activity and EEG changes.
Figure 2.1 Polysomnogram Multiple channels of an overnight polysomnogram, showing recruitment of the respiratory musculature, nasal airflow, pulse oximetry, heart rate, skeletal muscular activity (to change position and restore airway patency) and encephalography to show brain activity. The period of apnoea and microarousal are highlighted at the bottom of the figure. Adapted from Kapur et al. (2010).
Overnight PSG is usually performed during normal sleeping hours in a sleep centre. This test is expensive, uncomfortable and often inconvenient for patients. Some centres use a simplified take-home version which can be complicated for patients to set up themselves. Routine clinical practice often utilises overnight pulse oximetry alone to confirm or exclude OSAS, which is more practical as the device is simple to attach and switch on and can be performed in the patient's own home. Afterwards, data are downloaded from the device and the frequency of nocturnal oxyhaemoglobin desaturation (oxygen desaturation index; ODI) can be measured. At this stage, it is not possible to make a conclusive diagnosis of OSAS as central sleep apnoea (CSA) and mixed sleep apnoea (MSA) could provide similar oximetry. To distinguish between these, patients are issued with a positive airway pressure device for a trial period, with the final night involving concurrent pulse oximetry. This device uses pressurised air to splint the airway and prevent the previously observed desaturations (discussed further in section 2.8.1); a normal ODI is indicative of OSAS, an improved but abnormal ODI suggests MSA, and an unchanged ODI indicates CSA. The events per hour thresholds for mild, moderate and severe used for AHI are often shared by ODI in clinical practice, despite the two not necessarily being equivalent.

Several questionnaires have been devised to facilitate the identification of potential OSAS and to assess its impact on subjective tiredness. The Berlin Sleep Questionnaire is a useful tool for evaluating the OSAS risk by asking multiple choice questions that assess sleep habits, daytime tiredness and general health (blood pressure, BMI). Two or more positive categories are highly suggestive of OSAS. This questionnaire has been validated as a screening tool (Chung et al., 2008). The Epworth Sleepiness Scale (ESS) devised by Johns (1991) is a subjective questionnaire that quantifies patients' self-reported daytime somnolence. Patients are asked their likelihood of falling asleep in eight different situations (such as watching television or as a passenger in a car) by providing a response between 0 (not likely at all) and 3 (very likely). The scores for the eight domains are summed to provide an overall score - less than 10 is considered normal and 10 or above suggests the presence of a daytime sleepiness (and potentially a sleep disorder). The ESS has been shown to be valid in OSAS, although its reproducibility is unclear (Nguyen et al., 2006; Knutson et al., 2006).
2.4 Epidemiology

OSAS has received appreciable attention only in recent decades in part because of population-based prevalence studies in Sweden (Gislason et al., 1988), Australia (Bearpark et al., 1995) and the USA (Young et al., 1993; Kripke et al., 1997). These studies identified a higher-than-expected prevalence that stimulated interest in the causes and consequences of OSAS. Prevalence surveys suggest that 2% women and 4% of men at middle age are affected by this syndrome and it is becoming increasing common with the current obesity epidemic (Young et al., 2002b). Punjabi et al. (2008) put this number between 3 and 7% in the general public. Estimates of prevalence are varied as a result of different populations, in different countries, with different criteria used to define and measure SDB. Davies and Stradling (1996) reviewed 12 prevalence studies from across the world and concluded that 1-5% of community-dwelling adult men have SDB, and that prevalence increases with obesity. The prevalence of minimally symptomatic OSAS (i.e. without daytime symptoms) is estimated to be 20% in the adult population (Young et al., 2002b).

There is some evidence to suggest that female sex hormones have a protective effect in pre-menopausal women, with incidence in post-menopausal women being 3.5 times greater than peri-menopausal counterparts (Young et al., 2003). In further support, incidence has been shown to be similar between post-menopausal women and age- and BMI-matched men (Resta et al., 2003), and women receiving hormone replacement therapy (HRT) to be half as likely to have an AHI>15 events·h^{-1} than those not on HRT (Shahar et al., 2003). Although HRT would seem to offer benefit in reducing SDB, its long-term effects on cardiovascular disease are still unclear (Nabulsi et al., 1993; Hulley et al., 2002; Manson et al., 2003).

OSAS is an underdiagnosed condition and Young et al. (1997) estimated that 82% of men and 93% of women with moderate-to-severe OSAS remain undiagnosed - this was based on findings from 4925 American adults in employment. Moreover, a study of 5615 community-dwelling adults showed increasing prevalence of mild OSAS associated with age, but a plateauing of moderate or worse disease (approximately 20% in adults aged 60-69, 70-79 and 80-99 years), and an overall prevalence rate of 18% (defined as AHI>15 events·h^{-1}; Young et al., 2002a). The prevalence of moderate-
or-worse OSAS was 3 times larger (32 versus 10%) in the obese quartile (BMI>30.9 kg·m⁻²) versus the normal weight quartile (BMI<24.4 kg·m⁻²). Another finding of this study was that the odds ratio of having an AHI>15 events·h⁻¹ after a BMI increment of 5.3 kg·m⁻² (adjusted for sex and race) progressively decreased in a linear trend with age, from 2.0 [1.7, 2.4] to 1.3 [1.1, 1.5]. A small study showed a prevalence of 53% (38 of 72) undiagnosed OSAS sufferers in obese otherwise healthy participants recruited from the community (Yim-Yeh et al., 2010).

Despite the widely-reported high prevalence of occult OSAS, some studies have not controlled for the possibility of unsuspected non-symptomatic OSAS in their age- and BMI-matched control groups. As a result studies evaluating the impact of obesity alone on endothelial dysfunction may report findings that were distorted by or attributable to undiagnosed OSAS.

Prevalence of OSAS in children is unclear, and is thought to affect up to 2% of children (Ali et al., 1993; Gislason et al., 1995; Redline et al., 1999). Adenotonsillectomy can cure OSAS in between 75 and 100% of children, independently of obesity (Schechter et al., 2002). This suggests that the pathogenesis of childhood OSAS is simpler than in adults, although others consider reduced compensatory upregulation of vasomotor tone (Marcus et al., 2000). In further support of the inheritability of OSAS, a case-control study comparing 22 children with at least one parent with confirmed OSAS with age- and gender-matched controls showed higher incidence of paediatric OSAS in those with OSAS-afflicted parents (Lucas, 2013). These results suggested higher frequency of OSAS symptoms, and possibly the condition itself, in the children with OSAS and recommended future research to test this hypothesis.

2.5 Pathophysiology

Pathophysiological consequences of OSAS were not truly appreciated until the 1980s. OSAS is independently associated with cardiovascular comorbidity, probably caused by the transient episodes of hypoxia and hypercapnia, and the cyclical fluctuations in intrathoracic pressure. These mechanisms are summarised in Figure 2.1.
Figure 2.2 Potential mechanisms linking obstructive sleep apnoea and cardiovascular disease. Arrows with complete and dashed lines to be interpreted the same. This figure has been adapted from one appearing in Kent et al. (2011)
2.5.1 Oxidative stress

Hypoxaemia can be measured in a laboratory setting by measuring the partial pressure of oxygen in an arterial blood sample \( (P_{a}O_2) \); however this requires specialist equipment and an arterial blood sample, which is more problematic to collect than its venous counterpart. The most convenient alternative is to measure the oxygen content of arterial blood using pulse oximetry. The haemoglobin dissociation curve is sigmoidal, and consequently if saturation drops below 90% (or \( P_{a}O_2 <60 \text{ mmHg} \)) then small further decrements in \( P_{a}O_2 \) can lead to profound reductions in oxygen saturation. Oxygen relies on a pressure gradient both to bind to haemoglobin in the pulmonary circulation and to diffuse across the vascular endothelium into cells; therefore hypoxaemia can lead to hypoxic stress in the arterial wall.

Although endogenous ROS are produced naturally as a metabolic product, clearance mechanisms are in place to limit accumulation; if production significantly exceeds clearance then cellular damage is likely. The characteristic reoxygenation seen in OSAS after apnoea-induced hypoxaemia increases the production of ROS, in a similar manner to reperfusion injury seen after a period of ischaemia (Zweier et al., 1988). In vivo markers of oxidative stress (such as 8-hydroxy-2’-deoxyguanosine excretion in urine) have been used in the quantification of oxidative stress in OSAS. Yamauchi et al (2005) showed an independent association between OSAS and oxidative stress and that ODI correlated better with oxidative stress than AHI. Interestingly, superoxide dismutase pre-treatment reduced ROS formation during reperfusion of an ischaemic heart (Zweier, 1988). Hypoxaemia also increases expression of intercellular (ICAM) and vascular adhesion molecules, which increases adhesion of leukocytes to the vascular endothelium increasing local oxidative stress (Walzog & Gahtgens, 2000).

Hypoxaemia can impede aerobic energy production. Circulating 2,3-diphosphoglycerate has been shown to be elevated in OSAS (McKeon et al., 1990). This by-product of glycolysis increases the dissociation of oxygen from haemoglobin, which probably improves tissue oxygen delivery during hypoxaemia. The ratio of uric acid to creatinine is elevated in OSAS caused by hypoxaemia, and is successfully normalised with continuous positive airway pressure (CPAP) therapy (McKeon et al., 1990; Sahebjami, 1998). Although initially dismissed as a useful index of treatment efficacy
(McKeon et al., 1990), this possibility has since been reiterated (Sahebjami, 1998). Oxidative stress has also been implicated in the pathogenesis of obesity and metabolic disease, which themselves are risk factors for cardiovascular disease (CVD). It seems astute to assume prolonged periods of non-treatment will probably result in cardiovascular morbidity.

2.5.2 Sympathoexcitation

Persons with OSAS have chronic daytime sympathoexcitation (Carlson et al., 1993) the cause of which is currently unknown. Repeated episodes of increased muscle sympathetic nerve activity (MSNA) measured using microneurography have been observed during apnoea (Hedner et al., 1995). Episodes of nocturnal sympathoexcitation have been linked to activation of the peripheral sympathetic chemoreflex by the hypoxaemic/hypercapnic stimuli as the increased sympathetic traffic is curtailed by administration of a hyperoxic breathing gas (Leuenberger et al., 1995). Patients often suffer from OSAS for years before receiving a diagnosis and treated so it is possible that the pathogenesis of chronic sympathoexcitation is due, in part, to long-term activation of the peripheral chemoreflex during nocturnal desaturations. Further to this, Greenberg et al. (1999) demonstrated the exaggerated sympathetic response to chemoreflex stimulation in a rat model. Narkiewicz et al. (1998) demonstrated increased MSNA in obese OSAS patients but not in obese OSAS-free controls. Hypoxaemia is detected by specialised chemoreceptors in the carotid body, which to a lesser extent also detect hypercapnia and acidosis. Activation of the carotid chemoreceptors stimulates an increase in sympathetic outflow. This causes peripheral vasoconstriction and subsequent increased peripheral resistance and blood pressure. Experimental evidence suggests that the sympathetic nervous system-induced vasoconstriction is the most important contributor to the development of hypertension in OSAS.

Sympathetic nervous system activity can also be investigated experimentally by measuring catecholamine concentrations. Although urinary catecholamines have been consistently shown to be elevated in OSAS (Stenlof et al., 1996; Dimsdale et al., 1995) there is mixed evidence for plasma catecholamines (Carlson et al., 1993; Hedner et al., 1995; Dimsdale et al., 1995). Sustained elevated sympathetic drive is thought to be
hypoxygena-driven, and not mediated by hypercapnic stimuli. Although there is an acute sympathetic response to the apnoea-induced hypoxygenaemia, evidence suggests that chronic activation of this pathway can lead to a state of daytime sympathoexcitation (Somers et al., 1995; Carlson et al., 1993). Hypoxaemia and increased sympathetic nerve activity can cause oxidative stress and endothelial dysfunction, which in turn promote the development of cardiovascular disease (Kuniyoshi et al., 2010; Ip et al., 2004). Some studies have reported reductions in MSNA after treatment, however evidence suggests short-term therapy is insufficient to reverse the sympathetic hyperactivity (Jennum et al., 1989; Waradekar et al., 1996), although longer treatment periods have shown significant reductions (Stenlof et al., 1996; Narkiewicz et al., 1999).

Experimental evidence has shown the acute post-apnoea systemic blood pressure spike to be mediated solely by activation of the sympathetic nervous system (Katragadda et al., 1997; Schneider et al., 2000). This activation has been demonstrated (experimentally, using supplemental oxygen therapy) to be caused by chemoreceptor activation and that the negative intrathoracic pressures are unlikely to be involved (Morgan et al., 1993).

### 2.5.3 Inflammation

There are several ways OSAS can promote a state of inflammation. Elevated sympathetic outflow increases peripheral vasoconstriction which increases systemic vascular pressure. This increases luminal shear stress which in chronic cases can cause damage to the vascular endothelium. This damage, as described in more detail later (section 2.5.5), triggers local endothelial cells into a pro-inflammatory state, which increases cytokine and chemokine secretion. Interleukin-6 is a cytokine released in response to tissue damage, which binds to receptors in the liver and triggers synthesis of C-reactive protein; CRP in turn can induce expression of ICAMs. Serum CRP concentration in healthy adults is stable at <1 mg·L⁻¹; however in proinflammatory states (such as myocardial infarction) these concentrations can increase exponentially. It has been shown that even modest increases in CRP are associated with increased risk of atherogenesis in otherwise healthy men (Ridker et al., 1997) and women (Ridker et al., 2000) and hence it is considered to be an independent risk factor for ischaemic
heart disease (Ridker et al., 1997; Lind et al., 2003). Serum CRP is elevated in patients with OSAS compared to non-OSAS controls (Shamsuzzaman et al., 2002). Mills et al. (2009) reported a diurnal relationship between CRP and time of day; higher between 10 p.m. and 6 a.m. versus 8 a.m. to 8 p.m. (P<0.001), perhaps resulting from the nocturnal physiological assault caused by repeated apnoea. Koc et al. (2011) suggested that a 1% increase in delta CRP (midday CRP minus 6 a.m. CRP) reflects a 58% increase in risk of cardiovascular events. Elevated plasma CRP almost exclusively indicates inflammation; however there is no way to ascertain the location, type or cause of inflammation from its measurement. Other circulating markers of systemic inflammation including plasma cytokines (Minoguchi et al., 2007), serum amyloid-A (Svatikova et al., 2003) and adhesion molecules (Ohga et al., 2003) are also elevated in OSAS.

### 2.5.4 Intrathoracic pressure oscillations

The presence of respiratory effort is the principal difference between OSAS and CSA. In OSAS, apnoeic events include repeated futile contractions of respiratory muscles despite no airflow through the conducting airway. This causes transient reductions in intrathoracic pressure, leading to reductions in left ventricular stroke volume, cardiac output and systolic blood pressure (caused by less blood entering the circuit). Once ventilation is restored, stroke volume and cardiac output return to normal, ejecting high pressure blood into a systemic circulation with increased resistance caused by sympathetic vasoconstriction. This causes a temporary elevation of systolic blood pressure that quickly subsides.

It has been reported that moderate to severe OSAS is associated with abnormal ventricular filling and impaired diastolic function (Otto et al., 2007), ventricular dysfunction (Romero-Corral et al., 2007) and left ventricular afterload (Arias et al., 2005). This causes decreases in intrathoracic pressure, which increases transmural pressure gradients on the chambers of the heart and thoracic conduit vessels (Buda et al., 1979) leading to myocardial remodelling (Chan et al., 2009) and haemodynamic instability (Somers et al., 1993).
2.5.5 *Endothelial dysfunction*

The vascular endothelium is the single-cell layer of the luminal surface of blood vessels, which until the early 1980s was thought to do little more than act as a barrier to separate circulating blood and tissue. Since then, a multitude of vital roles have been identified, which include the control of coagulation and vascular tone. Indeed, it could be considered to be the largest paracrine and endocrine structure in the human body (Änggård *et al.*, 1990). The healthy endothelium secretes substances that create an anti- or pro-thrombotic environment as required (Wu *et al.*, 1996). These include heparin sulphate and prostacyclin (PGI$_2$) that impair clot formation, and von Willebrand's factor and factor V which promote clot formation. Endothelial dysfunction disrupts normal coagulative processes, which may cause hypercoagulation under normal circumstances and hypocoagulation in response to haemorrhagic injury – increasing the possibility of infarction and uncontrolled blood loss, respectively.

The ability of blood vessels to self-regulate their tone is critical in the management of blood pressure (e.g. during critical blood loss) and controlling the distribution of blood flow (e.g. during exercise). The endothelium secretes vasodilators such as nitric oxide (NO), PGI$_2$ and endothelium-derived hyperpolarising factor (EDHF) that induce relaxation of vascular smooth muscle cells (VSMCs) in the adventitial layer of blood vessels. This relaxation increases luminal diameter, which reduces intravascular pressure and increases blood flow (as a fluid in a high pressure circuit will always favour the path of least resistance). Conversely, secreted vasoconstrictors such as endothelin and angiotensin-converting enzyme (ACE) stimulate contraction of VSMCs and reduce luminal diameter, increasing vascular pressure and reducing blood flow. Dysregulation of vasomotor tone caused by endothelial dysfunction impairs the ability to alter local blood flow in response to a local stimulus. For example, a sudden increase in arterial blood flow that increases blood pressure and shear stress on the endothelium would not be addressed by sufficient vasodilation. This can have further adverse effects on the endothelium, and contribute to further dysfunction as described later, a physiological response measurable in the flow-mediated dilatation test.
The endothelium is semi-permeable to a plethora of substances that can enter or exit the blood stream using diffusion or active transport through membrane channels, pumps and gap junctions. Ineffective transendothelial solute transport between circulating blood and respiring tissues will impede effective clearance of cellular and metabolic waste products (e.g. carbon dioxide and potassium) and delivery of oxygen and nutrients. Dysfunction of these mechanisms is thought to be a precursor for atherogenesis (Libby, 2006).

Endothelial function can be assessed in vivo using non-invasive techniques such as arterial Doppler ultrasonography, laser-Doppler flowmetry (LDF) and strain-gauge venous plethysmography, often coupled with a provocation stimulus to test vascular reactivity. Invasive alternatives do exist, such as endothelial cell harvesting, which involves catheterisation of a vein and collecting endothelial cells directly using a guidewire. Assessment of endothelial function is particularly relevant to obstructive sleep apnoea patients because endothelial dysfunction in the peripheral vasculature is a key early event that precedes or accelerates the development of atherosclerosis (Ross, 1999). Furthermore, it is thought that early identification of endothelial dysfunction could predict future cardiovascular events (Anderson et al., 1995; Perticone et al., 2001) and provide opportunity for pre-emptive action.

NO is an important vascular cell signalling molecule and its bioavailability directly correlates with endothelial function. NO is a heterodiatomic product of the conversion of L-arginine to L-citruline in the presence of endothelial nitric oxide synthase (eNOS). Therefore, the limiting factors in NO production are the bioavailability of the enzyme (eNOS) and the substrate (L-arginine). L-arginine can be biosynthesised, or absorbed in the diet from common food groups such as dairy and meat. Expression of eNOS has been measured in OSAS studies before; Jelic et al. (2008) proposed that the long term oxidative stress in untreated OSAS reduces eNOS enzymatic activity by suppressing eNOS phosphorylation (Tanaka et al., 2005). Jelic et al. (2008) provided evidence for vascular inflammation in OSAS, with an upregulation of the endothelial expression of cyclo-oxygenase-2 (COX-2) in harvested venous endothelial cells. This research group went on to identify significantly lower concentrations of eNOS and phosphorylated-eNOS (P-eNOS) in normal weight and obese OSAS patients versus BMI-matched OSAS-free control groups (screened for undiagnosed OSAS) (Jelic et al., 2010).
Endothelial function has been assessed in OSAS patients before (Ip et al., 2004; Trzepizur et al., 2009; Yim-Yeh et al., 2010). B-mode Doppler ultrasonography can be used to measure flow-mediated dilatation (FMD) in conduit arteries after a distal arterial occlusion (Celermajer et al., 1992). This technique assesses endothelial function in the macrovasculature by measuring the change in brachial artery diameter after a period of forearm ischaemia. Conversely, LDF can be used to assess microvascular function by measuring changes in skin blood flow following provocation (Turner et al., 2008); which can include a proximal cuff occlusion (that induces a post-occlusion reactive hyperaemia; PORH), local heating (that induces local thermal hyperaemia; LTH) or administration of vasoactive compounds (such as acetylcholine (ACh)) which can be delivered intra-venously, intra-arterially (Kato et al., 2000), subcutaneously or iontophoretically. FMD, although requiring substantially more training, is currently the preferred index of vascular endothelial function because of its specificity, standardised protocol, reproducibility and non-invasive nature. A meta-analysis (Inaba et al., 2010) estimated that for every 1% increase in FMD there is a concordant 13 [9, 17]% reduction in future risk of cardiovascular events.

PORH index is a sensitive indicator of CVD in high-risk populations (Yamamoto-Suganuma & Aso, 2009) and is considered a generalised test of microvascular function as there is conflicting evidence in the literature as to the role of NO in the prolonged hyperaemic response. Some studies have identified the response as being partially NO-mediated (Tagawa et al., 1994), whereas some have noted there is no contribution from NO (Wong et al., 2003), consequently, prudence should be exercised when interpreting PORH results. The LTH response has two phases: an initial peak, primarily mediated by a local sensory nerve axon reflex (Minson et al., 2001); and, a secondary rise and plateau which is primarily NO-mediated (Kellogg et al., 1999; Minson et al., 2001). Dhindsa et al. (2008) identified a modest-at-best relationship between PORH and FMD in measuring vascular reactivity suggesting they involve different mechanisms. Further work should assess the clinical relevance of provocation techniques such as PORH and LTH compared to the validated measurement of FMD. Nieto et al. (2004) suggested that vascular endothelial dysfunction could represent a premature aging of the vascular network. Obesity is independently associated with endothelial dysfunction and can be normalised with weight loss (Williams et al., 2005),
although Hashimoto et al. (1998) proposed OSAS has a stronger association with visceral rather than subcutaneous adiposity. Endothelial-dependent FMD has been shown to be impaired in OSAS, although some researchers have reported no impaired FMD response in large- (Chami et al., 2009) and small-scale (Kato et al., 2000) studies. Dysfunction can be reversed with successful CPAP treatment (Ip et al., 2004; Jelic et al., 2008), although nightly therapy is important because improvements in endothelial function with CPAP are reversed after withdrawing treatment for one week (Ip et al., 2004).

Obesity is thought to contribute to endothelial dysfunction, but some studies have identified reduced FMD responses that are independent of obesity (Namtvedt et al., 2013; Jelic et al., 2010) or EDS (Kohler et al., 2008). Resting brachial artery diameter has been shown to be larger than OSA-free controls (Chami et al., 2009; Yim-Yeh et al., 2010) although others have not (Kato et al., 2000; Oflaz et al., 2006). Larger baseline diameter in OSAS could be attributable to Glagov’s phenomenon; during the development of atherosclerotic plaque in the arterial wall there is a local compensatory dilatation of the vessel in an attempt to maintain adequate downstream perfusion. Higher baseline diameters in OSAS groups could represent more advanced atherosclerotic state.

The extent of the endothelial dysfunction has been shown to be associated with age (Yim-Yeh et al., 2010) and OSAS severity (Chung et al., 2008; Jelic et al., 2008); and ODI rather than AHI seems to have a greater association with FMD (Chung et al., 2008). Oflaz et al. (2006) demonstrated FMD to be lower in the morning than the evening in overweight normotensive adults and overweight OSAS patients alike, suggesting the presence of OSAS affects only the absolute values, and not the diurnal variation. This is unlikely to impact practice as the majority of FMD assessments occur in the morning after an overnight fast.

### 2.6 Consequences of obstructive sleep apnoea syndrome

There are several consequences of OSAS that merit discussion. These include physiological consequences of repeated nocturnal apnoea, the psychological effects of
fragmented sleep and the economic impact of OSAS including its direct and indirect costs.

2.6.1 Cardiovascular morbidity and mortality

The structure and function of the cardiovascular system is affected by several aspects of the repeated obstructive events associated with OSAS. OSAS has been linked to a variety of cardiovascular complications including hypertension, myocardial infarction and stroke and is considered to be an independent cardiovascular risk factor (Doherty et al., 2005; McNicholas et al., 2007). It is difficult to independently dissociate the contribution of OSAS to its associated morbidities from other (often present) risk factors such as obesity, dyslipidaemia, hypertension and physical inactivity. Considering the numerous cardiovascular risk factors present in the typical OSAS patient, it is perhaps unsurprising that certain pathologies are common. Overall, untreated OSAS is independently associated with cardiovascular mortality, which can be reversed with CPAP (Marin et al., 2005). For healthy adults, the risk of sudden cardiovascular events is highest in the late morning, between 6 a.m. and midday (Atkinson et al., 2010); in untreated OSAS the period of highest cardiovascular risk is overnight during sleep (Wilcox et al., 1998)

2.6.1.1 Hypertension

Pressure within the circulatory system is dynamic and changes in response to posture, exercise and medication. The normal range for systolic blood pressure (SBP; i.e. pressure in the arterial tree at the end of the systole phase of the cardiac cycle) is 100 to 140 mmHg. If resting SBP persistently exceeds 140 mmHg, then blood pressure is considered to be high, and a diagnosis of hypertension can be made. Hypertension increases the risk of blood vessel rupture, which if it were to occur in the coronary (cardiac tamponade) or cerebral (haemorrhagic stroke) circulations it could have fatal consequences.

The association between hypertension and OSAS was first introduced by small-scale (Fletcher et al., 1985; Nieto et al., 2004) and medium-to-large scale (Bixler et al., 2000) cross-sectional studies and ratified in the Wisconsin Sleep Cohort (Peppard et al., 2000b). Peppard et al. reported a proportional relationship between the severity of
OSAS and the incidence of hypertension, which was not supported by evidence from another prospective cohort study (O’Connor et al., 2009). Peppard et al. (2000b) reported that moderate-to-severe OSAS is independently associated with a 3-fold increased risk of developing systemic hypertension. The Sleep Heart Health Study assessed the five-year risk of developing hypertension in 2470 normotensive middle-aged and older adults with sleep apnoea at baseline and reported that after controlling for baseline BMI, no significant relationship was found. The causal relationship between OSAS and hypertension is further supported by numerous trials reporting attenuated blood pressure after treatment (Fletcher et al., 1985). Some authors hypothesise that OSAS could contribute to the development of drug-resistant hypertension (Logan et al., 2001). Purported mechanisms for the development of hypertension in OSAS include hyperactivity of the sympathetic nervous system secondary to apnoea-induced hypoxaemia, which increases catecholamine secretion from the adrenal glands (Hedner et al., 1995) and vascular smooth muscle tone.

2.6.1.2 Coronary artery disease and stroke

In the developed world, coronary artery disease (CAD) is the leading cause of mortality and is defined as the presence of atherosclerosis in the coronary arteries that can lead to infarction of the myocardium (Bogaert et al., 2005). Atherosclerosis is a chronic build-up of fatty substances, foam cells and macrophages between the tunica intima and tunica media, which steadily increase resulting from localised endothelial dysfunction and mild inflammation (Hansson et al., 2005). The impact of atheroma depends on its size and location as it can cause physiological insult in several ways. The presence of atheroma increases turbulent blood flow in the artery and coupled with endothelial dysfunction can increase the risk of thrombosis. Atheroma increase in size steadily and subsequent stenosis can eventually reduce blood flow enough to cause downstream ischaemia or completely occlude blood flow causing infarction and acute (heart attack) or chronic (angina pectoris) chest pain symptoms. Thrombosis can also impede organ perfusion in a similar way. If these events occur in the coronary or cerebral circulation they can cause irreparable damage to vital cardiac or neural tissue, which can cause debilitating long-term complications or death.
Observational studies have shown a high proportion of male (Mooe et al., 1996a) and female (Mooe et al., 1996b) patients with CAD to have comorbid OSAS. The odds ratio was 4.1 [1.7, 9.7] (P<0.01) for females, which was higher than for hypertension and smoking. Chronic intermittent hypoxia (CIH) has been shown to induce atherosclerotic plaque formation in the presence of a high cholesterol diet in a murine model (Savransky et al., 2007) highlighting the increased risk of coronary artery disease in OSAS and the importance of regulating dietary lipid intake. This paper also identified elevated lipid peroxidation markers suggesting additional CIH-induced cellular damage.

2.6.1.3 Other cardiovascular sequelae

Intrathoracic pressure changes can impact on the structure and function of both cardiac ventricles. Stroke volume is transiently impeded during apnoea (Tolle et al., 1983) and long term hyperactivity of the sympathetic nervous system could induce left ventricular hypertrophy and/or dysfunction (Corea et al., 1983; Alchanatis et al., 2002), which can be reversed with CPAP (Alchanatis et al., 2002). The incumbent increased resistance in the pulmonary circuit (caused by a combination of sympathetic nervous system-induced vasoconstriction and local vasoconstrictor response to hypoxaemia) can result in pulmonary hypertension, or cor pulmonale (Chauvat et al., 1996). Over time this condition causes right ventricular hypertrophy because the right heart must work harder to pump blood into the hypertensive pulmonary circuit. The hypoxaemic episodes in OSAS have been shown to induce myocardial ischaemia (identified using electrocardiography) in patients with ischaemic heart disease (Franklin et al., 1995).

The acute effects of apnoeic events (especially when in high frequency) can contribute to interruptions of the cardiac rhythm, termed arrhythmia. Intrathoracic pressure oscillations can distort the shape of the gross structures of the heart (Condos Jr et al., 1987), reduce oxygen supply to the myocardium (Schäfer et al., 1997) and cause irregular autonomic nervous system activity. The most common nocturnal arrhythmia observed in OSAS is characterised by bradycardia during apnoea followed abruptly by a brief period of tachycardia, it is termed cyclic variation in heart rate. This arrhythmia is not observed in patients with tracheostomy (whom would not experience obstructive apnoea; Guilleminault et al., 1983), in patients treated with atropine or in patients with autonomic dysfunction (Guilleminault et al., 1983). Atropine is a
parasympatholytic which reduces the action of the parasympathetic nervous system, which is upregulated during periods of rest and lowers heart rate and blood pressure (removal of this parasympathetic "brake" would increase the frequency of sinoatrial depolarisations towards the intrinsic rate (approximately 100 times per min). Common arrhythmias such as atrial fibrillation (AF) are also prevalent in OSAS – Gami et al. (2004) reported a higher incidence of OSAS in AF patients than non-AF patients referred to a cardiology (49% [41, 57]% vs. 32% [27, 37]%; P<0.001). This study used the Berlin questionnaire to identify OSAS instead of objective physiological measures - to address this they subtracted the false positive rate (measured internally using PSG in 44 cases) from the OSAS count.

2.6.2 Daytime somnolence

Daytime somnolence is often the presenting intrinsic symptom for most patients being referred to sleep clinics. Frequent nocturnal arousals fragment sleep and prevent OSAS sufferers from reaching the rapid-eye movement (REM) phase of sleep. Although the purpose of REM sleep is not well understood (roles in learning and memory consolidation have been proposed), some implications of preventing it have been reported (Sutton et al., 2001). The most severe OSAS is associated with over a hundred microarousals per hour - more than one every minute on average. This can leave patients with EDS - the most common symptom of OSAS. This can cause untreated OSAS sufferers to fall asleep for very short periods of time (micro-sleeps) during the day giving rise to potentially dangerous consequences, most notably by falling asleep while driving (Barbe et al., 1998). In the UK, it is the responsibility of the driver to ensure they drive in accordance with the Road Traffic Act (1988) by informing the Driver Vehicle Licensing Association and their respective motor insurance provider about their diagnosis and to remain compliant with treatment to prevent daytime sleepiness.

Aguillard et al. (1998) suggested that the EDS experienced in OSAS could reduce inclination for patients to start or continue exercise regimens due to excessive fatigue in their leisure time, and suggested that after treatment activity levels may increase – which could lead to weight loss and improvement of OSAS. This has since been refuted by West et al. (2009) who reported that successful CPAP therapy and alleviation of
daytime somnolence does not improve daily activity levels in OSAS. Many obese OSAS patients probably have negative attitudes towards physical activity, hence the presence of obesity in the first place. Therefore there are underlying psychological factors and barriers that should be addressed in this population.

2.6.3 Exercise capacity

Exercise capacity can be broadly defined in two ways: the ability to increase cardiopulmonary effort in response to incremental exercise; and, the ability to maintain a given exercise intensity for an extended duration. There is dispute concerning the impact of OSAS on exercise capacity. Some studies have reported impaired exercise capacity in OSAS (Lin et al., 2006; Aguillard et al., 1998) and others have reported no decrement (Alameri et al., 2010) compared with age- and BMI-matched control groups. Rizzi et al. (2010) showed no deficit in exercise capacity in lean OSAS patients. Because of these uncertainties, the effect of CPAP (discussed further in section 2.8.1) on exercise in this population is also unclear (as there may not be an exercise capacity deficit to reverse). As presiding symptoms for OSAS include EDS and fatigue, Hong and Dimsdale (2003) proposed these as limiting factors for regular physical activity levels in this population, and hence to the detriment in exercise capacity. It is often suggested that if CPAP can reverse the daytime fatigue experienced in OSAS that patients could find it easier to introduce lifestyle changes (such as improving diet and physical activity levels) that could address underpinning causes such as obesity. Billings et al. (2013) reported no significant differences in physical activity levels between patients with high CPAP compliance, low CPAP compliance and CPAP intolerant patients.

Alameri et al. (2010) assessed exercise capacity in 55 OSAS patients using the 6-minute walk test and detected no difference in capacity compared to 32 obese controls and 30 lean controls. Despite no difference in distance walked, there was increased perceived dyspnoea in the OSAS group compared to both control groups, suggesting OSAS is associated with increased breathlessness. This may have been caused by the narrower-than-normal airway often observed in OSAS (Schwab et al., 2003). Alonso-Fernandez et al. (2006) published a randomised controlled cross-over trial that identified
impairments in cardiac output and stroke volume responses to exercise in untreated OSAS were reversed after 3 months of CPAP therapy.

2.6.4 Economic impact

Several studies have tried to estimate the financial cost of sleep disorders, not only in terms of diagnosis and management, but also in terms of lost working hours and tiredness-related accidents. Hillman et al. (2006) estimated that undiagnosed sleep disorders cost the Australian economy almost $2 billion United States Dollars (USD) (~£1.3 billion) in work-related injuries, $800 million USD (~£498 million) in private motor vehicle accidents and $1.2 billion USD (~£747 million) in productivity losses. Total financial cost was estimated to be $7.5 billion USD (~£4.8 billion).

Using this estimate from 2004, AlGhanim et al. (2008) extrapolated this figure (based on a population of approximately 20 million) to the United States of America, which has an approximate population of 300 million. This prediction is approximately $103 billion USD. AlGhanim and colleagues (2008) also reported the estimated 2002 economic cost of chronic obstructive pulmonary disease (COPD) and diabetes as $32 billion USD (Mannino et al., 2007) and $132 billion USD (American Diabetes Association, 2002), respectively. Although AlGhanim et al. (2008) suggested the total financial burden of OSAS to be of a similar impact to diabetes; the reported figures would suggest the proportion is approximately 75-80%. Applying this percentage to the UK healthcare setting, Hex et al. (2012) reported the total economic burden of diabetes to be £24 billion in 2010/11, which would put the estimate of the total economic burden of OSAS to be approximately £20 billion. Further costing studies have placed the financial cost of OSAS-related motor vehicle accidents at almost $16 billion USD (~£10 billion) in the year 2000 (Sassani et al., 2004). The authors also forecast that treatment for those involved would cost $3.2 billion USD (~£2 billion) and potentially save more than $11 billion USD (~£7 billion), and reduce OSAS-related vehicle deaths by about 70%.

Although these methods involve a lot of conjecture and little certainty can be placed in the estimates, what can be ascertained is that OSAS is associated with enormous direct and indirect costs, and considering the steady increase in incidence, these costs are only likely to increase further. These financial implications support the need to
increase OSAS awareness and investigate alternative treatment strategies that address the underlying causes of OSAS as opposed to long-term management. The latter is particularly important in recognition of the large number of CPAP-resistant patients.

2.7 Obesity, metabolic syndrome and obstructive sleep apnoea syndrome

There is an established and undisputed relationship between the presence of obesity and OSAS, however more recently a causative link between OSAS and obesity has been proposed. This suggests the relationship between OSAS and obesity is bidirectional and more complicated than it first appears. Obesity is fundamentally the effect of sustained positive energy balance arising from an augmented energy intake, diminished energy expenditure, or a combination of both; however the causes of this energy imbalance are unclear. This promotes energy storage, usually in the form of adipose tissue. Fat distributions differ between sexes which could explain the differences in prevalence.

Obesity is associated with increased collapsibility of the airway. Magnetic resonance imaging (MRI) studies and computer tomography have shown decreased intraluminal calibre in obese OSAS patients. Excess cervical adiposity increases the structural load on the airway and increased peri- and parapharyngeal fat deposition reduce the concentration of structural smooth muscle and overall luminal size (Li et al., 2012). These factors, coupled with natural nocturnal reduction in airway muscle tone increase the collapsibility of the upper airway during sleep. Additionally, excessive thoracic fat and abdominal fat are implicated in the pathogenesis of SDB by impeding respiratory dynamics. When supine, surplus weight on the thorax increases the work done by inspiratory muscles (principally the diaphragm) to create the negative intra-thoracic pressure required to draw air in. When the diaphragm contracts it moves toward the abdomen, increasing intra-abdominal pressure. With increasing abdominal obesity, there is a proportional increase in abdominal resistance and a decrease in lung compliance. These contribute to a reduced lung volume and a tendency towards rapid shallow breathing similar to that in chronic restrictive pulmonary disease and obesity hypoventilation syndrome. Reduced lung volumes are associated with closure of
smaller distal airways, which creates a ventilation perfusion mismatch (i.e. perfusing alveoli downstream of a small airway collapse) and widening of the alveolar-arterial PO₂ difference.

Leptin is an adipocyte-derived hormone which plays a central role in energy balance by regulating appetite and metabolism (Blüher & Mantzoros, 2009). Leptin binds to receptors in the mediobasal hypothalamus to inhibit appetite (and in principle, food intake). As it is secreted from adipose cells, circulating leptin increases proportionally with increasing obesity (Enriori et al., 2006). It has previously been suggested that sustained hyperleptinaemia could lead to insensitivity to its effects, appetite dysregulation and increased energy intake. Independent of obesity, OSAS has been shown to be associated with increased serum leptin (Philips et al., 2000) - supporting a contributory link between SDB and obesity. CPAP therapy can reduce serum leptin in OSAS patients, suggesting that its production is stimulated by the physiological consequences of apnoea (Saarelainen et al., 1997; Harsch et al., 2003). Hyperleptinaemia and leptin resistance have been implicated in the development of CVD (Wallace et al., 2001).

Ghrelin is a hormone that works antagonistically to leptin - higher concentrations stimulate appetite (Arvat et al., 2001). It is secreted from the P/D1 cells of the stomach fundus and from epsilon cells in the pancreas. Acylated ghrelin binds centrally to receptors in hypothalamus (Bagnasco et al., 2003) and stimulates hunger (usually resulting in behaviours that increase energy intake). Indeed, circulating ghrelin has been shown to be highest immediately prior to mealtimes (Pinkey & Williams, 2002). The relationship between OSAS and ghrelin is unclear; some have shown it to be increased (Ursavas et al., 2010) and reduced with CPAP (Takahashi et al., 2008), while others have shown no difference (Ciftci et al., 2004). It is possible that changes in the production of, or physiological response to, leptin and ghrelin could offer novel therapeutic targets for obesity and, by association, OSAS.

Insulin is a pancreatic hormone responsible for the conversion of glucose to glycogen (a more efficient storage molecule). It also reduces lipolysis of adipocytes into serum fatty acids. It is an essential hormone in the maintenance of glucose homeostasis. Insulin resistance is one aspect of the metabolic syndrome. The association between
insulin resistance and OSAS is currently unclear (Peled et al., 2007; Ip et al., 2002; Young et al., 1993; Gruber et al., 2006) and requires further investigation.

2.8 Treatment paradigms

2.8.1 Continuous positive airway pressure

Continuous positive airway pressure is the standard treatment for patients with moderate or worse OSAS (Sullivan et al., 1981). It provides a steady stream of pressurised air into the airway via a nasal or facial mask, which increases the intrapharyngeal pressure and prevents airway collapse and the ensuing sequelae (episodes of hypoxaemia and intrathoracic pressure swings). The air pressure of the CPAP device is determined by titrating pressures overnight during simultaneous polysomnogram or oximetry. The pressure is usually fractionally higher than the pressure required to prevent an apnoea. A minority of patients experience residual EDS despite adequate CPAP treatment, although this is not thought to be associated with the increased cardiovascular risk seen in untreated OSAS patients (El-Solh et al., 2010). Patients are prescribed CPAP therapy to be used at all times during sleep; however, patients are variably compliant with its use for a variety of physical and social reasons (Basner, 2007; Kribbs et al., 1993). Several studies have assessed the contributory factors to successful adherence to CPAP and there is evidence to suggest the main physiological indicator for long-term CPAP compliance is the oxygen desaturation index (ODI) and not symptoms of EDS (Krieger et al., 1996; Kohler et al., 2008).

The effectiveness of CPAP therapy in normalising the apnoea frequency during sleep has been demonstrated in studies that report reduced daytime hypersomnolence after a short period of therapy (Robinson et al., 2006; Jenkinson et al., 1999; Engleman et al., 1998). Furthermore, there is strong evidence to suggest overnight CPAP therapy significantly reduces mean nocturnal blood pressure, perhaps unsurprisingly as the apnoea it prevents are the primary causes of high blood pressure secondary to OSAS. Robinson et al. (2006) conducted a randomised controlled crossover trial comparing therapeutic CPAP to sham CPAP. The authors did not identify any significant reductions in 24-hour ambulatory blood pressure during sleep or wakefulness in a group of obese
non-sleepy patients. The impact of CPAP on blood pressure in hypertensive patients with OSAS seems clearer (Becker et al., 2003). Many studies are unclear when describing the status of participants’ antihypertensive therapy (whether continued or discontinued before and during the trial, and if discontinued, how long for?) and some have used prescription of antihypertensive medication alone as evidence of hypertension. This is perhaps hasty as some General Practitioners may adopt a preemptive approach to the treatment of ailments more common with progressing age (e.g. hypercholesterolaemia and hypertension) and may prescribe medication as a preventative measure based on borderline clinical measures. Moreover, it is possible that previous hypertension had since subsided as a result of improved modifiable risk factors, such as obesity, and that patients were unnecessarily undergoing antihypertensive therapy. Thus, the use of an existing prescription for antihypertensives as inclusion criteria seems weak. The possible dose-response relationship between OSAS severity and incident hypertension is unclear, and Dempsey et al. (2010) suggest this may be due to the use of AHI as the metric of choice for disease severity. Indeed, the apnoea frequency does not necessarily represent the intensity, duration or frequency of hypoxaemic episodes.

Despite several short-, medium- and long-term studies assessing endothelial dysfunction in OSAS patients receiving CPAP versus no treatment (Imadojemu et al., 2002; Lattimore et al., 2006; Bayram et al., 2009; Nguyen et al., 2010), there is limited evidence that quantifies the extent to which low compliance (i.e. undertaking occasional CPAP therapy) attenuates the increased endothelial dysfunction caused by the OSAS, compared to persons that are highly compliant (> 80% nightly use, 4 h per night). A study by Jelic et al. (2008) showed that vascular endothelial dysfunction can be significantly reversed with four weeks of effective CPAP treatment and that ineffective treatment elicits no marked reversal. These results are ambiguous as the researchers grouped persons who refused treatment and had low compliance together, limiting interpretation of the effect of low compliance on FMD.

Other factors that can influence the effect of CPAP on reversing the negative effects of OSAS include treatment compliance, which is not always reported. However, when compliance data are presented, they are commonly presented as hours per night or percentage of nights > 4 h. This is perhaps inadequate as a patient could be excluded
for using CPAP for 3 ½ hours every night when they only sleep 4 hours per night, and another could be included for using their CPAP for 4 ½ hours per night when they sleep for 9 hours on average (including a mid-afternoon nap without CPAP). Furthermore, a patient who uses their CPAP for 9 hours on alternate days could still yield an “acceptable” compliance level (9 hours x 4 days = 36 hours, divided by 7 days = 5 hours per day = compliant?). These confounding circumstances are very difficult to determine, measure and control, however, their potential impact on published data should be acknowledged. Possibly a more subjective metric of patient compliance could be determined by an experienced healthcare professionals who would utilise quantitative data from the CPAP memory card and qualitative experiences of the patient would be more suitable?

### 2.8.2 Oral appliances

The overarching aim of oral appliance therapy is to modify the architecture of the oral cavity and/or upper pharyngeal airway to reduce the frequency and duration of obstructive events and the negative sequelae that follow them. Mandibular advancement devices (MAD) have been proposed as non-surgical alternatives to CPAP therapy. A MAD sits in the mouth upon the upper and lower teeth. It pushes the mandible in an anterior direction which also moves forward the connected anatomy of the upper airway, principally the glossus. Evidence suggests that MADs are much more suited to the treatment of non-obese OSAS patients as apnoea within this group tend to arise from collapse of the anterior wall of the airway. Gotsopoulos and colleagues (2004) reported significant reductions in daytime diastolic blood pressure and daytime systolic and diastolic blood pressure, albeit modest changes at <4 mmHg.

Tongue stabilising devices (TSD) allow the position of the tongue to be controlled to reduce the likelihood of airway collapse. Deane et al. (2009) conducted a randomised crossover design that compared TSD therapy with MAD therapy. There was an overwhelming preference for MAD therapy despite both treatments having similar effects on key outcomes. Tsuiki et al. (2012) described a case study where such a device had successfully reduced AHI by 75% in a lean 54 year old female with severe OSAS. Such devices are in their infancy and research into treatment effectiveness and cost effectiveness is needed to assess suitability in the routine management of OSAS.
There is increasing demand for pragmatic alternatives to the efficacious yet poorly-tolerated CPAP machine. The advantages of CPAP include a huge array of oral, nasal and full-face mask interfaces to suit all face shapes and the reusable nature of the machine and much of its tubing. Unfortunately, oral appliances are not a standard provision on the NHS and require self-funding. MADs in particular require moulding to the shape of the patient’s upper and lower teeth to ensure effectiveness. This makes them bespoke in nature and the assessment of their effectiveness and tolerability on a patient-to-patient basis expensive to discern. It has been suggested that oral appliances are best suited in the treatment of patients who are CPAP-intolerant and have mild-to-moderate OSAS (Kushida et al., 2006).

2.8.3 Surgical intervention

In certain circumstances surgical intervention may the best treatment, and such procedures have been reported in the literature for as long as CPAP (Fujita et al., 1981). Caples et al. (2010) reviewed this topic and concluded that a lack of robust trials, especially for newer techniques, makes it difficult to determine which patients would most likely benefit from surgical intervention. There are two types of surgery that can be used in the treatment of OSAS: surgery to alter the anatomy of the airway (e.g. by excising soft tissue structures) to reduce propensity for obstruction and to reduce airway resistance (Fleisher & Krieger, 2007) and surgery to address obesity by facilitating weight loss and reducing the obesity-related airway collapse (Lojander et al., 1998). Riley et al. (1993) assessed outcomes of surgical intervention in OSAS. Procedures included uvulopalatopharyngoplasty (UPPP; for obstructions involving the soft palate) and genioglossus advancement with hyoid myotomy-suspension (for obstructions at the base of the tongue); these procedures are most often indicated in patients with especially narrow airways. The overall success rate was determined to be 77% for phase I surgery. As obesity is a major contributory factor for the development of OSAS, bariatric surgery can facilitate significant weight loss and the associated reduced pressures on the upper airway. General anaesthesia and major surgery carry significant peri- and post-operative risks, which need to be considered in the overall clinical picture of the patient,
2.8.4 Supplemental oxygen and pharmacological interventions

To address the periods of nocturnal oxyhaemoglobin desaturation, some researchers have investigated the effects of supplemental oxygen therapy on several key outcomes. Norman et al. (2006) concluded that although supplemental oxygen improves mean oxyhaemoglobin concentration, it did not improve daytime or nighttime blood pressure. This finding was supported by Loredo et al. (2006) who also reported no improvement in daytime somnolence with supplemental oxygen therapy.

An abundance of pharmacotherapies have been investigated in OSAS including oestrogen therapy, medroxyprogesterone acetate (MPA; a progesterone analogue) and tumour necrosis factor alpha (TNF-α) antagonists. To address the increased incidence of OSAS in post-menopausal women (as described in subsection 2.4) Manber et al. (2003) investigated the effects of oestradiol plus placebo versus oestradiol plus progesterone in a single group placebo-controlled design. They observed approximately 50% improvement in AHI and non-REM AHI after oestradiol-only therapy, but not when coupled with progesterone. Although promising, these findings should be treated as preliminary because of the small sample size and non-randomised design. Polo-Kantola et al. (1999) reported reduced occurrence and frequency of nocturnal apnoea in post-menopausal women; however this was based upon only one participant that was apnoeic at baseline. Volunteers were excluded from participating in this trial if they had any history of suspected OSAS – on average the sample was overweight (27±3 and 27±5 kg.m⁻²). It is therefore difficult to conclude what effect oestrogen therapy could have in improving OSAS. Unfortunately, no prospective study has reported a randomised controlled trial of hormone replacement therapy on AHI in post-menopausal women with moderate to severe OSAS. The effects of MPA therapy in OSAS are also unclear. Some studies have reported no meaningful improvements in OSAS (Block et al., 1981; Cook et al., 1989), however evidence suggests MPA could be useful for subpopulations such as hypercapnic (Strohl et al., 1981) or alcoholic patients (Collop, 1994). Etanercept (a TNF-α antagonist) improved AHI, daytime sleepiness and plasma IL-6 levels in a study with eight obese participants (Vgontzas et al., 2004). Although the findings are encouraging, they cannot be generalised to the OSAS population because of the small sample size. Sibutramine is an oral anorexant and has
been investigated in OSAS, however due to potentially fatal side-effects it has since been withdrawn from the market.

### 2.8.5 Other therapies

The implicated role of impaired smooth muscle tonus in the upper airway in the development of OSAS has motivated researchers to investigate the effects of neuromuscular stimulation of the upper airway muscles on subjective sleepiness and disease severity. In 2006 a quite novel intervention was reported in the British Medical Journal. Puhan et al. (2006) assessed the impact of regular didgeridoo playing on daytime sleepiness and OSAS severity in non-obese adults with moderate disease. Their trial was randomised in nature and elicited significant reductions in ESS (-2.8 [-5.4, -0.2]; P=0.04) and AHI (-6.6 [-13.3, -0.1]; P=0.05), after adjusting for baseline disease severity and changes in body mass during the study period. These data are limited by the study design which was small scale (n=25) and involved baseline PSG data from up to a year prior to enrolment. Other devices that manipulate the internal architecture of the oral cavity and oropharynx have been developed to offer viable alternatives to CPAP therapy and to reduce the monopoly that such devices have in the sleep medicine market.

### 2.9 Adjunct therapies for obstructive sleep apnoea syndrome

Several alternative medical therapies have been proposed and explored for their feasibility and effectiveness at treating different severities of OSAS. These mostly include lifestyle intervention (e.g. modifying dietary intake and/or physical activity to promote weight loss) studies, and more uncommon approaches such as psychological interventions (e.g. behavioural therapy and hypnotherapy) and respiratory muscle training, which could be incorporated into lifestyle intervention or used independently. Motivated by the sub-optimal tolerance of CPAP and its ineffectiveness at curing OSAS, these studies have often investigated CPAP or non-CPAP patients, but often not both in a parallel design.
2.9.1 Dietary intervention

Suratt et al. (1992) delivered a very low energy diet (VLED) in eight obese patients with severe OSAS. This study preceded the routine use of CPAP in clinical practice (data were collected in the early 1980s) and the intervention produced mixed results for weight loss and change in AHI. Patients that improved at the end of the VLED had regressed at 2-year follow-up. Lojander et al. (1998) evaluated the impact of a hospital-based nurse-delivered dietary intervention in 24 (96% male) obese newly diagnosed OSAS patients. The intervention consisted of a six-week VLED and behavioural management (consisting of group meetings and remote contact). The intervention was safe, well-tolerated and caused a 5 kg·m⁻² reduction in BMI at the end of the VLED phase and >50% improvement in SDB indices, which were maintained at 1 year follow-up. Furthermore, unlike most studies of this nature, the authors reported the cost-effectiveness of the intervention and compared it with the cost of CPAP therapy. The cost of delivering CPAP for one year was estimated at $3000 USD, which was almost double the cost of delivering the one-year VLED and behavioural management intervention for the same period. Although this study was based in Finland, the direct costs of both are unlikely to differ greatly in other affluent countries.

Kajaste et al. (2004) conducted a randomised trial that compared effects of a 6-week VLED (500 kcal per day) followed by 24 months of cognitive-behavioural therapy (CBT) with and without preceding CPAP therapy for six months. The CBT aspect was based on guidelines by Brownell (1989) and was bespoke to each participant and included the provision of counselling on a weekly/fortnightly basis, which reduced to monthly after 6 months and bimonthly after one year. This reduction in support is intended to promote participants’ independence and hopefully reflects the reduced requirement of regular support. This tapering of contact is common in counselling-related interventions. This study concluded that there was no association between an initial 6 months of CPAP and the uptake of a VLED intervention including CBT. Although significant reductions in body mass and ODI were not observed in either arm of the trial at six months, they were once the groups were merged. This improvement steadily worsened over the following 18 months but was still significantly better than
baseline. This regression is common and represents the major challenge of lifestyle intervention. The authors lauded two aspects of their intervention. Firstly, the individualised counselling approach they used and suggested that one-on-one contact may offer additional benefits in obese patient populations. Secondly, the flexible approach they took to the timing of appointments, which likely improved attendance by working around the participants’ other commitments.

Johansson et al. (2009) reported a single-centre, randomised, controlled trial of 63 obese men (mean BMI: 35 kg·m⁻²; mean age: 49 y) with OSAS who had been undergoing CPAP therapy for >6 months. These men were randomised into a VLED intervention and a usual diet control group. The intervention consisted of a liquid VLED (550 kcal·day⁻¹) for 7 weeks, with gradual introduction of normal food over the following 2 weeks (to 1500 kcal·day⁻¹). To support the changes in diet fortnightly group meetings were arranged with a research nurse and two dieticians. Compared with the control group, the intervention group improved body mass by 20 kg (-18%) and AHI by 23 events·h⁻¹. There was a modest relationship between the amount of weight lost and the improvement in AHI, suggesting other mechanisms besides weight loss are implicated. 87% of patients on the intervention improved their OSAS severity stratus, with 46% improving by two strata (i.e. severe to mild or moderate to OSAS-free). Overall, 17% were in complete remission of OSAS. Improvement in OSAS was proportional to baseline AHI.

The longer term effects of this study were reported in a prospective observational follow-up study in 2011 (Johansson et al., 2011). The 31 control participants from the previous study were invited to start the 9-week dietary intervention. They also completed end-point assessment and both groups underwent 4-weekly assessments for a further 43 weeks. Firstly, the second cohort underwent similar improvements in body mass and AHI to the first cohort (-20 kg vs. -19 kg; - 21 vs. -25 AHI) and a similar relationship in improvement in that the largest improvers lost the greater amount of weight, and had the higher AHI at baseline. Ultimately, the improvements in AHI from a 9-week VLED were largely maintained after one year (-58% vs. -47%); 48% no longer needed CPAP therapy and 10% were in complete remission.

Typically fixed-term hypoenergetic diets that induce an initial rapid weight-loss phase and support this with education, counselling and recommendations of how to maintain...
the weight-loss long term. These hypoenergetic diets are typically between two and eight weeks in duration and have a prescribed daily energy intake of approximately 400-800 kcal (VLED) or 800-1200 kcal (LED). In obese individuals, this can induce a daily energy deficit of more than 2000 kcal, which can easily induce weight loss in excess of 15 kg, but unfortunately no high-efficacy strategy currently exists to avoid weight regain in the medium to long-term (6 months to 5 years post intervention).

2.9.2 Exercise intervention

Exercise has been proposed as both a supplementary therapy and an alternative treatment modality for OSAS. The justification for exercise therapy is multifactorial and includes improving the impaired exercise capacity reported by some groups (section 2.6.3), reducing the inherent health risk caused by a sedentary lifestyle and promoting negative energy balance to facilitate weight loss and perhaps reduce the obesity-related collapse of the airway.

Netzer et al. (1997) were the first to report a study investigating the effect of exercise in the management of OSAS. Their open trial recruited 11 patients (91% male) with mild-to-severe disease onto a six-month exercise program involving twice-weekly two-hour sessions delivered by physical therapists. The intervention lasted 6 months with two 2-hour exercise sessions a week, the first of which was sports-based (i.e. jogging, games and gymnastics) and the second weights-based (repetitive light weight-lifting). This study showed no changes in body mass and significant reductions in RDI, although the absolute improvement was minimal and unlikely to be of clinical benefit. Extended results from the same trial were reported in Giebelhaus et al. (2000). Participants' BMI ranged from 23.6 to 32.8 kg·m² including normal-weight, overweight and class I obese participants. This was a German community-based study and the main findings were that this type of intervention was safe, feasible and able to reduce mean RDI by 28% (32.8 to 23.6 events·h⁻¹) without marked changes in body mass or physical fitness. These data suggest that exercise could beneficially impact on SDB via alternative mechanisms to weight loss. These findings must certainly be interpreted with caution as the low-powered, non-randomised, uncontrolled design was not especially robust. The authors reported that funding for the six-month intervention came from public
health insurance different to the NHS currently in place in the UK, so alternative funding routes also need to be explored.

Ueno et al. (2009) conducted a four-arm prospective exercise intervention study in heart failure patients with OSAS, CSA and no sleep apnoea, and a group of healthy age-matched controls. After baseline assessment the heart failure patients underwent four months of no exercise, were reassessed, and then four months of exercise training, followed by a final reassessment. The exercise intervention involved three 1-hour training sessions, including approximately 25-40 min of cycle ergometry and 10 min of strengthening exercises. The main findings of this study were that exercise significantly improved MSNA in OSAS (66 to 31 bursts-min⁻¹; P<0.01) and CSA (62 to 32 bursts-min⁻¹; P<0.01) but not in OSAS-free controls (20 to 20 bursts-min⁻¹; P<0.01) and improved AHI by 36% in OSAS (P<0.01) with no improvement in the CSA group. As in the Giebelhaus et al. (2000) study, the improvements in severity index were independent of changes in body mass. This study again suffered from a small sample size with less than 10 participants in each arm of the trial.

Sengul et al. (2011) published a randomised controlled trial investigating the effects of breathing exercises and aerobic training in overweight and obese men with non-severe OSAS. The intervention involved 12 weeks of thrice-weekly supervised sessions lasting up to 90 min consisting of breathing exercises (15 to 30 min) and cycle ergometry (45-60 min). The main finding of this study was that the intervention reduced AHI by 28% (15.19 ± 5.43 to 11.01 ± 5.28 events-h⁻¹; P=0.02) despite no changes in anthropometric variables. This study again included a small sample size, and recruited only middle-aged men (40 to 65 years), which limits the generalisability of the results. Furthermore, the study sample did not include patients with severe OSAS or many (if any) patients with extreme obesity (inferred from the BMI characteristics; mean BMI 30 ± 3 kg·m⁻²). Although this study was probably investigating the role of exercise in non-CPAP treated mild-to-moderate OSAS, it is unclear whether the patients included were pre-CPAP, CPAP-resistant or CPAP compliant.

This ambiguity was not present in a randomised controlled trial conducted by Ackel-d’Elia et al. (2011). This study evaluated the effects of exercise training coupled with CPAP therapy versus CPAP only in patients with at least moderate OSAS (AHI>15 events-h⁻¹) up to a BMI of 35 kg·m⁻². In this trial, exercise involved 1-hour sessions,
thrice weekly for two months and consisted of treadmill walking and running. The intensity ranged from 85% of the speed at anaerobic threshold to 40 min above the original anaerobic threshold towards the end of the study. The CPAP plus exercise group improved several aspects of quality of life compared to CPAP-only, perhaps unsurprisingly considering the widely reported benefits of exercise. General health perception, physical functioning and vitality scores using the Short Form-36 tool were improved significantly more in the exercise group after treatment and also after washout. There were improvements in some markers of physical fitness (+14% maximum speed in exercise test; \( P<0.01 \)) but not others (+10% \( \text{VO}_{2\text{max}} \); \( P>0.05 \)), perhaps due to the small number of participants (\( n=12 \) in intervention). These authors did not report any benefits of adjunct exercise therapy on AHI on nights with CPAP, on the first night without CPAP and after a 1-week washout period off CPAP, compared to the CPAP only group. Of note, after the washout period ESS increased in both groups; the increase in the CPAP only group made it similar to pre-CPAP levels, whereas the exercise plus CPAP group remained significantly improved. This evidence suggests treadmill-based exercise has no impact on OSAS severity or sleep architecture. This study also integrated a period of sleep hygiene education before the CPAP delivery, although not explicitly discussed it seems that the advice-only nature of the sleep hygiene had no beneficial impact on any outcome measures, with the exception of the general health perception aspect of the SF36 questionnaire.

Kline et al. (2011) conducted a randomised controlled trial investigating the effects of aerobic exercise training in overweight and obese patients with moderate OSAS. Their design incorporated a stratified randomisation process and a placebo control group (whom still underwent an intervention, but this was based on static stretching only). They reported 38% improvement in AHI (-12 events-h\(^{-1}\); \( P<0.01 \)) in favour of the exercise group with no significant change in body mass (-0.3 kg net change) and slight improvement in total body fat (-1.4% net change). There was also a modest net improvement in maximum inspiratory pressure that failed to reach statistical significance but was characterised by a small-to-moderate effect size (+15.9 mmH\(_2\)O; \( P=0.06 \); \( g=0.43 \)). This observation suggests that exercise can certainly stimulate changes in the anatomy and physiology of the respiratory system. The precise mechanisms underlying improvements in objective measures of OSAS in the absence
of meaningful weight loss are unclear. It has been suggested that upper airway smooth muscle tone, which is largely withdrawn during sleep in OSAS, could undergo a training effect similar to the effect of resistance training on skeletal muscle. This “training effect” could be stimulated by increased airflow through the airway. The increased activation of airway smooth muscle during exercise could increase basal tone, and increase nocturnal tone also. Improved tonus increases the structural integrity of the airway and reduces its propensity for collapse. Another possibility is the rostral fluid shift; lower limb exercise or physical activity activates the skeletal muscle pump to aid venous return and could reduce or prevent the development of oedematous legs during the day - thereby reducing the potential for rostral shift. The Kline et al. (2011) study also observed excellent participant adherence to the intervention and suggested this was because of the careful progression of the intervention. That aside, they expected they may have recruited a motivated sample relative to the population, which is difficult to avoid in exercise-based randomised controlled trials that involve obtaining written informed consent and where it is not possible to blind groups to treatment allocation. This study also showed that exercise can ameliorate deficits in daytime functioning such as depressive symptoms and quality of life. The generalisability of these results is limited for three key reasons. This sample was fairly homogenous for severity, with principally moderate severity OSAS patients included. The relationship between exercise and severity of OSAS is bidirectional (Giebelhaus et al., 2000; Peppard & Young, 2004) and probably reversible.

2.9.3 Combined dietary and exercise intervention

Dietary intake and physical activity are the principal governors of energy intake and energy expenditure, and consequently energy balance. Common sense would suggest that addressing both in tandem should exert greater influence on the energy balance equation and avoid compensatory shifts in energy intake and physical activity to maintain energy homeostasis. This has been shown to be the case in obesity (Shaw et al., 2006). Peppard et al. (2000a) showed that reducing body mass by 10% was predictive of a 26% reduction in the severity of OSAS. Several studies have incorporated such approaches in OSAS with mixed results.
Norman et al. (2000) built on the study by Netzer et al. (1997) by conducting a similar trial incorporating dietary counselling. Eleven obese patients with mild-to-severe OSAS were recruited, of which 9 completed (89% male) the study and were included in the analyses. This included patients that were \( n=5 \) and were not \( n=4 \) undergoing nasal CPAP therapy. The main findings were significant reductions in body mass (110.9 to 104.7 kg; \( P=0.001 \)), neck (\( \Delta -1.8 \text{ cm}; P=0.002 \)), waist (\( \Delta -4.7 \text{ cm}; P=0.004 \)) and hip circumferences (\( \Delta -2.9 \text{ cm}; P=0.016 \)), systolic (\( \Delta -8.8 \text{ mmHg}; P=0.016 \)) and diastolic (\( \Delta -8.1 \text{ mmHg}; P=0.001 \)) blood pressure and AHI \( (21.7 \pm 9.0 \text{ to } 11.8 \pm 6.8 \text{ events-h}\(^{-1}\); \( P=0.002 \)). The authors did not identify any correlation between amount of weight lost and reductions in AHI \( (r=0.35; P=0.35) \), strengthening the hypothesis that exercise can independently improve OSAS. They also split their group up into CPAP and non-CPAP users to detect any treatment effect on key outcomes, but none were noted.

Barnes et al. (2009) conducted a cohort study investigating the effects of a 16-week VLED and structured exercise in 12 obese, middle-aged adults. The VLED component was steadily withdrawn over the course of the intervention (from three study meals per day to two, then to one) as was the supervised exercise component (from three per week to one-to-three per week. Participants were asked to maintain a fixed frequency of five training sessions per week consisting of hospital-based sessions and independent home-based sessions. The exercise was 100% resistance training-based for the first four weeks to limit muscle atrophy during the rapid weight loss phase, after which an aerobic component was added. The cohort reduced body mass by 12.7 ± 9.6 kg at 16 weeks, however all participants suffered weight regain at 12-month follow-up which reduced weight loss from baseline to 8.3 ± 7.7 kg. The weight reduction at 16-weeks corresponded to an approximate 25% improvement in mean AHI \( (24.6 \pm 12 \text{ to } 18.3 \pm 11.9 \text{ events-h}\(^{-1}\)) \). This improvement was not statistically significant \( (P=0.19) \) perhaps because three of the twelve participants' AHI increased by \( >5 \text{ events-h}\(^{-1}\). In this study, the intervention reduced daytime sleepiness, daytime blood pressure, 24-hour blood pressure, total cholesterol, LDL, triglycerides, CRP, Insulin and gamma-GT (all \( P<0.05 \)) at 12 weeks.

Tuomilehto et al. (2009) conducted a randomised controlled trial to examine the effects of a VLED, nutritionist-delivered counselling and advice to increase physical activity on body mass and OSAS severity in 81 patients with mild OSAS. At the one year
follow-up there were significant improvements in body mass, waist circumference, plasma insulin concentration and AHI, compared to the advice only group. This study also identified strong correlations between weight loss and improvement in OSAS. Longer-term follow-up of this cohort was conducted 2 years after enrolment (Tuomilehto et al., 2010). The intervention group improved AHI further (Δ -4.1 [-7.6, -0.7] events-h⁻¹; P=0.049) despite having regained 50% of the weight initially lost during the intervention, which promotes the hypothesis of exercise-induced improvements in OSAS independent of weight change. Furthermore, 26 [3, 48]% of those in the intervention group were clinically cured of OSAS; an outcome not associated with CPAP intervention. It is worth reiterating that this study did only include those with mild OSAS. Recently, Tuomilehto et al. (2013) published impressive five-year follow-up data for this cohort. Weight loss from baseline was still marked in the intervention group (Δ -5.5 kg) compared with the control group (Δ 0.6 kg). Changes in AHI from enrolment were 5.0 [0.8, 9.2] and -0.8 [-3.3, 0.8] events-h⁻¹, which was still significantly different (P=0.04).

In 2009, Foster et al. (2009) reported a prospective cohort study as part of the Look AHEAD (Action for Health in Diabetes) study; the Sleep AHEAD study. To date, this is the largest randomised trial assessing weight loss in OSAS. The study recruited 264 participants with type 2 diabetes and compared the effects of two interventions; diabetes support and education and intensive lifestyle intervention. The former involved thrice-yearly group sessions aimed at promoting behaviours to effectively manage diabetes, and the latter was a behavioural weight loss program involving prescribed LED and 175 min of moderate exercise per week. The provision of LED prepared meals was reduced after four months. The sample included good representation of different severities of OSAS (approximately 40% mild, 35% moderate and 25% severe) and included only 5% CPAP users at follow-up. After one year, the intervention group showed improved body mass (-10.8 ± 0.7 kg) compared with the support and education (-0.6 ± 0.7 kg) group (P<0.001), while AHI reduced by 5.4 ± 1.5 in the intervention group and increased by a similar amount in the support and education group (P<0.001). It is likely that these improvements were more profound after four months however these data were not collected. It is therefore difficult to assess the weight regain pattern and its association with improvements in AHI.
Alarmingly there was a significant increase in AHI for the support and education arm of the trial (+4.2 ± 1.2 events-h⁻¹), despite no increase in body mass (-0.6 ± 0.7 kg; \( P<0.05 \)). The authors proposed this could be due to a rapid natural progression of OSAS in older adults, although other contributing factors to apnoea were not assessed (e.g. alcohol/sedative use). There is limited evidence for or against this hypothesis and it will be interesting to see how this trend continues in the four-year follow-up due within the next 12 months. The authors reported that the major findings of the study were unchanged after reanalysis excluding CPAP users.

Papandreou et al. (2012) conducted a randomised trial assessing the impact of CPAP therapy, unsupervised encouragement to increase physical activity, and either a Mediterranean diet (high in fish, vegetables and non-refined cereals) or a prudent diet (high in red meat and poultry) in 21 obese apnoeics. After six months the intervention group had reduced body mass by 10.8 ± 3.8 kg, along with proportional reductions in BMI, waist circumference and body fat percentage. The study reported significant reductions in a lipid peroxidation marker (thiobarbituric acid) suggesting that both diets beneficially improved oxidative stress. Reductions in AHI were only significant in the Mediterranean diet group, although the magnitude of improvement was similar in both groups (Mediterranean: 47 ± 34 to 35 ± 32; prudent: 46 ± 32 to 37 ± 29 events-h⁻¹; \( P<0.05 \)).

EDS is a hallmark characteristic of OSA, and Basta et al. (2008) showed that a lack of regular exercise is a significant predictor for degree of EDS in men. Exercise training has been shown to benefit many medical conditions and often offers an economically viable alternative which addresses not only the conditions at hand but also offers a protective effect against a wide range of other health problems. Exercise can increase NO bioavailability and decrease the inflammatory response; however the mechanism of action has not yet been identified (De Meirelles et al., 2009). Rizzi et al. (2010) reported that functional capacity was not impaired in lean OSAS patients suggesting that the impaired exercise capacity of obese OSAS patients is independently obesity-related. Currently there are routine rehabilitation clinics for patients with COPD and post-myocardial infarction patients, and are intended to improve functional capacity and long-term outcomes. Other less specific referral schemes exist for patients referred from their GP are available in some parts of the UK.
2.9.4 Summary

There is considerable evidence to support the role of lifestyle management in the overall management of OSAS, especially because a significant proportion of OSAS patients are intolerant to CPAP therapy. However, with evidence coming from interventions of varying duration and design (e.g., combined VLED and exercise or LED and behaviour change counselling), and from specific subsets of the OSAS population, for example those with a specific disease severity (i.e., mild, moderate or severe) or a comorbid condition (e.g., heart failure or diabetes), it is difficult to generalise these findings to the OSAS population. The ways in which weight loss can improve OSAS seem to be clear; increased airway calibre and decreased load-induced collapse. The precise mechanisms implicated in the exercise-induced improvement in OSAS (independently of changes in body mass) are unclear. Improved airway smooth muscle and reduced rostral fluid shift could be responsible.

2.10 Assessing the impact of clinical trials

Empirical data in scientific research are most often quantitative and scalar in nature. This can make the determination of not only the magnitude, but the simple presence of a significant change difficult. Karl Raimund Popper (b. 1902) was a 20th Century philosopher responsible for promoting the concept of falsifiability; where an experiment is characterised by a testable hypothesis. In fact, Ronald Fisher (1935) suggested that the only hypothesis that should be scrutinised is the null hypothesis, which states that there is no effect. This inferential method provides a probability, or \( P \) value, that the observed difference was due to chance. Rather arbitrarily, the threshold probability is almost universally considered to be 1 in 20, or 5%. Probabilities lower than this are considered to be significant (e.g., a change with \( P=0.024 \) is significant and not attributable to chance alone). A single \( P \) value is usually produced from a statistical test when comparing different sets of data and it can be generated from a number of statistical tests. These tests, or more correctly their selection, are much more fallible than ideal, with subjective assessments of the characteristics of the data (e.g., does it fit a Gaussian distribution?) as key decisions on the path to test selection. The underpinning mathematics of many methods, such as analysis of
variance (ANOVA), are robust to minor violations in key assumptions but quantifying the amount of violation that makes a test unsuitable has not yet been proposed. Although hypothesis testing, and the sacred $P$ value, provide a quantifiable likelihood of an observed effect being attributed to chance, there is no information about the direction or magnitude of this effect (Bland & Peacock, 2002). Jacob Cohen proposed using an effect size, $d$, to quantify the magnitude of the effect (or difference) (Cohen, 1992). This is calculated by dividing the difference between means by the pooled standard deviation. Thresholds of 0.2, 0.5 and 0.8 are considered to be small, moderate and large effects, respectively. A caveat, this approach is suitable only for parametric data; alternative statistics such as Cliff’s $\delta$ and Hedge’s $g$ should be calculated for datasets that profoundly violate normality.

More recently, confidence intervals around the difference (between groups, over time, or between groups over time) have been endorsed as an estimate of the effect and its error. Confidence intervals provide a range of values that are 90, 95 or 99% likely to include the true population parameter of interest. Both significance testing and confidence intervals are heavily influenced by the number ($n$) of cases included. As $n$ increases the standard error of the mean (SEM; equal to the standard deviation divided by the square-root of $n$) decreases - this reduces the breadth of confidence interval. Similarly, as $n$ increases, the probability that the observed difference is attributable to chance alone decreases, because as more cases are included a higher proportion of the population is included in the sample and more confidence can be placed in the test statistic. This phenomenon can contribute towards making seemingly small changes statistically significant.

Most clinical trials report results that are statistically significant with no effort to ascertain the clinical meaningfulness of the results. The minimum clinically important difference (MCID) is an a priori threshold specified by the researcher that is clinically meaningful; a change from baseline - or difference between groups - of greater than the beneficial MCID (positive or negative, depending on the variable) can be inferred to affect the clinical status of the sample (and possibly the population). MCIDs are population- and disease-specific; for example a reduction in serum cholesterol of 1 mmol$\cdot$L$^{-1}$ is probably of clinical benefit to group whose baseline was 5 mmol$\cdot$L$^{-1}$, but not to a hypercholesterolaemic group whose baseline was 10 mmol$\cdot$L$^{-1}$. The MCID can
be calculated using anchor-based (i.e. pilot data) or distribution-based (i.e. a pre-determined effect size or distribution proportion) methods. The latter is often calculated as a proportion of the standard deviation at baseline (e.g. one fifth, one quarter or one third) and is therefore dependent on baseline characteristics. For example, if by chance (or design) all participants on a diabetes study are between 120 and 130 kg, the standard deviation may be as low as 2 kg, with one quarter of that as 500 g – this proposed MCID could be feasibly observed before and after a single trip to the toilet, and is unlikely to be of clinical benefit to patients. Distribution-based methods are not founded with any clinical metric (such as answers to “Do you feel better?”) so it is perhaps more prudent to refer to these values as minimal important differences (MIDs) instead.

Batterham and Hopkins (2006) proposed a trichotomous scale to compare confidence intervals for the difference between groups over time. These three regions describe effects as harmful, trivial or beneficial. The thresholds between harmful and trivial, and trivial and beneficial were defined as one MCID (or MID) either side of 0. The authors also recommended reducing the confidence interval to 90% as they considered 95% to be too conservative – this allows each tail of the interval to be equal to alpha (usually 0.05). Moreover, Sterne & Davey-Smith (2001) also promote the use of 90% confidence intervals, but their justification was to increase distinction between confidence intervals and alpha thresholds to minimise misinterpretation. Depending on the proportion of the 90% confidence interval that sits within each domain, the effect can be attributed a simple, plain-language inference based on ratios previously published (Hopkins, 2002). The particular usefulness of a confidence interval and its meaningfulness is apparent in health economics.

### 2.11 Rationale

With a strong epidemiological link between obesity and OSAS, and the obesity epidemic rife in the developed world, there is likely to be a marked increase in diagnoses of OSAS over the next decade. Although CPAP has formed the bedrock of OSAS treatment for two decades, patient compliance to its use is sub-optimal and hence identifies a need for alternative therapeutic approaches to be investigated with
robust randomised controlled trials, and for the effects of low compliance with CPAP on key health outcomes to be better understood.
Section 2 – Lifestyle intervention in obstructive sleep apnoea syndrome – a randomised controlled feasibility study
Chapter 3 – Introduction

Despite the effectiveness of nightly CPAP therapy, it simply manages OSAS (by increasing intra-pharyngeal pressure and reducing airway collapsibility) and does not itself offer hope of a cure. Especially for those diagnosed in their early adult life, it can be a quite daunting prospect to know they may sleep with a facemask for the remainder of their life. This can be especially complicated for those who travel regularly, spend time away from electricity or are starting a new relationship. Consequently, intervention must take place for there to be any chance of reducing syndrome severity significantly enough that CPAP is no longer necessary. This can probably act as a reliable motivator.

Therapies delivered in parallel to CPAP can induce marked reductions in body mass that have been shown to improve OSAS. These can be broadly grouped into dietary interventions, exercise interventions, surgical interventions, pharmacological interventions and hypnotherapeutic interventions. Indeed, diet and exercise are commonly addressed in tandem (as they both contribute to energy balance) and hence termed lifestyle-based interventions. Also, they can be supplemented with other approaches such as behaviour change counselling or Orlistat therapy. Such strategies could provide efficacious and cost-effective options for the holistic management of OSAS. Reductions in obesity and improvements in cardiopulmonary fitness can provide significant health benefits and although there are substantial recommendations in the public domain to make such improvements self-directed uptake appears to be poor. Furthermore, NHS-supported programmes are limited to cardiac and pulmonary rehabilitation services.

With continuous treatment likely unless significant changes in the underlying aetiology are achieved, it is obvious that strategies to address causation are required. Exercise plays a role in the management of many chronic conditions and has been proposed in OSAS to not only stimulate weight loss, but to address the greater risk inactivity is thought to play in the development of cardiovascular disease (Blair, 2009). Previous exercise-based interventions have been poorly designed with small sample sizes (Ueno et al., 2009; Sengul et al., 2011), uncontrolled designs (Norman et al., 2000; Giegelhaus
et al., 2000; Barnes et al., 2009) and homogenous samples (Ueno et al., 2009). There is a need for robust randomised controlled trials investigating the effects of pragmatic lifestyle interventions in obstructive sleep apnoea.

This longitudinal study aimed to investigate the feasibility of a pragmatic lifestyle intervention incorporating exercise, dietary advice and behaviour change counselling delivered to obese obstructive sleep apnoea patients in Sheffield whom were compliant with CPAP therapy. The underlying reason for recruiting highly-compliant CPAP patients is two-fold: because this group constitutes the most common sub-group of treated OSAS patients; and, CPAP-compliant patients are likely to be compliant with adjunct lifestyle intervention. To our knowledge this is the first UK-based trial of its kind. The objectives of this study are to design and conduct a combined lifestyle intervention that could be deliverable in a healthcare setting and to collect provisional data to evaluate its feasibility and its effects on important health outcomes.

The research questions are:

- Is a pragmatic lifestyle intervention feasible in CPAP-compliant OSAS patients?
- Can a lifestyle intervention improve body mass and composition, cardiovascular risk profile, health-related quality of life and exercise capacity?

The original hypotheses are:

- The lifestyle intervention will be acceptable to patients which will be reflected in a favourable recruitment rate of at least 10% (in line with other studies from our centre)
- The lifestyle intervention will improve key health outcomes including body mass, serum cholesterol and exercise capacity
Chapter 4 – Methods

4.1 Study design

This was a non-blinded, parallel group, randomised controlled feasibility study. The terms feasibility and pilot in reference to trial design are often used interchangeably and the difference between the can be difficult to discern. Arain et al. (2010) discussed the key differences between the two and advocated the use of National Health Service Evaluation, Trials and Studies Coordinating Centre (www.netscc.ac.uk/glossary) definitions. A feasibility study investigates the viability of a study design/methods, such as key participation rates (such as recruitment, retention and compliance), the practicality/design of study commitments, provisional data on principal outcome measures and the overall practicality and value of recreating the study on a larger scale. Feasibility studies will often identify areas of weakness in the design of a study which should be addressed before proceeding to a larger trial. A pilot study is a small version of an intended larger study that is conducted to ensure the main processes of the main study, such as recruitment, randomisation and the intervention are appropriate and integrate effectively. If the pilot study is successful the collected data could be used in the larger study, hence becoming an internal pilot. Regardless of their “classification” these small-scale studies tend not to be reported, despite their value in contributing towards study design for researchers in similar fields (Lancaster et al., 2004; Arain et al., 2010). Preliminary studies are usually conducted to refine study design and are often developed into randomised controlled trials (RCTs). An RCT is the gold-standard design in health research and allocates a sample randomly to either a treatment group or a control group. The treatment could be a new drug, behaviour, device or procedure and the control is usually either the current preferred treatment or no treatment at all. In this study, our treatment was a lifestyle intervention, and our control was basic lifestyle advice.

Pilot work is important to ensure subsequent trials are feasible in design and to provide provisional clinical evidence on treatment efficacy. Data yielded from pilot work can contribute to a sample size calculation for a definitive trial, providing specific
aspects of the design remain unchanged. This study was sponsored by Sheffield Teaching Hospitals NHS Foundation Trust (STH-15488) and jointly funded by Sheffield Hospitals Charitable Trust and the Sleep Apnoea Trust Association. This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and ethics approval was provided by South Yorkshire Research Ethics Committee (09/H1310/74). This study was registered on an international database (http://www.clinicaltrials.gov; NCT01546792)

4.2 Recruitment

4.2.1 Strategy

Participants were recruited from sleep clinics at the Royal Hallamshire Hospital, Sheffield, and the Northern General Hospital, Sheffield. Potentially eligible participants were identified at first pass using three basic criteria:

- at last check were using CPAP for >4 hours on >65% nights over at least a 6 month period
- body mass index (BMI) >28 kg·m⁻²
- no obvious contraindications to any study procedures
- no obvious exclusion criteria

This criteria were intentionally more inclusive than the inclusion and exclusion criteria because physiological parameters that could exclude someone (e.g. BMI=29 kg·m⁻²) who had since put weight on and was now eligible. Potentially eligible participants were sent a recruitment pack that contained a recruitment letter (appendix 1), participant information sheet (appendix 2), response slip and an addressed prepaid envelope (to return their response slip). Whether interested or not, patients were asked either to call the study researcher (and leave a message if unable to get through) or return the reply slip (that included space for their name, phone number and two check boxes to indicate interest or disinterest). After four weeks, those who did not provide a response were presumed to have not received the recruitment letter and to ensure equal opportunity were followed up with a telephone call.
4.2.2 Familiarisation

Participants that responded with interest were invited to our laboratory at Sheffield Hallam University to meet a member of the research team. The researcher briefly outlined the activities that the Centre for Sport and Exercise Science undertakes gave a tour of our laboratories. A thorough medical history (appendix 3) was taken and compared with a detailed list of inclusion and exclusion criteria (section 4.3 and 4.4). Patients' compliance to CPAP therapy was also discussed. Ineligible patients were informed of their unsuitability at this point and thanked for showing an interest in the study. Patients considered potentially eligible were referred to Dr Stephen Bianchi (Consultant Physician in Respiratory Medicine, Northern General Hospital, Sheffield, UK) for final approval.

The researcher then explained in detail the purpose of the study, the procedures involved in assessments, the randomisation process and the potential benefits and risks of participation. The difference between the two groups in the study and the different requirements these groups had was also discussed. The difference in time commitment was emphasised and patients were encouraged to enrol only if they intended to fulfil the requirements of whichever group they were randomised. At this point a demonstration of the principal equipment and procedures involved with the study was given. The patient was then encouraged to ask any questions and express any concerns they may have had. Once questions had been answered to the satisfaction of the patients they were asked if they would like to enrol on the study. Those wishing to enrol provided written informed consent (appendix 4). The medical history taken at this stage was compared with a more extensive list of inclusion and exclusion criteria.

4.3 Inclusion criteria

- Obstructive sleep apnoea confirmed with single-/multi-channel polysomnography or overnight pulse oximetry. OSA was defined as an AHI of $>10\text{ events}\cdot\text{h}^{-1}$ or an ODI of $>10\text{ desaturations}\cdot\text{h}^{-1}$
- BMI $>30\text{ kg}\cdot\text{m}^{-2}$
• Compliant to CPAP therapy (>4 hours per night and >75% of nights) for six months prior to enrolment
• Men and women aged 18 to 85 years

4.4 Exclusion criteria

• Current participation in regular purposeful physical activity (>30 minutes on >3 days per week for six months prior to enrolment); based on self-report
• Contraindications to exercise testing and training, such as unstable angina, uncontrolled hypertension
• Individuals unable to provide written informed consent
• Patients unable to travel to the testing facility at Sheffield Hallam University
• Recent participation in medical research (<6 months)

4.5 Lifestyle intervention

4.5.1 Overview

The lifestyle intervention involved 24 supervised sessions delivered with decreasing frequency over 12 consecutive weeks that took place within a dedicated exercise facility at Sheffield Hallam University, Sheffield, UK. The sessions were based on supervised exercise and included components of dietary advice and behaviour change counselling. As the study was designed as a pilot, the overall objective was to collect data that could inform the design of a larger definitive trial (outcomes described further in section 4.6). Consequently, the intensity and duration of the intervention were designed with both efficacy and pragmatism in mind. We were conscious not to over-burden participants with a high-intensity intervention with which they were likely to struggle, but to have it intensive enough to elicit clinically meaningful changes in secondary outcome measures. The intervention was designed so that it could be integrated into NHS practice, with sessions lasting approximately an hour (similar to most hospital appointments) and arranged at a time mutually convenient for staff and participants (as is the practice in the sleep clinics from which we were recruiting from).
4.5.2 Role of supervision

The role of the supervisor in the exercise sessions was three-fold: instructor, educator, and counsellor. Participants were coached on the safe and correct way to use the exercise machines involved in the exercise sessions (instruction). They were given full tuition on the correct technique for free-weight dumb-bell exercises and Swiss ball exercises selected to strengthen the major muscle groups of the body. Participants were taught in simple terms the benefits of exercise as understood in current scientific literature (education). This helped to address any misconceptions participants may have had from their own education and experiences over the decades previous. The researcher discussed with participants their habits and behaviours and encouraged them to make improvements wherever possible (counselling). This aspect of the study is discussed further in section 4.5.5.

4.5.3 Exercise

4.5.3.1 Safety and considerations

Recruiting to exercise trials from clinical populations brings with it an increased level of risk. These risks are especially present when obese adults are involved as obesity is associated, amongst other things, with an increased load on the heart during rest, and during exercise. This can be caused by increased intrapericardial fat and increased peripheral blood vessel resistance.

Comparing our inclusion criteria with the ACSM risk stratification (Armstrong, 2006), all participants recruited onto this study were of at least moderate risk (because of the presence of obesity and physical inactivity) and consequently, all exercise training and testing were conducted by individuals with at least Immediate Life Support training, access to life support equipment such as a defibrillator/oxygen and knowledge of the current departmental standard operating procedures for dealing with serious adverse events (such as cardiac arrest). Sessions involving exercise to volitional exhaustion were likely to involve the highest risk and were conducted in the presence of two qualified individuals.
4.5.3.2 Principles of exercise prescription

The exercise sessions were bespoke in design for each participant depending on comorbidities, physical ability and patients' preference. Exercise can be defined by the acronym FITT; frequency, intensity, time, type.

- **Frequency**: How often the exercise occurs.
- **Intensity**: How hard the exercise is perceived.
- **Time**: How long the exercise lasts.
- **Type**: How the exercise is delivered.

This intervention involved a fixed frequency of three exercise sessions per week. This allowed for alternate training days and resting days through the working week (supervised sessions took place during working hours to comply with health and safety protocols and reflect the circumstances with which it could be delivered in a healthcare setting). After 4 weeks the number of weekly supervised sessions was reduced to 2 with the third weekly session being completed unsupervised at a remote location. The structure of this session was planned in advance on a home exercise sheet (appendix 5), which also left space to put the achieved exercise. At week nine the number of unsupervised sessions increased to two per week, with one still supervised at Sheffield Hallam University. The intensity of prescribed exercise was controlled using the Borg rating of perceived exertion 6-20 scale (RPE; Borg, 1982) and heart rate. The RPE scale is a subjective measure of exercise intensity from the participant’s point of view. The researcher carefully explained this tool at the start of the intervention using a pre-defined explanation. Heart rate (HR) was also used to control the intensity of exercise – this is an objective physiological measure free from conscious bias (unlike RPE). Heart rate was assessed using a chest-mounted heart rate monitor (F4, Polar Electro, Finland) and a wristwatch receiver (Polar Electro, Finland).

Heart rate reserve (HRR) was calculated for each participant using estimated maximum heart rate ($HR_{\text{MAX}} = 220 - \text{age}$) and resting heart rate ($HR_{\text{REST}}$; measured after 20 minutes in a supine position during the baseline assessment) using a standardised equation ($HR_{\text{MAX}} - HR_{\text{REST}}$). Percentage of HRR (%HRR) was determined (decimalised proportion $\times$ HRR + $HR_{\text{REST}}$). The use of HRR for exercise prescription was used only in patients not taking medication that can affect heart rate (such as β-blockers).
Exercise sessions started with a light 5-min warm-up (i.e. RPE 9/10; light) and concluded with a 5-min cool-down (i.e. RPE 9/10) in accordance with ACSM guidelines (Armstrong, 2006). The intensity was initially set low (equivalent 50% HRR, RPE<12; moderate) to allow participants to become accustomed to any unfamiliar equipment and techniques. After one week, once participants were comfortable, the intensity was increased to 60-80% HRR and RPE 13-15. Over the course of the intervention the intensity was increased in accordance with decreased heart rate and RPE (indicative of improved exercise tolerance). The time spent on each piece of equipment was initially between 8 and 12 minutes and also increased as the programme progressed. Sessions initially targeted 30 minutes of aerobic exercise and steadily increased to 45 minutes. The types of aerobic exercise include treadmill walking, cycle ergometry, rowing ergometry and elliptical-training. An example of a participant in a training session is depicted in Figure 4.1.
Figure 4.1 Participant exercising during a training session.
Interval training was commonly used in the delivery of aerobic exercise (i.e. 2 min cycling at 80 W/1 min cycling at 110 W) because it has greater health benefits than moderate-intensity exercise (Tjønna et al., 2008). Also, alternating hard and light intervals allowed participants to be interactive with their exercise sessions, possibly reduced repetitiveness and boredom and provided opportunity for discussion and counselling with their supervisor (during the light intervals). Participants who could not exercise at intensities equivalent to hard intervals performed continuous exercise at their highest capacity. There was also dedicated time spent on flexibility (dynamic and static stretching) and resistance training. Resistance training consisted of 2-3 sets of 8-12 repetitions using free-weights that were challenging, yet achievable (correctly selected weights would be very challenging towards the end of the final set). Target muscle groups included biceps brachii, triceps brachii, deltoids, pectoralis major, rectus abdominus, quadriceps femoris and rectus femoris.

4.5.4 Dietary advice

Unlike the aforementioned VLED interventions, the dietary aspect of our study was designed to be direct, pragmatic and deliverable in a healthcare setting. Advice was basic, delivered by an exercise scientist and integrated into the supervised exercise sessions (either before the session, during light exercise, during recovery periods or after the session). The dietary advice was delivered using three key points based on addressing the quality (dietary imbalance and food labelling) and quantity (portion sizing) of food consumed:

**Dietary balance**: using the Eat-well Plate - a pictorial representation of the five major food groups in a pie chart format (Food Standards Agency, www.eatwell.gov; Figure 4.2) - with size of each segment relative to the proportion of a healthy diet that food group represents. Particular emphasis was placed on the "fruit and vegetables" slice and the concept of 5-a-day (five portions of fruit and vegetables per day). This tool has been incorporated into interventions previously (Ford et al., 2010).

**Food labelling**: although traffic-light labelling is mandatory for pre-packaged foods in the UK on packaged food, manufacturers are keen to present their product in the best
light to improve sales. As a result, the perceived “healthiness” of products is easily overestimated. A systematic review by Campos et al. (2011) suggested regular use and understanding of food labels is sub-optimal. Participants were taught how to read and understand food labels to facilitate their ability to make informed choices.

Portion sizing: participants were advised about the concept of energy balance and the role of portion sizing in regulating daily energy intake. Plate choice, the amount of food prepared during mealtimes and snacking habits were also discussed.

To support these ideas, participants kept a three-day diet diary (appendix 6) during the first week to assess baseline eating habits. This facilitated the identification of dietary imbalance and overeating. The diet diary was used in the setting of short- and long-term goals to support the concurrent behaviour change. A British Heart Foundation resource "So... you want to lose weight for good" was used as a take home resource. The study researcher discussed the contents with participants with particular emphasis on key topics such as planning mealtimes ahead of time (e.g. making a shopping list and sticking to it) and identifying eating patterns and triggers for unhealthy choices.
Figure 4.2 The EatWell Plate. A basic pictorial representation of the main food groups and they proportion of a balanced diet each should occupy. Food Standards Agency, www.eatwell.gov
**4.5.5 Behaviour change**

Behaviour change counselling aimed at promoting regular physical activity and healthy eating (referred to hereafter as lifestyle change) was integrated into the supervised exercise sessions. The intention was to provide participants assigned to the lifestyle intervention group with the necessary knowledge and the psychological skills and tools to sustain changes in their exercise and dietary behaviours. This trial used the Trans-Theoretical Model (TTM; Prochaska & Norcross, 1999) as the guiding model of behaviour change.

Information elicited from health screening and during the first couple of sessions helped the researcher to assess which stage of change participants were at (pre-contemplation, individuals with no intention of lifestyle change; contemplation, individuals intending to change their lifestyle; preparation, individuals who have made steps towards lifestyle change without regularity). Individuals in the action (have sustained lifestyle change for six months) and maintenance (have sustained lifestyle change for more than six months) stages of change had already been screened out at the familiarisation stage and were not enrolled on the study. In line with TTM, weeks 1–4 focussed on cognitively based intervention strategies such as cognitive reappraisal and consciousness-raising.

During weeks 5–8, more behaviourally-based interventions were introduced, for example, goal-setting, self-monitoring and finding social support. Participants followed a broad, structured curriculum of topics over the course of the intervention. Detailed descriptions of the strategies and techniques used are outlined in appendix 7. Such an approach has demonstrated efficacy in other clinical populations (Daley *et al.*, 2004).

This aspect of the intervention was delivered by an exercise physiologist (James Moss) and supervised by a Chartered Psychologist (Dr Robert Copeland).

**4.6 Primary outcome measures - feasibility**

Feasibility trials assess study design and identify changes that should be made before reproducing the study on a larger scale. This should safeguard against major wastage of resources, time and effort. Despite their importance, pilot trials are under-
represented in leading medical journals (Arain et al., 2010). Thabane et al. (2010)) suggested that pilot studies address four key aspects of research design:

Process:

- Recruitment: how willing will participants be to volunteer for the research? What factors limit recruitment (i.e. childcare, work commitments, transport difficulties?)
- Retention: how many participants will withdraw from the study? Why have participants withdrawn?
- Compliance: how compliant will participants be with the commitments of the study? Will compliance to study activities dwindle as time goes on?
- Eligibility: are the eligibility criteria appropriate? Are they too inclusive (confounding results) or exclusive (limiting recruitment)?

Resources:

- Capacity: how many participants can the study handle at any one time? What changes could be made to increase participant throughput?
- Equipment: is the equipment valid, does it promote reproducible measures and does it measure the desired variable?

Management:

- Financial management: was the projected budget accurate? Were there and unexpected costs?
- Data collection: can assessments be completed on time?
- Data management: can the data stored safely and backed up regularly? Was any data lost?
- Variability of outcome measures: how variable were the outcome measures of interest?

Scientific

- Safety: were there any adverse or serious adverse events? How can they be avoided in the future?
• Treatment intensity: was the intensity of the intervention appropriate?
• Estimate of the treatment effect: were any changes of the direction and magnitude anticipated?
• Variability of the treatment effect: how variable was the treatment effect?

4.6.1 Recruitment

Two recruitment rates were calculated as screening occurred at two points during the recruitment process. Some participants were obviously unsuitable and not sent a recruitment pack (screened out at first-pass). Some patients were then ineligible upon a more in depth screening after showing an interest in participating (second-pass).

The overall recruitment rate was determined by dividing the number of patients recruited by the number of patients invited to participate (multiplied by one hundred to yield a percentage). The expected recruitment rate was calculated by inferring the rate of ineligibility in the interested patients existed in the original sample (once those who had already been identified as ineligible were accounted for). This produced a smaller denominator and a higher recruitment rate. The reasons participants were not interested in participating were recorded, if offered, to identify potential factors that limited recruitment.

4.6.2 Retention

Participant retention rate was calculated by dividing the number of patients completing the study by the number of patients that enrolled (expressed as a percentage). The reasons for participants withdrawing from the study were recorded, if offered, to identify factors that were influencing participant drop-out.

4.6.3 Compliance

Compliance was calculated for the assessment sessions (for participants in all arms of the trial), for exercise sessions (for the intervention group only) and for all study visits (including familiarisation, assessments and exercise sessions (if applicable)). Compliance with assessments was calculated by dividing the number of completed assessments by the total number of possible assessments (multiplied by one hundred to yield a percentage). Exercise sessions compliance was calculated by dividing the
number of attended exercise sessions by the total number of possible exercise sessions (multiplied by one hundred to yield a percentage). Total study compliance was calculated by dividing the number of attended study sessions by the number of scheduled visits. All compliance rates were calculated post-hoc using attendance data from participants who completed the study only.

4.6.4 Safety

Any adverse events (AEs) or serious adverse events (SAEs) were recorded in the study site file and reported in accordance with Sheffield Teaching Hospitals NHS Foundation Trust procedure. The nature of the event, how it was managed, and what action was to be taken in the future to avoid it was included.

4.7 Secondary outcome measures - efficacy

Assessment sessions took place at varying times of the working day (8 a.m. to 6 p.m.) depending on convenience principally for participants but also researchers. Subsequent assessments took place at the same time of day as the baseline assessment. For shift workers subsequent assessments occurred at the same stage of their shift pattern (e.g. two days after finishing night shifts). For pre-menopausal female participants subsequent assessments occurred at the same stage of their menstrual cycle.

Participants were asked to abstain from exercise for 48 hours, from smoking for 12 hours, from caffeine for 12 hours and from food for 4 hours before all assessments. Ideally, all assessments would take place in the morning after a 12-hour overnight fast, however we were concerned this would have an adverse effect on those in full-time work and consequently their willingness to participate.

4.7.1 Anthropometry

Stature was measured (in cm to 1 decimal place) using a fixed stadiometer (Harpenden, Holtain Ltd, Crymmych, Wales). Participants were asked to align the posterior aspect of their scapulae to a backboard, with the posterior aspect of their heels to a heel board. The head was positioned so that the Frankfort plane was parallel
to the floor. Participants were asked to inhale deeply while adhering to the aforementioned criteria. Body mass was measured (in kg to 2 decimal places) on a calibrated balance beam scale (model 424; Weylux; Hallamshire Scales Ltd, Sheffield, UK). Minimally dressed participants were asked to stand upright while the researcher used sliding weights to counter balance their mass. Once an equilibrium was met the position was held for >10 s to ensure accuracy. Body fat percentage was assessed using bioelectrical impedance (Bodystat® Quadscan 4000, Bodystat ltd., IM99 1DQ). Participants were asked to lay supine with two adhesive cathodes attached to the volar aspect of the wrists and anterior aspect of the ankles on the right hand side of their body. Anodes were placed 3 cm distal to the cathode. Neck, waist and hip circumference were measured using an inelastic anthropometric tape. The locations for measurement were covered with as little clothing as possible. Neck circumference was defined as the smallest measureable circumference between the clavicle and the mandible on the anterior aspect, and the first and seventh cervical vertebrae on the posterior aspect. Abdominal circumference was defined as the circumference of the abdomen at the level of the omphalion, parallel to the floor (Eston & Reilly, 2009). Hip circumference was defined as the circumference of the pelvis at the point of greatest posterior protuberance, parallel to the floor (Eston & Reilly, 2009). The mean of three measurements was used at each site.

4.7.2 Blood-borne biomarkers

Participants were asked to fast for at least six hours prior to their visit. Blood was drawn using the BD VacuTainer® system from a vein in the antecubital fossa using aseptic techniques. Blood was collected into gel serum-separator tubes lined with a clot-activator compound. The tubes were inverted and reverted six times to allow thorough mixture of the blood and preservatives and given 30 minutes to clot. The clotted samples were then centrifuged at 3000RCF (Heraeus Labofuge 400R, Germany) for 9 minutes. Once separated, 4 mL of serum were extracted using a 1000 µL Gilson pipette and stored in 1 mL Eppendorf tubes. These Eppendorf tubes were immediately placed in a -80 °C freezer. Time between venepuncture and freezing was <1 hour (which limits ex-vivo glycolysis and subsequent inaccurate measurement of serum glucose; Fernandez et al., 2012). Once the study was complete, samples were
transported to the Blood Sciences laboratory at Leeds General Infirmary (Old Medical School, Leeds, LS1 3EX) in a polystyrene container with dry ice. Once there, they were transferred to another freezer. Samples were thoroughly defrosted at room temperature and analysed in two batches on consecutive days in January 2012. Full lipid profile, glucose and high-sensitivity CRP were assessed using an ADVIA 2400 and insulin concentrations on an ADVIA Centaur® XP (Siemens, 511 Benedict Ave, Tarrytown, NY 10591). The CRP concentrations that exceeded 10 mg·L⁻¹ were considered indicative of an acute infection/inflammatory response (Ridker et al., 2003) and all blood results for that participant at all time-points were discarded.

4.7.3 Microvascular function

Microvascular function was assessed by measuring changes in skin blood flow in response to iontophoresis of vasoactive compounds. Skin blood flow was assessed non-invasively using laser Doppler flowmetry (LDF; PF 5010 laser Doppler perfusion monitoring system, Perimed AB, Järfälla, Sweden). This technique uses a low-energy laser beam (780 nM) transmitted from one or more probes positioned on the surface of the skin. The beam penetrated the skin to a depth of approximately 1 mm and was deflected back to a photosensor built into the probe. Some returned light underwent a wavelength shift (caused by the Doppler effect) proportional to the number and velocity of erythrocytes in the penetrative field. Unfortunately, LDF cannot measure actual blood flow in traditional units (volume per time) as there is no way to determine microvessel diameter (and consequently volume) – flux is calculated as an index of erythrocyte concentration and velocity and is expressed in arbitrary values. LDF has been used to investigate microvascular function in OSAS. (Yim-Yeh et al., 2010; Trzepizur et al., 2009; Kheirandish-Gozal et al., 2010). We used a thermostatic iontophoresis probe (PF 481-1, Perimed AB) to make skin blood flux measurements (Figure 4.3). This probe consisted of a single-fibre laser surrounded by a copper heating disc that was connected to the PF 5010 unit and a heating unit (PF 5020, Perimed AB).
Figure 4.3 Equipment used in iontophoresis. Top left: Perimed PF 481-1 iontophoresis probe; right: Perilont power supply; bottom left; PF 383 iontophoresis electrode.
The iontophoresis probe was affixed to a drug delivery electrode (PF 383, Perimed AB) to allow simultaneous iontophoresis and skin blood measurement. Iontophoresis is the non-invasive transcutaneous delivery of ionic compounds using an appropriately charged electrical current (i.e. a positively charged current will cause delivery of a positively charged compound; Figure 4.4).
Figure 4.4 Iontophoresis of a positively charged molecule
The iontophoresis electrode consists of a transparent window (for the LDF laser beam), surrounded by a small sponge in a well, which can be soaked in an ionic compound to be delivered (Figure 4.3). Behind this sponge is a copper disc that extends from the back of the electrode in an insulated arm. A device capable of providing a time-measured current (PF 382b Perilont power supply, Perimed AB; Figure 4.3) was attached to the drug delivery electrode and a dispersive electrode (PF 384, Perimed AB) placed 10-15 cm away. The total charge (current × time) was minimised in favour of an extended duration to prevent any non-specific (charge-related) vasodilation. The electrode has a large adhesive surface to facilitate secure placement of the probe (and to ensure the sponge makes contact with the skin (and complete the circuit)).

All vascular assessments were performed in a temperature-controlled room (22–24 °C) with participants supine and their experimental arm (usually left) extended laterally at the level of the heart. Heart rate and blood pressure (Dinamap Dash 2500, GE Healthcare, USA) were measured on the contralateral arm to avoid occlusion-related alterations in blood flow in the experimental arm. Participants rested for at least 15 min before starting data acquisition during which the probe was calibrated using the manufacturer’s motility standard. During this time a suitable site for the LDF iontophoresis probe was identified using the following criteria:

- hypopigmented glabrous skin
- no visible superficial blood vessels
- no dermal damage (e.g. burns, dermatitis, psoriasis)
- stable, resting perfusion of 2-8 APU
- stable skin temperature (set at 32 °C)
- stable total backscatter reading

Once identified, the site was cleaned using a disposable alcohol wipe to remove any latent skin cells and natural skin oils which could distort the signal or compromise the electrode adhesion. As appropriate, excessive arm hair was gently shaved at the earliest opportunity to allow any microtrauma-related hyperaemia to subside.

Skin blood flow data were recorded using PeriSoft for Windows 2.5 (PSW 2.5; Perimed AB) software. Data capture commenced once the participant was at rest and all
experimental conditions had been met. A 5-minute recording of resting skin blood flow was taken; if this was stable the first iontophoresis dose was usually delivered at 5 minutes.

4.7.3.3 **Endothelium-dependent vasodilation**

Twenty mg of acetylcholine chloride (ACh; Miochol-E, Novartis, Stein, Switzerland) were dissolved in 2 mL of Ringer's solution to produce a 1% solution. One hundred μL of this solution were then soaked into the circular sponge on the drug-delivery electrode. ACh is an anionic neurotransmitter and was delivered in seven 30-second doses, 30 seconds apart, using a delivery current of 0.1 mA·s\(^{-1}\) (Morris & Shore, 1996). Blood pressure was measured during each dose on the upper part of the contralateral arm. The experimental set-up is depicted in Figure 4.5.

The ACh delivery increased the activity of endothelial nitric oxide synthase (eNOS) that in turn increased nitric oxide NO production. NO is a potent vasodilator that activates cyclic guanosine monophosphate (cGMP), which opens calcium-dependent potassium channels (KCa). Potassium effluxes the cell, hyperpolarizing the cell membrane, closing voltage-gated calcium channels, inducing smooth muscle relaxation, vasodilation and increasing blood flow. ACh-dependent vasodilation is endothelium-dependent as it relies on the presence of eNOS to convert l-arginine to NO. This is supported by evidence that ACh will only stimulate vasodilation in the presence of the vascular endothelium (Furchgott, 1980).
Figure 4.5 Experimental set-up (study 1)
4.7.3.4 Endothelium-independent vasodilation

Endothelium-independent vasodilation can be assessed by iontophoresing sodium nitroprusside (SNP; Nitroprussiat Fides, Reig Jofré Group, Barcelona, Spain). Fifty mg of SNP were dissolved in 5 mL of Ringer’s solution to produce a 1% SNP solution. Once again, 100 µL of this solution were then soaked into the circular sponge on the iontophoresis electrode. SNP is a NO-donor, which causes vasodilation without involvement of the vascular endothelium. SNP is cationic and was delivered in four 30-second doses, 150 seconds apart, using a current of 0.2 mA·s⁻¹ (Morris & Shore, 1996). Blood pressure was measured during each dose on the upper part of the contralateral arm.

4.7.3.5 Offline analysis of laser Doppler flowmetry scans

Raw LDF recordings were analysed offline using PeriSoft (PeriMed AB, Sweden) after all study data had been collected. Areas of interest were highlighted on each trace and averaged using software-based algorithms. Any artefacts assumed to be caused by movement of the participant during data collection were interpolated to ensure data reflected changes in skin blood flow only.

The ACh traces had eight areas of interest; one area represented resting skin blood flow and seven “dose” areas followed each iontophoresis dose (indicated by vertical event markers on the trace; Figure 4.6). The SNP traces had five areas of interest; the first represented resting skin blood flow and remaining four areas represented peak blood flow after each dose. Resting skin blood flow was defined as a period >2 min before iontophoresis during which blood flow, total backscatter and temperature were stable and within range (each dose area was defined as the 30 seconds of continuous recording during each dose/rest cycle, which showed the highest mean blood flow). Once each trace was marked up, a report was generated to calculate mean values for each of the highlighted areas of interest. Mean values were divided by the mean arterial pressure recorded during that period to transform data into cutaneous vascular conductance (CVC) (mV/mmHg).
Figure 4.6 Example laser Doppler flowmetry recording. Highlighted areas are averaged to provide peak perfusion values for each region of interest for acetylcholine chloride (top) and sodium nitroprusside (bottom) iontophoresis. The top trace represents skin blood flow (in PUs); the middle trace shows the total backscatter (TB); and, the bottom trace shows temperature (°C)
4.7.4 Quality of life

The EuroQol-5D-3L quality of life assessment tool assessed health-related quality of life (appendix 8). The questionnaire consists of five questions pertaining to mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each question has a three-level response indicating no presence, some presence and high presence of disability. There is also a visual analogue scale which represents participants’ self-reported health. It involve interception a 0-100 scale at the appropriate level, with 0 representing worst health state imaginable, and 100 being best health state imaginable. Participants were not given any advice about interpreting and completing the questionnaire additional to that included in the official version. On recurring assessments, investigators and participants were blinded to previous responses. This tool was registered online with EuroQol.

4.7.5 Exercise capacity

Choosing appropriate assessment tools for exercise capacity in clinical populations is often difficult because of the multi-factorial nature of disease. Typically, an incremental protocol is used that starts off at a very light intensity and gradually increases allowing a large measurable range for the test that should - if appropriate - include the best and worst performer in the sample.

In a surgical setting, a pre-operative cardiopulmonary exercise test (CPET) is increasingly being used to assess functional capacity in high-risk patient groups. CPET performance strongly correlates with peri- and post-operative outcomes (Simpson et al., 2009) as exercise increases cardiopulmonary demand in a similar fashion to major surgery. The CPET involves assessment of expired gas volumes and gas fractions to determine the volume of oxygen and carbon dioxide being utilised and produced, respectively. These measurements are typically made on a breath-by-breath basis during an incremental test on a treadmill or cycle ergometer. Maximal oxygen uptake \( \dot{V}O_2 \text{max} \) can be measured if a CPET test is performed to patient exhaustion, otherwise peak oxygen uptake is recorded \( \dot{V}O_2 \text{peak} \). It is common to perform maximal tests on a cycle ergometer to reduce the risk of a falling injury or to wear a harness connected to a “dead man’s switch” (a pressure sensor that instantly stops the treadmill belt if a threshold force is applied to it - typically if a participant was to lose balance and fall).
With the equipment and expertise required, CPET can be an expensive way to assess functional capacity and simpler tests have been devised.

The Bruce protocol is an incremental treadmill test devised by Robert A. Bruce (Bruce et al., 1963). This protocol was designed to address the lack of standardised test to investigate cardiopulmonary function in exercising patients. The Modified Bruce protocol introduces two easier stages before the original first stage to allow even the most immobile patients to safely undertake the test.

The six-minute walk test (6MWT; Balke et al., 1963) utilises a 10-, 20- or 30-metre shuttle course which patients are asked to walk as far as possible at a self-selected pace in the allotted six minutes. This is a useful test for patients with moderate to severe physical impairment and requires no specialised equipment or venue. 6MWT distances (6MWD) have a moderate-to-high correlation with VO\textsubscript{2peak} in heart failure patients (Faggiano et al., 1997)). Its specificity and utility as a clinical trial endpoint in some populations has been questioned (Schoindre et al., 2009).

In this study, exercise capacity was assessed using the incremental shuttle walking test (ISWT; Singh et al., 1992). This test involved completing circuits of a 10 metre shuttle course. Shuttle timing was dictated by an audio signal broadcast through wall mounted speakers. Participants wore a chest-mounted heart rate monitor (F4, Polar Electro, Finland) allowing for continuous monitoring during the test. The ISWT was terminated under the following conditions:

- The participant completed the test (102 shuttles, 1020 metres)
- The participant fell more than 1 metre behind the audio signal for two consecutive shuttles
- The participant electively discontinued the test for symptoms such as breathlessness, fatigue or pain
- The researcher considered the participant to be having an adverse reaction to the exercise stimulus

Participants were asked to continue to the test for as long as possible and encouraged to continue during the latter stages. The ISWT is more commonly used in COPD populations, but has recently been validated in obese OSA patients (Billings et al., 2013). An alternative test would have been the 20-metre shuttle running test (Léger,
1982), however this protocol starts at 8 km·h⁻¹ and probably would have exceeded the capacity of the most limited participants. Participants were told to use any type of locomotion they wished to maintain pace with the audio signal for as long as possible, including running. Immediately after the test, heart rate, blood pressure, RPE and the primary reason for not completing additional stages of the test.

4.8 Statistical analyses

Reseland et al. (2001) reported a significant reduction in body mass index between groups over time (Δ -2.1 kg·m⁻²; P<0.001) using a similar intervention (supervised exercise and dietary advice). Using these data a sample size estimate was calculated using nQuery; at least 25 participants in each group were required (α= 0.1; β=80%). As we were unsure of potential dropout rates and the variability of our outcome measures we included an additional 20% and aimed to recruit 60 patients (30 per group). A sample size of 60 is further supported by the general rule of “at least 30 participants could be used to estimate a parameter from pilot trials” referred to in Browne (1995) and Lancaster et al. (2004). The sample size calculation in Johansson et al. (Johansson et al., 2009) aimed for 30 patients per group, to detect an improvement in AHI of 15 events·h⁻¹; although the current study did not assess AHI, we have recruited a sample large enough to detect a meaningful improvement. The principal reasons for not using AHI as an outcome in the current study was because of financial constraints (the monetary cost of acquiring the necessary equipment and consumables) and the added inconvenience for participants to make additional, albeit brief, visits to the study centre.

Mean and SD values described continuous variables at all time-points. Changes between groups over time were assessed using ANCOVA using change scores from baseline as the dependent variable and group and baseline values as independent variables. This model produced an adjusted mean difference that accounted for the impact of variation at baseline. The mean difference in change between groups was used as the main treatment effect with 90% confidence intervals and effect sizes using an adapted Cohen's $d$ method (Morris, 2008) were calculated. These confidence intervals were compared with the estimated minimal important difference (MID)
which was defined as one third of the pooled standard deviation at baseline (Yost & Eton, 2005). Effect sizes >0.33 were considered clinically meaningful. These comparisons were attributed qualitative inferences to facilitate interpretation (Batterham & Hopkins, 2006). Key relationships were explored using Spearman’s correlations; strong relationships were explored further using repeated measures within-subjects modelling (Bland & Altman, 1995).

Statistical significance was set at $P<0.05$. All analyses were done on an intention-to-treat basis with previous observations carried forward where necessary. A per-protocol analysis was also done for exploratory purposes. Data were analysed using the SPSS 21.0 statistical package (SPSS, IBM Corporation, Armonk, NY, USA).

4.9 Reliability of methods

4.9.1 Analysis outline

It is important to assess the reliability of experimental techniques. This requires assessment of both the skill of the investigator in performing the measurement and the technique itself. The reliability of a technique includes its validity (does it measure the required variable with adequate precision?) and reproducibility (are repeated measurements consistent?). Inter-day reproducibility can be assessed. Validity is critical for novel techniques and measurements will usually be compared to the current state-of-the-art method. Reproducibility can be measured on an inter-day intra-observer or inter-observer basis. Both examine the skill of the investigator and the former also assesses the stability of the measurement, which is especially useful for measuring biological variables (e.g. cholesterol).

Statistical methods exist to scrutinize reliability using the test-retest method. Intraclass correlation coefficient (ICC), coefficient of variation (CV) and technical error of measurement (TEM) were be used to assess inter-day reproducibility in this thesis.

4.9.2 Results

The reproducibility of methods used in study one is outlined in Table 4.1. Nine participants were tested twice at the same time of day approximately seven days apart. The reproducibility of the blood-borne biomarkers investigated in this study was
not assessed. This was deemed an unnecessary cost because the equipment used to perform the analysis was in a working hospital laboratory and undergoes quality control and assurance at multiple points through the day. Furthermore, omitting repeat blood samples reduced risk to participants.

Table 4.1 Inter-day intra-observer reproducibility of secondary outcome measures

<table>
<thead>
<tr>
<th></th>
<th>CV (%)</th>
<th>ICC</th>
<th>TEM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>0.27</td>
<td>1.00</td>
<td>0.4</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>0.07</td>
<td>1.00</td>
<td>0.1</td>
</tr>
<tr>
<td>Body fat percentage (%)</td>
<td>1.85</td>
<td>0.99</td>
<td>2.2</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>0.45</td>
<td>0.99</td>
<td>0.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.67</td>
<td>0.99</td>
<td>0.9</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>0.65</td>
<td>1.00</td>
<td>0.7</td>
</tr>
<tr>
<td>Resting heart rate (beats·min⁻¹)</td>
<td>3.95</td>
<td>0.82</td>
<td>4.6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>1.83</td>
<td>0.89</td>
<td>2.6</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>2.91</td>
<td>0.84</td>
<td>3.8</td>
</tr>
<tr>
<td>Post-ex RPE</td>
<td>5.78</td>
<td>0.60</td>
<td>8.2</td>
</tr>
<tr>
<td>Post-ex heart rate (beats·min⁻¹)</td>
<td>3.22</td>
<td>0.95</td>
<td>4.2</td>
</tr>
<tr>
<td>Post-ex systolic BP (mmHg)</td>
<td>3.92</td>
<td>0.83</td>
<td>7.1</td>
</tr>
<tr>
<td>Post-ex diastolic BP (mmHg)</td>
<td>7.57</td>
<td>0.59</td>
<td>9.2</td>
</tr>
<tr>
<td>ISWD (m)</td>
<td>4.38</td>
<td>0.97</td>
<td>4.9</td>
</tr>
<tr>
<td>CVCR_Est (ACh; mV/mmHg)</td>
<td>17.8</td>
<td>0.69</td>
<td>17.9</td>
</tr>
<tr>
<td>CVCMAX (ACh; mV/mmHg)</td>
<td>25.3</td>
<td>0.33</td>
<td>30.4</td>
</tr>
<tr>
<td>CVCR_Est (SNP; mV/mmHg)</td>
<td>14.5</td>
<td>0.54</td>
<td>17.1</td>
</tr>
<tr>
<td>CVCMAX (SNP; mV/mmHg)</td>
<td>26.0</td>
<td>0.61</td>
<td>33.3</td>
</tr>
</tbody>
</table>

BP: blood pressure; ex: exercise; RPE: rating of perceived exertion; ISWD: incremental shuttle walk distance; CVC: cutaneous vascular conductance; ACh: acetylcholine; SNP: sodium nitroprusside.
Chapter 5 – Results

5.1 Baseline characteristics

Sixty patients with CPAP-controlled OSAS were recruited from local sleep clinics and randomised equally into the intervention group and the control group. Baseline characteristics are described in Table 5.1. The intervention group were 56 ± 12 years old, 73% male, normotensive (systolic: 127 ± 11; diastolic: 72 ± 8 mmHg) and had a BMI of 40 ± 7 kg·m⁻². Similarly, the control group were 55 ± 10 years old, 80% male, normotensive (systolic: 132 ± 15; diastolic: 75 ± 9 mmHg) and had a BMI of 39 ± 7 kg·m⁻².

Despite randomisation there was higher prevalence of coronary artery disease (16 vs. 3%), ACE-inhibitor usage (23 vs. 13%) and beta-blocker usage (23 vs. 3%) in the control group compared with the intervention group. Other key comorbidities identified during history taking were similar including hypertension (40 vs. 50%), type 2 diabetes mellitus (20 vs. 16%) and smoking status (current: 6 vs. 10%; previous: 43 vs. 50%). Furthermore, other key medications were also similar (calcium channel antagonists: 20 vs. 13%; diuretics: 30 vs. 23%; statins: 36 vs. 33%; and, platelet inhibitors: 16 vs. 20%).

Post-hoc verification of CPAP compliance from three months prior to study enrolment to the completion of the follow-up assessment was assessed using data from the CPAP machine memory card that was downloaded routinely within a year of their participation. These data showed that although median compliance was excellent there were 13 of 60 participants (22%) across both groups who were unexpectedly below the compliance threshold despite reporting regular usage (>4 hours per night on >75% nights) to the research team during the screening process (Control: 95 [65, 98]%; intervention: 95 [81, 99]% nights>4 hours·night⁻¹).
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56 ± 12</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>22 / 8</td>
<td>24 / 6</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>118 ± 22</td>
<td>117 ± 24</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>172 ± 8</td>
<td>174 ± 9</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>40 ± 7</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Resting heart rate (beats·min⁻¹)</td>
<td>65 ± 14</td>
<td>67 ± 11</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127 ± 11</td>
<td>132 ± 15</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72 ± 8</td>
<td>75 ± 9</td>
</tr>
<tr>
<td>CPAP usage (% nights &gt;4h)</td>
<td>95 [65, 98]</td>
<td>95 [81, 99]</td>
</tr>
<tr>
<td>Serum cholesterol (mmol·L⁻¹)</td>
<td>4.6 ± 0.9</td>
<td>5.0 ± 1.3</td>
</tr>
<tr>
<td>Serum HDL (mmol·L⁻¹)</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Serum triglycerides (mmol·L⁻¹)</td>
<td>1.8 ± 0.7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Serum LDL (mmol·L⁻¹)</td>
<td>2.7 ± 0.7</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>Serum C-reactive protein (mg·L⁻¹)</td>
<td>3.0 ± 2.3</td>
<td>2.9 ± 2.5</td>
</tr>
<tr>
<td>Serum glucose (mmol·L⁻¹)</td>
<td>5.7 ± 1.6</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>ISWT distance walked (m)</td>
<td>414 ± 162</td>
<td>588 ± 157</td>
</tr>
</tbody>
</table>

**Co-morbidities**

- Coronary artery disease: 5 (16) | 1 (3)
- Hypertension: 12 (40) | 15 (50)
- Diabetes: 6 (20) | 5 (16)
- Current smoker: 2 (6) | 3 (10)
- Ex-smoker: 13 (43) | 15 (50)

**Medication**

- Beta-blocker: 7 (23) | 1 (3)
- ACE inhibitor: 7 (23) | 4 (13)
- Calcium channel antagonist: 6 (20) | 4 (13)
- Diuretic: 9 (30) | 7 (23)
- Statin: 11 (36) | 10 (33)
- Platelet inhibitor: 5 (16) | 6 (20)

CPAP: continuous positive airway pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ISWT: incremental shuttle walk test; ACE: angiotensin-converting enzyme. Data are presented as mean ± SD, median [IQR], or frequency (%).
5.2 Primary outcome measures - feasibility

5.2.1 Recruitment

In accordance with the Consolidated Standards of Reporting Trials (CONSORT) a flow chart depicting the flow of participants through the study is shown in Figure 5.1. Of 550 patients considered, 69 (12.5%) were overtly unsuitable either not meeting inclusion criteria such as BMI (26; 4.7%) or CPAP usage at the last compliance check (18; 3.2%), or having comorbid medical conditions that compromised participant safety or the fidelity of results (9; 1.6%). These data were gleaned from patient files within the sleep clinics, including consultation notes and referral letters.

The remaining 481 provisionally eligible patients were formally invited to participate. 123 (25.6%) responded with interest. Of these, 35 (28.5%) were ineligible upon further screening; including 11 (8.9%) who did not meet the inclusion criteria and 24 (19.5%) who met at least one exclusion criterion. Assuming all patients contacted were eligible to participate, our recruitment rate was 12.5%. However, pre-enrolment medical screening during familiarisation identified an ineligibility rate of 28.5%. Inferring this rate to the whole sample, 157 of the 550 patients initially considered would have been ineligible. This number of remaining patients inferred to be eligible (397) used as the denominator to calculate recruitment rate gives an inferred rate of 15.3%.

Of those interested, 88 (71.5%) were eligible to participate. Twenty patients were unable to integrate the demands of the study into their current commitments (e.g. employment and childcare) and 8 patients became unreachable by the research team. The remaining 60 enrolled giving a recruitment rate of 15.3%. Recruitment was completed within the proposed timeframe of 18 months.
Figure 5.1. CONSORT flowchart showing the flow of participants through the study. BMI: body mass index; CPAP: continuous positive airway pressure; PIS: participant information sheet
5.2.2 Retention

Six participants withdrew from the study (10%) and 54 (90%) completed the follow-up assessment (90%). Withdrawals were evenly split between groups (intervention: 3 vs. control: 3). Two participants cited a change in their health unrelated to their involvement in the study (one participant had recurrence of a previous chronic back problem, brought about by a fall during a weekend and another had acute exacerbation of COPD and although these participants wished to continue they were excluded by the research team for safety reasons). Indeed, this made the elective dropout rate from the intervention group one in thirty (3%). Two participants withdrew because of a change in their professional commitments (one participant was relocated by his employer to elsewhere in the UK and the other was given an alternative position at a different site making travelling to the University unfeasible). Both participants reported they would not have enrolled had known about the impending changes in their circumstances. Two participants declined to provide a reason for discontinuing their participation although they expressed mild dissatisfaction at their group allocation.

5.2.3 Compliance

Participants in the intervention group who completed the study (n=27) attended 98% (79 of 81) of assessment sessions compared with 96% (78 of 81) in the control group (n=27). Reasons for missing an assessment session included work commitments (n=3) and ill health (n=3). The last observed value, when available, was carried forward for missing data points. The intervention group attended 620 of 648 (96%) University-based exercise sessions and completed 312 of 324 (96%) independent exercise sessions. Overall compliance with scheduled sessions was 96% (777 of 810).

5.2.4 Safety

In over 650 hours of supervised exercise and 300 hours of independent exercise there were no reported AEs or SAEs. Participants in the intervention arm occasionally reported muscle soreness and joint ache resulting from the exercise. In these circumstances the researcher amended the training stimulus to prevent further
discomfort and limit the risk of long-term injury without, where possible, reducing the load.

5.3 Secondary outcome measures – efficacy

Changes in continuous variables were assessed using ANCOVA with the dependent variable set as the change score between time-points (e.g. delta follow-up is equal to follow-up score minus baseline score). Scatter plots and histograms were subjectively assessed to evaluate the assumptions of linearity, independence, homoscedasticity and normality as outlined in chapter 4.

5.3.1 Anthropometry

Data for anthropometric outcome measures are shown in Table 5.2. There was a modest reduction in body mass from baseline in the intervention group compared with the control group at 13 weeks (Δ -2 [-3, -1] kg; P=0.007) and 26 weeks (Δ -2 [-3, -1] kg; P=0.010). The MID for changes in body was estimated as ±7.7 kg (Table 5.3) - comparison of the 90% CI of the change between groups over time suggests that the change, albeit statistically significant, was not clinically meaningful (Figure 5.2). Moreover, the effect sizes for changes at end-point and follow-up were very small (d=0.08 and d=0.09, respectively). There were also significant reductions in percentage body fat, which was reduced by 1% ([1, 0]; P=0.002; d=0.12) at end-point; this improvement was maintained at follow-up (Δ -1 [-1, 0] %; P=0.002; d=0.14). Similar observations were also made for changes between groups over time for hip circumference (Δ -2 [-3, 0] cm; P=0.026; d=0.13; clinically trivial) at follow-up. Changes in hip circumference at 13 weeks were not significant (P=0.102; d=0.07) and deemed clinically trivial. There were no changes in neck circumference and waist circumference at end-point or follow-up (all P>0.05, d<0.20 and deemed clinically trivial). In addition to changes in body mass, changes in all other anthropometric outcomes were also deemed trivial (Figure 5.2).
### Table 5.2 Anthropometric outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>BL</th>
<th>EP</th>
<th>FU</th>
<th>BL</th>
<th>EP</th>
<th>FU</th>
<th>Δ (90% CI)</th>
<th>P</th>
<th>d</th>
<th>Δ (90% CI)</th>
<th>P</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>60</td>
<td>118 ± 22</td>
<td>118 ± 21</td>
<td>118 ± 21</td>
<td>117 ± 24</td>
<td>115 ± 24</td>
<td>115 ± 24</td>
<td>-2 (-3, -1)</td>
<td>0.006</td>
<td>0.08</td>
<td>-2 (-3, -1)</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>60</td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>39 ± 7</td>
<td>38 ± 7</td>
<td>38 ± 7</td>
<td>-1 (-1, 0)</td>
<td>0.002</td>
<td>0.12</td>
<td>-1 (-1, 0)</td>
<td>0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>60</td>
<td>40 ± 9</td>
<td>40 ± 9</td>
<td>40 ± 9</td>
<td>39 ± 8</td>
<td>37 ± 8</td>
<td>38 ± 8</td>
<td>-1 (-2, 0)</td>
<td>0.044</td>
<td>0.14</td>
<td>-1 (-2, 0)</td>
<td>0.033</td>
<td>0.15</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>60</td>
<td>45 ± 5</td>
<td>45 ± 5</td>
<td>44 ± 5</td>
<td>44 ± 4</td>
<td>44 ± 4</td>
<td>44 ± 4</td>
<td>0 (-1, 0)</td>
<td>0.293</td>
<td>0.07</td>
<td>0 (-1, 1)</td>
<td>0.837</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>60</td>
<td>128 ± 14</td>
<td>127 ± 15</td>
<td>127 ± 15</td>
<td>125 ± 16</td>
<td>123 ± 16</td>
<td>123 ± 15</td>
<td>-2 (-3, 0)</td>
<td>0.117</td>
<td>0.11</td>
<td>-2 (-4, 0)</td>
<td>0.143</td>
<td>0.11</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>60</td>
<td>129 ± 14</td>
<td>128 ± 15</td>
<td>128 ± 15</td>
<td>125 ± 15</td>
<td>123 ± 15</td>
<td>122 ± 15</td>
<td>-1 (-2, 0)</td>
<td>0.093</td>
<td>0.07</td>
<td>-2 (-3, -1)</td>
<td>0.02</td>
<td>0.13</td>
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</table>

BL: baseline; EP: end-point; FU: follow-up; Δ: change; d: Cohen’s d. Data presented are frequency (n), mean ± SD and mean difference (90% confidence interval)
### Table 5.3 Distribution-based minimal important differences

<table>
<thead>
<tr>
<th>Metric</th>
<th>Baseline SD pooled</th>
<th>MID</th>
<th>Direction of improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>23.1</td>
<td>7.7</td>
<td>↓</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>6.9</td>
<td>2.3</td>
<td>↓</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>8.3</td>
<td>2.8</td>
<td>↓</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>4.5</td>
<td>1.5</td>
<td>↓</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>15.0</td>
<td>5.0</td>
<td>↓</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>14.6</td>
<td>4.9</td>
<td>↓</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
<td>12.2</td>
<td>4.1</td>
<td>↓</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>12.9</td>
<td>4.3</td>
<td>↓</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>8.1</td>
<td>2.7</td>
<td>↓</td>
</tr>
<tr>
<td>Serum cholesterol (mmol·L⁻¹)</td>
<td>1.1</td>
<td>0.4</td>
<td>↓</td>
</tr>
<tr>
<td>Serum HDL (mmol·L⁻¹)</td>
<td>0.3</td>
<td>0.1</td>
<td>↑</td>
</tr>
<tr>
<td>Cholesterol to HDL ratio</td>
<td>0.7</td>
<td>0.2</td>
<td>↓</td>
</tr>
<tr>
<td>Serum triglycerides (mmol·L⁻¹)</td>
<td>0.8</td>
<td>0.3</td>
<td>↓</td>
</tr>
<tr>
<td>Serum LDL (mmol·L⁻¹)</td>
<td>0.9</td>
<td>0.3</td>
<td>↓</td>
</tr>
<tr>
<td>Serum C-reactive protein (mg·L⁻¹)</td>
<td>2.4</td>
<td>0.8</td>
<td>↓</td>
</tr>
<tr>
<td>Serum glucose (mmol·L⁻¹)</td>
<td>1.4</td>
<td>0.5</td>
<td>↓</td>
</tr>
<tr>
<td>Serum insulin (mU·L⁻¹)</td>
<td>35.4</td>
<td>11.8</td>
<td>↓</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>11.7</td>
<td>3.9</td>
<td>↓</td>
</tr>
<tr>
<td>EQVAS</td>
<td>17.6</td>
<td>5.9</td>
<td>↑</td>
</tr>
<tr>
<td>ISWD (m)</td>
<td>159.7</td>
<td>53.2</td>
<td>↑</td>
</tr>
</tbody>
</table>

HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; EQVAS: EuroQol visual analogue scale; ISWD: incremental shuttle walk distance; SD: standard deviation; MID: minimal important difference. Data based on the 54 completing participants.
Figure 5.2. Changes in anthropometrics Comparison of the 90% confidence interval for the change between groups over time against the distribution-based minimal important difference (MID) at end-point (above) and follow-up (below). Green refers to beneficial change; yellow as trivial change; and, red as harmful change. BMI: body mass index; PBF: percentage body fat.
5.3.2 Cardiovascular risk

There were no statistical or clinically meaningful changes in systolic or diastolic blood pressure at end-point ($P=0.547$ and $P=0.590$) or follow-up ($P=0.893$ and $P=0.589$). Nine patients could not provide blood samples because of poor venous access. Of samples collected from 51 participants, eight individuals had raised CRP (>10 mg·L$^{-1}$) at one or more time-points. Those with raised CRP had their complete blood panel at all time-points excluded (as specified \textit{a priori}) and the remaining 43 complete data-sets were included in the analysis (21 control; 22 intervention). Three patients provided a small sample at one or more time-points which was insufficient for the insulin assay and therefore insulin was measured in 40 participants (20 control; 20 intervention). At baseline, both groups were classified as eucholesterolaemic, euglycaemic and hyperinsulinaemic.

There were no statistically significant changes in serum cholesterol or HDL at end-point or follow-up (Table 5.4). These data also had trivial effect sizes. However the cholesterol to HDL ratio increased by 0.2 [0, 0.4] at end-point and 0.1 [0, 0.3] at follow-up, as evidenced by small effect sizes ($d=0.30$ and $d=0.27$, respectively). Comparison of the 90% confidence interval of the change with the MID suggested a possibly harmful effect of the intervention (Figure 5.3). However, these changes were not statistically significant ($P=0.167$ and $P=0.205$, respectively) and could be attributed to chance. There were no changes in LDL at end-point or follow-up, or for serum triglycerides at end-point or follow-up.

CRP was significantly lower at end-point ($\Delta -1.3 [-2.2, -0.3]$ mg·L$^{-1}$; $P=0.028$) and was deemed to be a moderate effect ($d=0.53$). This reduction was maintained at follow-up ($\Delta -1.3 [-2.3, -0.3]$ mg·L$^{-1}$; $P=0.037$) along with the estimate of the effect size ($d=0.52$). Intervention-induced changes in CRP were deemed to be possibly beneficial by comparing the confidence interval for the change with the MID (Figure 5.3).

Serum glucose was also reduced at end-point and follow-up ($\Delta -0.3 [-0.8, 0.1]$ mmol·L$^{-1}$, and $\Delta -0.3 [-0.8, 0.2]$ mmol·L$^{-1}$, respectively). These changes were classified as non-significant using traditional hypothesis testing ($P=0.224$ and $P=0.267$, respectively) although the effect size estimates were small (both $d=0.24$). Serum insulin measurements had a large variability at all time-points with the standard deviation
often being similar in magnitude to the mean. There were no significant changes between time-points, however changes at end-point and follow-up had small effect sizes in a positive ($d=0.22$) and negative direction ($d=-0.20$), respectively. Changes in glucose and insulin were deemed to be probably trivial but possibly beneficial at end-point. At follow-up changes in glucose were still possibly beneficial and changes in insulin had become probably trivial, but possibly harmful.

The HOMA-IR scores for insulin resistance did not change baseline and end-point ($\Delta -1.2 [-6.9, 4.4]; P=0.720$) although this modest reduction was associated with a small effect size ($d=0.33$). The beneficial effect was lost at follow-up ($\Delta -1.8, -14, 11; P=0.808; d=0.03$). Improvements in HOMA-IR at end-point were probably trivial, and changes at end-point were unclear as the confidence interval for the change between groups over time spanned the harmful, trivial and beneficial regions of the graph.
### Table 5.4 Cardiovascular risk outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>BL</th>
<th>EP</th>
<th>FU</th>
<th>BL</th>
<th>EP</th>
<th>FU</th>
<th>Δ (90%CI)</th>
<th>P</th>
<th>Δ (90%CI)</th>
<th>P</th>
<th>Δ (90%CI)</th>
<th>P</th>
<th>Δ (90%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>60</td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>39 ± 7</td>
<td>38 ± 7</td>
<td>38 ± 7</td>
<td>-1 (-1, 0)</td>
<td>0.002</td>
<td>-1 (-1, 0)</td>
<td>0.002</td>
<td>0.14</td>
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<tr>
<td>SBP (mmHg)</td>
<td>60</td>
<td>127 ± 11</td>
<td>127 ± 13</td>
<td>131 ± 14</td>
<td>132 ± 15</td>
<td>130 ± 13</td>
<td>133 ± 16</td>
<td>0 (-4, 3)</td>
<td>0.893</td>
<td>0.02</td>
<td>0.534</td>
<td>0.14</td>
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<tr>
<td>DBP (mmHg)</td>
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<td>72 ± 8</td>
<td>72 ± 8</td>
<td>75 ± 8</td>
<td>75 ± 9</td>
<td>74 ± 9</td>
<td>76 ± 10</td>
<td>-1 (-3, 2)</td>
<td>0.59</td>
<td>0.09</td>
<td>0.539</td>
<td>0.12</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Serum cholesterol (mmol·L⁻¹)</td>
<td>43</td>
<td>4.8 [3.4, 6.2]</td>
<td>4.8 [3.2, 6.4]</td>
<td>4.4 [2.5, 6.3]</td>
<td>4.7 [3.6, 5]</td>
<td>4.8 [3.5, 6.1]</td>
<td>4.6 [3.6, 5.5]</td>
<td>0.1 (-0.1, 0.3)</td>
<td>0.575</td>
<td>0.06</td>
<td>0.645</td>
<td>0.09</td>
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<td>Serum HDL (mmol·L⁻¹)</td>
<td>43</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>0 (-0.1, 0)</td>
<td>0.569</td>
<td>-0.06</td>
<td>0.241</td>
<td>-0.24</td>
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<tr>
<td>Cholesterol to HDL ratio</td>
<td>43</td>
<td>4.0 ± 0.7</td>
<td>3.8 ± 0.7</td>
<td>3.8 ± 0.7</td>
<td>4.1 ± 0.7</td>
<td>4.1 ± 0.7</td>
<td>4.1 ± 0.8</td>
<td>-0.2 (-0.4, 0)</td>
<td>0.167</td>
<td>-0.30</td>
<td>0.205</td>
<td>-0.27</td>
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<td></td>
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<tr>
<td>Serum triglycerides (mmol·L⁻¹)</td>
<td>43</td>
<td>1.7 [0.6, 2.8]</td>
<td>1.6 [0.7, 2.5]</td>
<td>1.5 [0.5, 2.5]</td>
<td>1.8 [1.2, 2.4]</td>
<td>1.8 [1.2, 2.5]</td>
<td>1.7 [1, 2.4]</td>
<td>0 (-0.2, 0.3)</td>
<td>0.826</td>
<td>-0.04</td>
<td>0.517</td>
<td>0.04</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Serum LDL (mmol·L⁻¹)</td>
<td>43</td>
<td>2.7 ± 0.7</td>
<td>2.7 ± 0.8</td>
<td>2.6 ± 0.9</td>
<td>2.9 ± 1.1</td>
<td>2.9 ± 1</td>
<td>2.8 ± 0.9</td>
<td>0 (-0.1, 0.2)</td>
<td>0.731</td>
<td>0.04</td>
<td>0.814</td>
<td>0.04</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serum CRP (mg·L⁻¹)</td>
<td>43</td>
<td>2.9 [0.3, 5.5]</td>
<td>3.3 [0.1, 6.5]</td>
<td>3.2 [-2, 1.8]</td>
<td>2 [-0.1, 4.0]</td>
<td>1.4 [-0.7, 3.5]</td>
<td>1.8 [0.6, 3]</td>
<td>-1.3 (-2.2, -0.3)</td>
<td>0.028</td>
<td>0.53</td>
<td>0.037</td>
<td>0.52</td>
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</tr>
<tr>
<td>Serum glucose (mmol·L⁻¹)</td>
<td>43</td>
<td>5.7 ± 1.6</td>
<td>5.7 ± 1.9</td>
<td>5.7 ± 1.9</td>
<td>5.0 ± 1.1</td>
<td>4.8 ± 0.5</td>
<td>4.8 ± 0.8</td>
<td>-0.3 (-0.8, 0.1)</td>
<td>0.224</td>
<td>0.24</td>
<td>0.267</td>
<td>0.24</td>
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<td></td>
</tr>
<tr>
<td>Serum insulin (mU·L⁻¹)</td>
<td>40</td>
<td>29 [7, 51]</td>
<td>27 [7, 47]</td>
<td>27 [6, 48]</td>
<td>27 [4, 50]</td>
<td>22 [6, 37]</td>
<td>29 [-17, 75]</td>
<td>-8 (-20, 5)</td>
<td>0.306</td>
<td>0.22</td>
<td>0.491</td>
<td>-0.20</td>
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<td></td>
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<tr>
<td>HOMA-IR</td>
<td>40</td>
<td>7 [0, 14]</td>
<td>7 [2, 12]</td>
<td>6 [-1, 12]</td>
<td>5 [0, 11]</td>
<td>5 [1, 9]</td>
<td>6 [-5, 16]</td>
<td>-1.2 (-6.9, 4.4)</td>
<td>0.720</td>
<td>0.33</td>
<td>0.808</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; HOMA-IR: homeostatic model assessment for insulin resistance; BL: baseline; EP: end-point; FU: follow-up; Δ: change; d: Cohen's d. Data presented are frequency (n), mean ± SD and mean difference (90% confidence interval)
Figure 5.3 Changes in blood-borne biomarkers  Comparison of the 90% confidence interval for the change between groups over time against the distribution-based minimal important difference (MID) at endpoint (above) and follow-up (below). Green refers to beneficial change; yellow as trivial change; and, red as harmful change. Chol: cholesterol; HDL: high-density lipoprotein; C:H: cholesterol to HDL ratio; Trigs: triglycerides; LDL: low-density lipoprotein; CRP: C-reactive protein; HOMA: Homeostatic model assessment for insulin resistance.
5.3.3 Vascular function

Changes in skin blood flow in response to iontopheretic ACh and SNP delivery are depicted in Figure 5.4 and Figure 5.5, respectively. There were no significant between-group differences for resting or maximal cutaneous vascular conductance at any time-point (all $P > 0.05$). Similarly, there were no significant within-group changes for resting or maximal CVC at end-point or follow-up. There was a large amount of variability in measurements at all time-points, with the standard deviation being equal to $33 \pm 6\%$ of the mean value for resting observations and $68 \pm 20\%$ of the mean for all dosed observations.
Figure 5.4 Cutaneous vascular conductance (CVC) during ACh iontophoresis. CVC at rest and in response to seven incremental doses of acetylcholine chloride (ACh) iontophoresis at baseline (blue), end-point (red) and follow-up (green) for the control group (above) and intervention group (below). Data presented are means with SD error bars.
Figure 5.5 Cutaneous vascular conductance (CVC) during SNP iontophoresis.
CVC at rest and in response to four incremental doses of sodium nitroprusside (SNP) iontophoresis at baseline (blue), end-point (red) and follow-up (green) for the control group (above) and intervention group (below). Data presented are means with SD error bars.
There were no significant changes in four of the five health domains of the EQ-5D survey at either time-point (Table 5.5). There was improvement in the proportion of patients reporting less problems performing usual activities (such as vacuuming or shopping; \( P=0.044 \)). There was no significant change in self-perceived health (EuroQol visual analogue scale; EQVAS) score at end-point (\( \Delta 3 [-3, 9]; P=0.385; d=0.17 \)). Despite this, there was a significant improvement in EQVAS at follow-up of 9 [3, 14] points (\( P=0.017 \)) that was associated with a small-to-moderate effect size (\( d=0.49 \)). The change at end-point was deemed probably trivial and at follow-up was probably beneficial (figure 5.6).
### Table 5.5 EQ-5D Quality of life outcomes

<table>
<thead>
<tr>
<th>Domain</th>
<th>Change at end-point</th>
<th></th>
<th>Change at follow-up</th>
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<th></th>
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</thead>
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<tr>
<td></td>
<td>Control Intervention</td>
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<td>Control Intervention</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>P</em></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mobility</td>
<td>Worst</td>
<td>1 (3.3)</td>
<td>2 (6.7)</td>
<td>0.099</td>
<td>0 (0)</td>
<td>1 (3.3)</td>
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<tr>
<td></td>
<td>Same</td>
<td>28 (93.3)</td>
<td>22 (73.3)</td>
<td></td>
<td>28 (93.3)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td></td>
<td>Better</td>
<td>1 (3.3)</td>
<td>6 (20.0)</td>
<td></td>
<td>2 (6.7)</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td>Self-care</td>
<td>Worst</td>
<td>3 (10.0)</td>
<td>1 (3.3)</td>
<td>0.509</td>
<td>3 (10.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Same</td>
<td>26 (86.7)</td>
<td>27 (90.0)</td>
<td></td>
<td>25 (83.3)</td>
<td>28 (93.3)</td>
</tr>
<tr>
<td></td>
<td>Better</td>
<td>1 (3.3)</td>
<td>2 (6.7)</td>
<td></td>
<td>2 (6.7)</td>
<td>2 (6.7)</td>
</tr>
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<td>Usual activities</td>
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<td>1 (3.3)</td>
<td>0.044</td>
<td>3 (10.0)</td>
<td>3 (10.0)</td>
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<tr>
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<td>Same</td>
<td>24 (80.0)</td>
<td>23 (76.7)</td>
<td></td>
<td>24 (80.0)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td></td>
<td>Better</td>
<td>1 (3.3)</td>
<td>6 (20)</td>
<td></td>
<td>3 (10.0)</td>
<td>7 (23.3)</td>
</tr>
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<td>Pain &amp; discomfort</td>
<td>Worst</td>
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<td>2 (6.7)</td>
<td>0.936</td>
<td>2 (6.7)</td>
<td>3 (10.0)</td>
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<td>Same</td>
<td>24 (80.0)</td>
<td>23 (76.7)</td>
<td></td>
<td>23 (76.7)</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td></td>
<td>Better</td>
<td>4 (13.3)</td>
<td>5 (16.7)</td>
<td></td>
<td>5 (16.7)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Anxiety &amp; depression</td>
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<td>1 (3.3)</td>
<td>2 (6.7)</td>
<td>0.838</td>
<td>6 (20.0)</td>
<td>2 (6.7)</td>
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<td>Same</td>
<td>25 (83.3)</td>
<td>24 (80.0)</td>
<td></td>
<td>19 (63.3)</td>
<td>24 (80.0)</td>
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<td>4 (13.3)</td>
<td>4 (13.3)</td>
<td></td>
<td>5 (16.7)</td>
<td>4 (13.3)</td>
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</tbody>
</table>

Data presented are count (percentage)
5.3.5 Exercise capacity

The intervention group lowered resting heart rate by 6 beats per minute compared to the control group at end-point (Δ -5 [-8, -3] beats-min⁻¹; P=0.002; Table 5.6), which was deemed possibly beneficial and a small effect (d=0.46). The magnitude of change from baseline was reduced at follow-up, no longer statistically significant (Δ -2 [-5, 1] beats-min⁻¹; P=0.165; d=0.27) and considered almost certainly trivial.

Although data were collected for all participants, seven post-ISWT datasets (post-exercise RPE, SBP, DBP, HR and ISWD) were excluded because the participant walked the entire 1020 metres at one or more time-points (four at baseline and three at end-point). This ceiling effect was unexpected and dilutes the true treatment effect – consequently these patients were excluded from the analysis.

Despite randomisation there was a large difference between groups at baseline for distance walked in the ISWT (164 m; 639 vs. 475 m; Table 5.1). The increased exercise capacity of the intervention group was also reflected in baseline post-exercise measurements of heart rate (148 ± 21 vs. 131 ± 28 beats-min⁻¹) and blood pressure (systolic: 192 ± 31 vs. 169 ± 29 mmHg; diastolic: 93 ± 11 vs. 86 ± 15 mmHg). Despite this, the perceived exertion from participants was equal (15 ± 2 vs. 15 ± 2). ISWD improved by 94 [58, 132] m (P<0.001) at end-point and by 132 [97, 168] m (P<0.001) at follow-up (Figure 5.7). These improvements were associated with moderate (d=0.58) and large (d=0.82) effect sizes, respectively. Additionally, comparing the 90% confidence interval for the change with the estimated MID suggests that changes at end-point and follow-up are almost certainly beneficial. Changes in ISWD were in the absence of any significant changes in post-exercise physiological measures (heart rate, systolic blood pressure and diastolic blood pressure) or perceived exertion (RPE) at both end-point and follow-up (all P>0.05) when the improvement in ISWD was used as a covariate. Despite no significance small effect sizes were identified for changes in post-exercise RPE at end-point (Δ 0.6 [-0.4, 1.7]; P=0.303; d=0.32) and post-exercise heart rate at follow-up (Δ -5 beats-min⁻¹ [-14, 4]; P=0.335; d=0.21).
Figure 5.6 Changes in ISWT data and EQVAS
Comparison of the 90% confidence interval for the change between groups over time against the distribution-based minimal important difference (MID) at end-point (above) and follow-up (below). Green refers to beneficial change; yellow as trivial change; and, red as harmful change. Ex RPE: post-ISWT rating of perceived exertion; Ex HR: post-ISWT heart rate; Ex SBP: post-exercise systolic blood pressure; Ex DBP: post-ISWT diastolic blood pressure; ISWD: incremental shuttle walk distance; EQVAS: EuroQol visual analogue scale.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Intervention</th>
<th>BL to EP</th>
<th>BL to FU</th>
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<tr>
<td></td>
<td>n</td>
<td>BL</td>
<td>EP</td>
<td>FU</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
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<td>65 ± 14</td>
<td>66 ± 12</td>
<td>64 ± 12</td>
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<td>Systolic BP (mmHg)</td>
<td>60</td>
<td>127 ± 11</td>
<td>127 ± 13</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>60</td>
<td>72 ± 8</td>
<td>72 ± 8</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Post-ex RPE</td>
<td>53</td>
<td>15 ± 2</td>
<td>14 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Post-ex heart rate (bpm)</td>
<td>53</td>
<td>131 ± 28</td>
<td>131 ± 26</td>
<td>130 ± 25</td>
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<td>Post-ex systolic BP (mmHg)</td>
<td>52</td>
<td>169 ± 29</td>
<td>167 ± 31</td>
<td>171 ± 30</td>
</tr>
<tr>
<td>Post-ex diastolic BP (mmHg)</td>
<td>52</td>
<td>86 ± 15</td>
<td>85 ± 14</td>
<td>85 ± 13</td>
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<tr>
<td>ISWD (m)</td>
<td>53</td>
<td>475 ± 240</td>
<td>475 ± 250</td>
<td>471 ± 240</td>
</tr>
</tbody>
</table>

BP: blood pressure; RPE: rating of perceived exertion; ISWD: incremental shuttle walk distance; BL: baseline; EP: end-point; FU: follow-up; Δ: change; d: Cohen's d. Data presented are frequency (n), mean ± SD and mean difference (90% confidence interval).
Figure 5.7 Changes in incremental shuttle walk distance (ISWD) Line plots showing changes in ISWD for the control group (blue) and the intervention group (red) over the course of the study. Intervention ISWD have been adapted to reflect the differences reported in ANCOVA. †: difference between groups; *: significant between groups change from baseline (P<0.001)
5.3.6 Correlations

Bivariate correlations were assessed on change scores for key outcome variables. Weak correlations were identified between delta scores for BMI and percentage body fat ($r_s=0.339; P=0.016$), ISWD and percentage body fat ($r_s=-0.308; P=0.045$) and ISWD and EQVAS score ($r_s=0.344; P=0.024$) at end-point (Table 5.7). Moderate relationships were also identified between delta scores for ISWD and BMI ($r_s=-0.628; P<0.001$), CRP and resting heart rate ($r_s=0.415; P=0.012$) and peak skin blood flow and resting skin blood flow during the ACh protocol ($r_s=0.361; P=0.010$).

Table 5.7. Correlation coefficients for key variables at end-point

<table>
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<th>Δ scores for variable 1 at end-point</th>
<th>Δ scores for variable 2 at end-point</th>
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<td>BMI</td>
<td>PBF</td>
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<td>---</td>
<td>---</td>
</tr>
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<td>BMI</td>
<td>1.000</td>
</tr>
<tr>
<td>PBF</td>
<td>1.000</td>
</tr>
<tr>
<td>HR</td>
<td>1.000</td>
</tr>
<tr>
<td>EQVAS</td>
<td>1.000</td>
</tr>
<tr>
<td>CRP</td>
<td>1.000</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.000</td>
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<tr>
<td>ISWD</td>
<td>1.000</td>
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<tr>
<td>ACh REST</td>
<td>1.000</td>
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<tr>
<td>ACh PEAK</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Δ: change; BMI: body mass index; PBF: percentage body fat; HR: heart rate; EQVAS: EuroQoL visual analogue scale; CRP: C-reactive protein; ISWD: incremental shuttle walk distance; ACh REST: resting skin blood flow before ACh iontophoresis; ACh PEAK: peak skin blood flow during ACh iontophoresis. *: $P<0.05$. Data presented are Spearman’s coefficient.

Correlations between changes in key variables at follow-up are displayed in Table 5.8. A significant moderate correlation was identified between changes in percentage body fat and BMI ($r_s=0.443; P=0.001$). Changes in resting heart rate at end-point were weakly correlated with changes in BMI ($r_s=0.324; P=0.017$) and percentage body fat ($r_s=0.291; P=0.033$). Changes in CRP at follow-up were correlated with BMI ($r_s=0.367; P=0.023$). Changes in ISWD had a moderate-to-strong correlation with changes in BMI ($r_s=-0.642; P<0.001$) and a moderate correlation with changes in percentage body fat ($r_s=-0.423; P=0.003$). Changes in resting skin blood flow had a strong correlation with
changes in CRP ($r_s=0.465$; $P=0.003$) and changes in peak skin blood flow were weakly correlated with changes in percentage body fat ($r_s=0.276$; $P=0.044$) and moderately correlated with changes in resting skin blood flow.

Table 5.8. Spearman correlation coefficients for key variables at follow-up

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>PBF</th>
<th>HR</th>
<th>EQVAS</th>
<th>CRP</th>
<th>Glucose</th>
<th>ISWD</th>
<th>AChREST</th>
<th>AChPEAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.000</td>
<td>.443†</td>
<td>.324*</td>
<td>-.162</td>
<td>.367*</td>
<td>.146</td>
<td>-.642†</td>
<td>-.126</td>
<td>-.097</td>
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<tr>
<td>PBF</td>
<td>1.000</td>
<td>.291*</td>
<td>-.086</td>
<td>.174</td>
<td>.269</td>
<td>-.423†</td>
<td>.038</td>
<td>.276*</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>1.000</td>
<td>-.160</td>
<td>-.025</td>
<td>.093</td>
<td>-.104</td>
<td>-.079</td>
<td>-.183</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQVAS</td>
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<td>.260</td>
<td>-.176</td>
<td>.283</td>
<td>.017</td>
<td>-.096</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.000</td>
<td>.254</td>
<td>-.216</td>
<td>.465†</td>
<td>.223</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1.000</td>
<td>-.200</td>
<td>.266</td>
<td>.093</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ISWD</td>
<td>1.000</td>
<td>-.114</td>
<td>-.068</td>
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<tr>
<td>AChREST</td>
<td>1.000</td>
<td></td>
<td>.445†</td>
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<td>AChPEAK</td>
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<td>1.000</td>
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</tbody>
</table>

$\Delta$: change; BMI: body mass index; PBF: percentage body fat; HR: heart rate; EQVAS: EuroQol visual analogue scale; CRP: C-reactive protein; ISWD: incremental shuttle walk distance; AChREST: resting skin blood flow before ACh iontophoresis; AChPEAK: peak skin blood flow during ACh iontophoresis. †: $P<0.001$; *: $P<0.05$. Data presented are Spearman’s coefficient.

The strong relationships between ISWD and EQVAS, BMI and ISWD, and BMI and CRP were explored further using repeated measures within-subjects modelling. The correlation coefficients for the control group were 0.04 ($P=0.774$), -0.35 ($P=0.008$) and -0.08 ($P=0.589$), respectively, and for the intervention group were 0.54 ($P<0.001$), -0.74 ($P<0.001$) and 0.14 ($P=0.334$), respectively.
Chapter 6 – Discussion

6.1 Overview

This research study aimed to investigate the feasibility of a pragmatic lifestyle intervention in patients being treated for OSAS. A secondary aim was to collect preliminary evidence on effects of the intervention on important health outcomes. The main finding of this study was that the intervention was feasible, evidenced by more than a quarter of those invited to participate showing interest in taking part, and those who took part being extremely compliant with the commitments of the study. This study also demonstrated preliminary evidence on the efficacy of the intervention; there were substantial improvements in exercise capacity and markers of systemic inflammation, however there were only modest improvements in body mass and composition. Changes in exercise capacity, systemic inflammation and body mass were maintained after a 3 months period of independence, which suggests that the benefits of the intervention extended beyond the period of supervision. Reductions in body mass of approximately 2 kg suggest that the dietary component of the intervention was ineffective and inadequate. Similarly, there were no meaningful changes in total serum cholesterol evoked by the intervention.

Moreover, all study procedures were completed in the absence of any adverse events (serious or otherwise) suggesting that the lifestyle intervention was safe to deliver. The intervention had a favourable impact on metrics of exercise capacity and systemic inflammation that were maintained at follow-up after a 12 week period of independence, although modest reductions in body mass were disappointing. These findings suggest that lifestyle interventions based on supervised exercise in OSAS require further investigation to maximise efficacy for weight loss and assess cost-effectiveness.
6.2 Characteristics

The characteristics of the recruited sample are in agreement with other studies in this population and with widely accepted prevalence rates. Our sample were obese with a mean BMI of 40 kg·m$^{-2}$ – obese individuals constitute an estimated 70% of OSAS patients (Malhotra et al., 2002) suggesting our sample was representative of the majority of OSAS patients. Similarly, despite not stratifying recruitment for age or sex, our sample had a mean age of 55 years and largely consisted of men (77% male). These characteristics match the typical OSAS patient which is thought to be a late middle-aged man. The cardiovascular risk profile was also matched to two recent studies: mean resting blood pressure was 132/75 mmHg (compared with 126/77 (Barnes et al., 2009) and 130/80 mmHg (Tuomilehto et al., 2010); fasting serum cholesterol was 4.8 mmol·L$^{-1}$ (compared with 5.3 (Barnes et al., 2009) and 4.6 mmol·L$^{-1}$ (Tuomilehto et al., 2010); and, fasting glucose was 5.4 mmol·L$^{-1}$ (compared with 6.1 (Barnes et al., 2009) and 6.2 mmol·L$^{-1}$ (Tuomilehto et al., 2010). Although these data themselves do not suggest elevated cardiovascular risk, many participants were undergoing pharmacological therapy to manage conditions such as hypertension and hypercholesterolaemia. These proportions were nineteen and thirty-five per cent, respectively.

6.3 Feasibility

We recruited the target sample of sixty patients within the proposed timeframe of 18 months. This recruitment was done mostly in batches of between 8 and 20 patients. The main factor affecting the number of patients recruited per batch was the maximum number of patients (specifically in the intervention arm) that the researcher could accommodate on the study at any one time. This was deemed to be approximately 10 staggered over a recruitment window of up to six weeks. Other limiting factors included availability of laboratory space (to conduct assessment sessions) and of the researcher (who had other study-related activities to perform). It is worth noting that although availability of the University-based exercise facility did not directly impact recruitment during the course of this trial, if the trial had not
conveniently coincided with down-time in other studies that this may have been an issue. Although personnel is a factor easily addressed in future trials, the size and shared nature of the facility are more rigid limiting factors and should be acknowledged.

To recruit the required number of participants a total of 481 recruitment packs were posted to participants. These participants were identified using patients’ clinic notes and three basic criteria (BMI, CPAP compliance and general health) by a member of the primary care team. These notes were readily available (stored on-site, not within patients’ main notes packet) and allowed for a rapid initial assessment of suitability (less than 2 minutes per patient). The reviewed documents typically included a letter from the referring physician, which usually summarised the patients' health background and clinical picture, and the previous annual clinic assessment sheet (that usually included BMI and the last CPAP compliance data). An alternative method of screening would be to review patients’ full notes, which undoubtedly would offer a much clearer clinical picture of the patients. However the time and effort this would require was considered too labour intensive for the first stage of the recruitment process and rejected in preference of basic screening. The underreporting of feasibility and pilot studies in peer-reviewed medical journals means there is very little evidence for the advantages and disadvantages of different screening methods when recruiting OSAS patients. Typically in studies that report outcome measures, very little if any of the published manuscript is dedicated to the description and discussion of recruitment methods used. Recruitment to clinical trials is often considered to be the rate-limiting step for the progression of a study with one review reporting only 31% of studies achieved their initial recruitment rate, despite over half having conducted a pilot study beforehand (McDonald et al., 2006). Indeed, evidence suggests that patients are not averse to participating in such research and that they understand the importance of these studies and their role in improving healthcare (Jenkins et al., 2010); poor recruitment is likely to be borne out of discontent of the procedures involved (e.g. anything uncomfortable) or logistical issues (e.g. travelling to and from the research centre and taking time off work). Recruitment in this study was completed independent of routine sleep clinic activities such as annual compliance checks, which could have provided an ideal avenue for recruitment. It is recommended that future
studies try to utilise the routine clinical activities of the sleep department to facilitate the recruitment process. It is also possible that patients could be more motivated to change lifestyle behaviours after receiving lifestyle advice in sleep clinic appointments, especially if such advice is delivered after demonstrating weight gain in the patient since the previous appointment. The effects of such lifestyle advice with the provision of support have not yet been investigated.

Using the basic screening approach, 69 of 550 patients were overtly unsuitable, mostly due to patients’ last BMI measurement being significantly lower than the study entry criteria. The screening threshold was BMI > 28 kg·m⁻², which although lower than that required for study entry, allowed patients with “borderline” obesity (BMI > 30 kg·m⁻²) to still be offered the opportunity to participate. It is possible that the measurements were inaccurate or that patients had experienced weight gain since then making them potentially eligible. It was considered unlikely that patients below the 28-kg·m⁻² threshold could have gained enough weight for inclusion. Also, borderline CPAP compliances were used on the premise that usage may have increased following encouragement from respiratory physiologists to improve wherever possible. Eighteen patients were not invited to participate because of a sustained pattern of low compliance over several years with no reason to suspect recent improvement. Only nine patients (1.6%) were excluded for obvious comorbidities that contraindicated participation – a proportion much lower than that encountered further down the recruitment tree (19.5%). These data suggest that a more thorough pre-invitation screening would reduce the number of ineligible patients initially approached (and improve the recruitment rate accordingly), however the additional time this would take and the benefit it would bring should be considered.

Two recruitment rates were reported; 12.5% and 15.3%. The former is the actual recruitment rate and the latter is the recruitment rate from those patients inferred to be eligible. These rates are similar to recruitment rates experienced by similar intervention studies from our centre (Tew et al., 2009; Bourke et al., 2011; Tew et al., 2012) and studies in other populations with high cardiovascular risk (Courneya et al., 2003; Sniehotta et al., 2011). Moreover, many of the exclusions for contraindicative comorbidity were for safety reasons and it is possible some of these could have been included in a clinical environment with medically-trained staff close at hand. This
would raise the recruitment rate closer to 20%. These recruitment rates are incredibly similar to other exercise-based RCTs in OSAS. Ackel d'Elia et al. (2011) reported a recruitment rate of 19.3% (47 of 244 patients invited to take part in their exercise intervention), Kline et al. (2011) recruited 43 of 400 patients (recruitment rate of 10.8%) invited to participate in their trial via telephone or mail, and, Tuomilehto et al. (Tuomilehto et al., 2009) recruited 12.9% (81 of 627) of patients onto their trial. The comparable studies report a range of between 10 and 20% into which both of our recruitment rates can be found. In most studies of this kind, ours included, participants have been self-selected (i.e. they have opted to enrol on the study, knowing that they were free to withdraw at any time without providing a reason). Although we did not collect data on the psychological, social or economic characteristics of our sample, the physiological characteristics seem matched to the typical OSAS population, and therefore our findings are likely generalisable. It is possible that the characteristics of patients enrolling onto OSAS intervention studies do so for one or more of the following reasons: patients motivated to lose weight or improve fitness; patients with ample time and nothing else to do; patients who previously enjoyed exercise and wanted an opportunity to get into it; particularly health-conscious patients who enrolled on a clinical trial for a thorough personal health assessment; and patients who have had a positive treatment experience and want to "give something back" to the field. Although these ideas are conjecture, there is no evidence in OSAS to suggest which facilitating factors determine whether a patient enrols, or does not.

Participant retention was excellent with 90% of participants completing the study. Of the six withdrawals, two were for changes in health status unrelated to the study. These participants were both in the intervention arm of the trial and were reluctant to withdraw themselves. They were excluded by the study researcher after reporting problems which could be exacerbated by the demands of the study. Two patients from the control arm were unable to attend the end-point or follow-up assessments due to changes in their professional commitments which made attending the University during working hours unfeasible. These participants enquired about the possibility of weekend assessments however this was not possible. Both participants admitted they would not have enrolled if they had been aware of the impending change in circumstances. Two participants declined to provide a reason for withdrawal but both
had expressed obvious disappointment to their randomisation. For the first four dropouts it is encouraging that none reported any aspect of the study as the reason for withdrawal. This lends considerable further evidence that the intervention was acceptable for OSAS patients. Retention rates in two similar studies were similar; Kline et al. (2011) retained 38 of 43 participants (88%) and Tuomilehto et al. (2009) retained 89% of their participants. The retention results for Ackel-d'Elia et al. (2011) were less convincing; only 13 of 25 participants randomised to the exercise arm of the trial, and 32 of 47 participants enrolled on the trial, completed the study. This equates to retention rates of 52 and 68%, respectively. The authors did not discuss these low rates beyond suggesting that the less motivated individuals withdrew from the study.

Participant withdrawal can provide researchers with key information to project whether any particular part of the study protocol influenced the staying power, and how the intervention might be accepted in a clinical setting. Our data, coupled with that from two similar studies (Tuomilehto et al., 2009; Kline et al., 2011) suggest that OSAS patients who show interest and enrol onto a lifestyle intervention are likely comply with its requirements.

Compliance to study activities was also excellent with >95% mean average attendance for assessment sessions, University-based and home-based exercise sessions. This is especially encouraging as 38 participants (63%) were in part- or full-time employment. Only one similar study has reported compliance with study activities; in the study by Kline et al., 87 ± 2% of intervention appointments were kept (Kline et al., 2011). This percentage is in keeping with the current study. These data not only suggest that patients felt they could integrate such an intervention into their daily routine, but that there may have been provision from their employers to participate (some participants described corporate Wellness programs designed to offer flexible working arrangements for health-promoting activities). Patients were offered sessions at times to suit their preference in the same manner as the NHS Choose and Book system (www.chooseandbook.nhs.uk). Some participants preferred sessions before or after their working day, some worked shift patterns and attended different times on different days each week and some utilised flexi-time. Participants were offered a long-term on-site parking permit which most reported as being a great facilitator of participation as on-street parking near our facility is especially challenging during the
Compliance on this study compares favourably with other studies from our centre suggesting that our participant-focussed approach promotes adherence. Additionally, the provision of a reasonably private non-commercial facility to conduct exercise in likely promoted adherence. Many participants described their apprehension towards introducing exercise into their lives without the support we provided and that they considered public gymnasiums an intimidating place. They also reported concern that in a non-research setting they wouldn’t know what to do – which they felt could cause embarrassment.

Our intervention involved a substantial time commitment both at the University and at home. The former required travel to and from the University which was subsidised at £2.50 a visit, irrespective of the distance travelled or actual costs incurred. This post-study reimbursement was likely insufficient to cover costs for most participants (except those eligible for free bus travel). This reduces the likelihood that participation and compliance were influenced by financial means. Sheffield is a sprawling city with a large catchment area for its hospitals – of all participants recruited onto the intervention, the furthest afield lived 50 miles away (estimated fastest route) and attended 100% of study commitments (balanced with married life and full-time employment).

We investigated the utility of using patients’ self-reported CPAP compliance to determine eligibility for participation using post hoc verification from CPAP compliance data downloaded from CPAP machines at the annual review following the end of the study. Although median usage was excellent in both groups, 13 of 60 patients were unexpectedly below the inclusion threshold. As participants were not actively encouraged to maintain high CPAP usage during the trial it is possible that the purpose of the study was misunderstood and patients electively discontinued or reduced their CPAP therapy. As this was a post-study observation, and completely unexpected, the reasons for inadequate were not recorded and all patients were still included in the analyses. We recommend using up-to-date objective CPAP data to assess eligibility in future studies and that continuous encouragement to use CPAP regularly and education on its importance be incorporated into any interventions. This could pose equipment- and software-related limitations for centres such as ourselves where the
bulk of research activities take place in a university setting; research centres in hospitals should find this straightforward. Moreover, the results of other studies that have used self-reported compliance with CPAP as an inclusion criterion should be treated with trepidation. An alternative strategy could be to introduce a lag period between enrolment and randomisation to specifically observe patients CPAP habits, as described by Ueno et al. (2009).

6.4 Efficacy

6.4.1 Anthropometry

There were modest reductions in body mass at intervention end-point of approximately 2 kg (less than 2% of original body mass) that were maintained at follow-up. Although much greater weight loss has been reported in trials using VLEDs, most intervention studies (especially those with an exercise focus) have reported limited weight loss. This contributes to a growing body of evidence that the weight loss is a greater challenge in the OSAS population than in BMI-matched non-OSAS counterparts.

The increased physical activity caused by high compliance with the exercise aspect of the study without accompanying marked weight loss could be explained by a compensatory increase in energy intake to counter increased energy expenditure. Although we identified this as a key point in the dietary advice aspect of the behaviour change counselling, it seems the delivery may have been ineffective or the adoption low. Another strategy investigated in this study was the efficacy of dietary advice and behaviour change counselling delivered by an exercise physiologist. Typically these aspects are delivered by individuals with specialist training and expertise; however with three specific aspects to the intervention (dietary support, exercise training and behaviour change counselling) it was not feasible to use a multidisciplinary team. Such an approach would have reduced the pragmatism of the study and complicated its delivery in a healthcare setting. The improvements in exercise capacity at end-point and follow-up and the absence of a detraining response suggest that the exercise aspect and exercise behaviour change aspect were effective. Unfortunately, the
present study lacks supportive empirical data such as actigraphy or qualitative data such as the Godin Leisure Score Index.

A previous research study in our centre included fortnightly healthy eating seminars in a pilot study that investigated lifestyle intervention in men with prostate cancer undergoing androgen deprivation therapy (Bourke et al., 2011). These researchers reported a 241 kcal reduction in daily energy intake (based on a four day diet diary) in the intervention group. The present study did not conduct post-intervention diet diaries, nor did it assess diet diaries for macronutrient intake. Diet diaries were used principally as a tool to promote awareness in participants and to identify overt dietary imbalance. It would be beneficial for future studies to conduct baseline, post-intervention and follow-up nutritional analysis.

Addressing obesity should be a key goal of any OSAS intervention as it has the potential to improve mechanical and anatomical contributors to airway collapse. An additional non-OSAS benefit of weight loss includes reduced stress on the skeleton and joints. Obesity is independently associated with arthritis in the weight-bearing joints of the lower limbs (i.e. ankles, knees and hips). Arthritis is potentially the most debilitating medical condition that affects older adults, and its development is undoubtedly exacerbated by obesity (Rejesky et al., 2010; Messier et al., 2004). Impaired mobility in older adults is a contributor to sedentary behaviour, which in turn increases the risk of cardiovascular disease.

6.4.2 Cardiovascular risk

This study evaluated several aspects of cardiovascular disease risk. Changes in resting systolic and diastolic blood pressure were negligible, as were changes in cholesterol, triglycerides and LDL. This is perhaps unsurprising as these variables were already comfortably within normal range and would not be expected to change. On the contrary, CRP was reduced at 13 weeks (Δ -1.3 [-2.2, -0.3] mg·L⁻¹) and 26 weeks (Δ -1.3 [-2.3, -0.3] mg·L⁻¹). This reflected a reduction of almost 50% from baseline values and effect sizes were moderate suggesting that changes were meaningful. The data presented were analysed using the LOCF principle, and after excluding elevated CRP values indicative of acute inflammation at any time-point. Reanalysis without these stipulations did not affect our findings. It has been demonstrated previously that
exercise training reduces circulating CRP in humans (Stewart et al., 2007), although others have suggested it does not (Hammett et al., 2004). CRP is an important marker of systemic inflammation and of risk to subsequent cardiovascular disease (Ridker et al., 2003). Previous research has shown serum CRP concentration to be proportional to the severity of OSAS (Shamsuzzaman et al., 2002). Furthermore, effective CPAP therapy has been shown to lower CRP (Yokoe et al., 2003). As the majority of our sample was compliant with CPAP therapy, any further reduction in CRP evoked by our intervention may reflect further reductions in cardiovascular risk. There was no relationship between change in CRP and percentage compliance.

It is possible that with a substantial proportion of participants not using CPAP on a regular basis that many patients were still experiencing apnoeic events on the nights they were not using CPAP. This should be taken into consideration when evaluating our results. Although there is no published evidence on what effects occasional CPAP use may have on the variables we explored in the current study, we suggest it may offer some degree of protective effect in a dose-response manner. This hypothesis is explored in study 2 (section 3).

The effect of the intervention on microvascular function is still unclear. As discussed in section 4.7.3 the reproducibility of LDF-assessed skin blood flow has been questioned, and often the utilisation of multi-fibre probes and of multiple probes to address spatial variability is recommended. The current study only used a single single-fibre iontophoresis probe. There was large between-participants variance in measurements at all time-points equal to between 50 and 80% of the mean value. Our results are in contrast to Trzepizur et al. (2009) who reported higher CVC in pre-CPAP OSAS patients than both of our groups. This is likely attributable to the difference is the iontophoresis protocols. Walker et al. 2009 showed that regular exercise is associated with higher endothelium-dependant vasodilation, however such improvements were not evident in the current study. A general assumption during the selection of a vascular reactivity test is that the vascular tissue being assessed (i.e. forearm skin resistance vessels, or limb-based conduit artery) is representative of other less-accessible vascular beds of interest, such as the coronary or cerebral circulations.
Despite randomisation, there was a large between-group difference at baseline for ISWD (Δ 174 m) and consequently in baseline post-exercise variables (perceived exertion, heart rate, systolic blood pressure and diastolic blood pressure). The reasons for the between-group discrepancy are unclear and three possible mechanisms could be implicated. The control group had a greater proportion of beta-blocker usage (7 vs. 2) which attenuates the exercise-induced tachycardia required to maximise cardiopulmonary function. Moreover, beta-blockers are often prescribed in the management of cardiovascular complaints which themselves could limit exercise capacity. Such a condition is coronary artery disease, which was more common in the history of the control group than the intervention group (5 vs. 2). Previous periods of ischaemia or infarction could contribute to impaired myocardial contractility which would reduce peak cardiac output and hence reduce exercise capacity. It is of course possible that independently of all other factors, participants in the control group just had a truly lower exercise capacity (in which case the randomisation process, on this occasion failed). Participants in the intervention made substantial improvements in ISWD at end-point, which were slightly improved upon further at end-point.

The improved exercise capacity was absent when assessing the control group data which had mean ISWDs of 414, 414 and 410 metres. This observation is in contrast to the findings of Fowler et al. (2005) who reported an improvement in ISWD between the first and second ISWT. Two important conclusions can be drawn from this data. Firstly, that it is reassuring to see that our approach to delivering the ISWT was sufficient to eliminate any potential learning effect. This included describing the protocol in detail, allowing participants to become accustomed with the course, and to play the audio signal briefly to highlight which sounds to listen out for and what they mean. Secondly, and perhaps more importantly, it seems that basic lifestyle advice alone is an insufficient stimulus for improving exercise capacity in OSAS patients.

Despite the aforementioned differences at baseline, the improvements in exercise capacity at end-point and follow-up should not be undermined. Overall, The ISWT data suggest that the intervention increased exercise capacity. There was substantial improvement in walking capacity over the course of the intervention, which was
improved further at 26 weeks (changes which were characterised by moderate and large effect sizes, respectively). The 16 [10, 22]% and 22 [16, 29]% improvement in ISWD in the intervention group at end-point and follow-up, respectively, is similar to the 20% improvement in aerobic capacity in the Barnes et al. (2009) study.

Exercise prescription in the current study was variable - each exercise session was bespoke to the participant with the design (frequency, intensity, duration and type) varied throughout the intervention period making direct comparison with existing data difficult. Anecdotally, patients reported increased daily activity and increased frequency and duration of purposeful physical activity, primarily gymnasium training and home-based exercise/walking. Additionally, our assessment of exercise capacity was the ISWT, a test more commonly used in chronic obstructive pulmonary disease. This tool was used to minimise equipment-related limitations (e.g. maximum weight tolerance) that restrict recruitment. The ISWT was recently validated by our group (Billings et al., 2013) and has been shown to be reproducible in other populations (Fowler et al., 2005). Unfortunately, seven participants completed the ISWT (a total distance of 1020 m in 12 min) at one or more time-points and hence their data were excluded. Although we were prepared for such an eventuality, it was nevertheless unexpected. The test was well tolerated by patients and the feasibility of adding additional levels to the protocol originally described by Singh et al. (1992) – the audio signal follows an arithmetic sequence, with each level lasting one minute and being divided into $n+2$ equal parts (where $n$=level number; i.e. level four has six shuttles of 10 s each).

As mentioned previously, improvements in “fitness” are thought to be more important than improvements in “fatness” (Blair, 2009). This study has demonstrated substantial improvements in exercise capacity are not only achievable in OSAS, but also maintainable in the medium-term. Higher exercise capacities are associated with improved quality of life (especially vitality). Fitness is also predictive of surgical outcomes; surgery presents an immense physiological assault on the body and can significantly increase the cardiorespiratory demands of the body at rest. The very nature of OSAS, its reputation for remaining undiagnosed for years and disrupting physiological mechanisms which can lead to chronic disease and the co-morbidity of
obesity make the need for a future surgical procedure plausible. Improving exercise capacity and habitual physical activity has numerous intrinsic benefits for patients; improved self-worth, improved sleep pattern and being able to meet new friends.

6.4.4 Quality of life

The underpinning purpose of all medical research is to improve patients' care and quality of life. OSAS in its untreated form can vastly reduce health-related quality of life by causing chronic tiredness that reduces capacity for work- and leisure-related activities. The current study demonstrated reduced problems in completing usual activities such as housework or shopping. Such improvements can contribute to the maintenance of independence, which in turn can allow patients to stay more active (physically and mentally) and to perform their professional and personal commitments. The EQ5D tool has been previously shown to lack sensitivity in sleep patients (Jenkinson et al., 1998), which could explain the lack of change in several health domains.

6.5 Limitations

There are several limitations in this study that need to be considered when interpreting these findings. The non-blinded nature of the study design introduces the possibility of bias into some measurements. Ideally, assessments are conducted by a separate researcher who is blinded to group allocation and has no acquaintance with participants – this makes the results more defendable. The method of bioelectrical impedance analysis used in this study (Bodystat) is not the current gold standard for assessing body composition. Use of dual-energy x-ray absorption (DXA) scanner would have been preferred, however such equipment is very expensive and few centres are fortunate enough to have access to them. Alternatively, a more advanced bioelectrical impedance analyser capable of measuring fat mass, skeletal muscle mass and bone mineral density should be considered. Such equipment would inform changes in overall body composition.

The effects of the intervention reported in this study may be confounded by the personalised, pragmatic nature of the intervention sessions. Exercise prescription was
variable between participants and was introduced and developed steadily at their own rates. Consequently, the exercise "dose" probably varied considerably. Another methodological limitation was the relatively short fasting period, which was shorter than the ideal 10 hours easily achieved overnight. This could impact on several outcome measures that are affected by fasting period such as lipid profile, glucose, body fat percentage and skin blood flow measurements. This limitation was recognised during study design and due to concerns that it may affect recruitment to the intervention (the primary outcome measure), we stipulated a minimum fasting period of six hours, which as assessments most often occurred in the morning was, in practice, longer.

6.6 Future research

Building on the limitations of this study, several changes should be made to the current protocol in future studies. The dietary advice component of the intervention should be re-evaluated; this could include incorporation of nutritionist- or dietician-delivered education and advice, or short-term hypoenergetic diet to induce a rapid weight-loss phase. Although the current study included no metric to assess behaviour change (other than changes in outcome measures between end-point and follow-up), future studies should assess longer term behaviours. Tools to assess this include free-living questionnaires and activity diaries, actigraphy, and diet diaries could be repeated at each assessment point and assessed for nutritional content. These will help researchers determine changes in behaviour. Perhaps also assessing psychological mediators that are thought to be linked to behaviour change, e.g. changes in exercise self-efficacy.

Future work should also incorporate an assessment of sleep apnoea severity, using either home-based PSG or pulse oximetry. These tests would need to be conducted at each assessment point, without simultaneous CPAP therapy and after a sufficient wash-out period. The ethical implications of withdrawing treatment would need to be considered; it would be acceptable, although not scientifically robust, to make this aspect of the study optional. It is likely that most patients would opt to do it to find out what impact the intervention has had on the severity of OSAS (with milder patients
probably hoping to have been cured). Also, a more sensitive metric for health-related quality of life in sleep disorders would be useful.

Overall, the results presented here merit conducting a larger, multi-centre phase three trial with changes in OSAS severity as the primary outcome measure. This study should also incorporate a longer term assessment point (perhaps at two years) and evaluate the cost-effectiveness of the intervention.

6.7 Summary

This study has shown that lifestyle intervention is an attractive opportunity to what is likely a motivated sub-set of the OSAS population. It can cause improvements in exercise capacity and serum CRP that are maintained after an extended period of independence, although no improvements were demonstrated in body mass or serum cholesterol. The high compliance rates reported suggest that integration of the lifestyle intervention is possible, despite the majority of commitments being during working hours. This study has identified some methodological issues that require addressing before advancing onto repeating the study on a larger scale.
Section 3 – Effects of obstructive sleep apnoea syndrome and its treatment with CPAP on macrovascular and microvascular function
Apnoea can evoke episodes of hypoxaemia and elevated sympathetic nerve activity that can augment oxidative stress and cause endothelial dysfunction and cardiovascular disease (CVD) (Ip et al., 2004; Kuniyoshi et al., 2010). Although CPAP represents the gold standard for treatment of OSAS, its uptake and daily utilisation is sub-optimal. It is thought 8-15% of patients refuse it after an initial trial (Weaver & Grunstein, 2008) and a population study in 38 men, only 11 (29%) were still undergoing CPAP after 6 months (Lindberg et al., 2006). It is unclear why compliance is so variable and researchers have reported physical (e.g. tolerance of the pressurised air), social (e.g. partner’s or family’s perceptions) and psychological (e.g. patients’ attitude toward change) reasons as important factors (Basner, 2007; Kribbs et al., 1993). As therapeutic CPAP normalises apnoeic frequency it can vastly improve sleep quality and reduce daytime somnolence, increase vitality and improve health-related quality of life. However, as not all patients are symptomatic the importance of nightly use can often be underestimated. This is likely to be a misunderstanding of the consequences of OSAS in the long term and a certain degree of ignorance from patients. A number of studies have assessed the contributory factors to successful adherence to CPAP and the main physiological indicator for long-term CPAP compliance is the ODI and not the symptoms of EDS (Krieger et al., 1996; Kohler et al., 2010).

Previous work has shown that endothelium-dependent vasodilation (NO-mediated) is impaired in OSA patients (Carlson et al., 1996; Kato et al., 2000) and that untreated OSA, rather than obesity, is a major determinant of vascular endothelial dysfunction (Jelic et al., 2010). Measuring FMD after a distal arterial occlusion can assess vascular endothelial function and LDF assessment of skin blood flow during PORH and LTH can assess general microvascular function (Cracowski et al., 2006). Despite a number of short-, medium- and long-term studies assessing endothelial dysfunction in OSAS patients receiving CPAP treatment versus no treatment (Imadojemu et al., 2002; Lattimore et al., 2006; Bayram, et al., 2009; Nguyen et al., 2010), there is limited evidence that quantifies the extent to which low compliance (10%< nightly use <60%)
attenuates the increased endothelial dysfunction caused by the OSA, compared to persons that are highly compliant (>80% nightly use, 4 h per night). A study by Jelic et al. (2008) showed that vascular endothelial dysfunction can be significantly reversed with 4 weeks of effective CPAP treatment and that ineffective treatment elicits no significant reversal. These results are ambiguous as the researchers grouped persons who refused treatment and had low compliance together, limiting interpretation of the effect of low compliance on endothelial dysfunction.

The objectives of this study are to design and execute a cross-sectional study to assess macro- and microvascular function in patients that have low compliance to CPAP therapy and how they compare with other OSAS populations and healthy matched controls.

The research question is:

- How effective is occasional CPAP use at ameliorating the vascular endothelial dysfunction previously reported in OSAS and how is it associated with anthropometric variables, cardiovascular risk, quality of life and exercise capacity?

The original hypotheses for this study are:

- Vascular endothelial function in low compliance OSAS patients will be impaired compared with high compliance patients and matched controls
- Vascular endothelial function in low compliance OSAS patients will be similar to untreated OSAS patients
Chapter 8 – Methods

8.1 Study design

This was a four-arm, cross-sectional study that investigated vascular function in four groups: (1) OSA patients established on CPAP therapy for at least 3 months with high compliance (>80% nightly use for ≥4 h per night, OSA-HC); (2) OSA patients established on CPAP therapy for at least three months with low compliance (10%< nightly use <60% for >4 h per night; OSA-LC); (3) untreated OSA patients (OSA-UN); and, (4) age- and BMI-matched controls (OSAS-free).

The primary comparison was the difference in percentage flow-mediated dilatation between the high-compliance group and the low-compliance group. The secondary comparison was between the low-compliance group and the untreated OSA group.

This study was sponsored by Sheffield Teaching Hospitals NHS Foundation Trust (STH-16309) and funded by the Sheffield Thoracic Institute. This research was carried out in accordance with the Declaration of Helsinki of the World Medical Association and ethics approval was provided by the Sheffield Research Ethics Committee (11/YH/0417). This study was registered on an international clinical trials database (http://www.clinicaltrials.gov; NCT01619748)

8.1.1 Outcome measures

The primary outcome measure was flow-mediated dilatation (FMD) of the brachial artery in response to forearm occlusion assessed using vascular ultrasound. Secondary outcome measures were: changes in skin blood flow in response to a local heating stimulus, measured using laser Doppler flowmetry; changes in skin blood flow in response to a 5-min proximal arterial occlusion, measured using LDF; exercise capacity using the ISWT; and, cardiovascular risk assessed by measuring established risk factors (e.g. blood pressure, cholesterol, HDL and smoking status). All outcome measures were assessed during one visit that lasted approximately 2.5 hours.
8.1.2 Sample size calculation

To our knowledge, FMD has not previously been studied in a homogenous low-compliance group. We anticipate FMD will be similar between low-compliance and untreated OSAS patients because of the acute effects of even one night without CPAP. Similarly, previous research has shown CPAP therapy to improve AHI to that of non-OSAS. Therefore, we based our sample size estimation on mean values and standard deviations for differences in percentage FMD reported by Tanriverdi et al. (2006). This study reported a group of pre-CPAP OSAS patients (our surrogate OSA-LC group) with an FMD of 4.57 ± 1.3% compared with OSAS-free controls (our surrogate OSA-HC group) with an FMD of 6.34 ± 0.83%. These groups had a common SD of 1.15%. Using nQuery sample size calculator (Statistical solutions Ltd., Cork, ROI), a 2-tailed study design, a power of 90% and an alpha value of 0.05, we estimated needing 10 participants in both primary groups. Because this calculation is based on surrogate data, we will conservatively double the sample size to \(n=20\) per group.

8.2 Recruitment

Two recruitment strategies were used. Strategy 1 populated the entire OSA-HC and OSA-LC groups and contributed to the population of the OSA-UN group. Strategy 2 contributed to the OSA-UN group and populated the entire OSAS-free group.

8.2.1 Strategy 1 - recruitment of patients from local sleep clinics

8.2.1.1 Untreated group

Pre-CPAP patients were recruited from the sleep clinic at Northern General Hospital, Sheffield, UK. They were initially approached during routine consultation with a respiratory physiologist after their overnight sleep test and before commencing CPAP therapy. The physiologist briefly assessed their eligibility (CPAP compliance, BMI, general health) and if suitable provided a quick overview of the research and a recruitment pack (invitation letter and participant information sheet; appendix 9 and 10, respectively). If the patient was interested they were referred to a study researcher to discuss the study in more detail. Patients who showed interest in participating but
could not attend the assessment session before starting CPAP therapy were re-assessed for eligibility after their 3-month trial.

8.2.1.2 High-compliance and low-compliance groups

Patients established on CPAP were recruited from the sleep clinic at Northern General Hospital, Sheffield, UK. They were initially approached during routine consultation with a senior respiratory physiologist after their 3-month CPAP trial. The physiologist briefly assessed their eligibility (CPAP compliance, BMI, general health) and if suitable provided a quick overview of the research. If the patient was interested they were given a participant information sheet and referred to the chief investigator to discuss the study in more detail.

8.2.2 Strategy 2 - recruitment of obstructive sleep apnoea syndrome-free control participants from the community

Study researchers contacted local interest groups in the vicinity of Sheffield Hallam University. Contacted groups included, but were not limited to, the Sheffield branch of the University of the Third Age (sU3A) and Sheffield branch of the National Association of Retired Police Officers (sNARPO).

Those who showed interest were contacted via telephone by a study researcher. They were given a brief overview of the study, given an opportunity to ask questions and asked some pre-screening questions to ensure they met the eligibility criteria. They were then posted an invitation letter (appendix 11), participant information sheet (appendix 12) and reply envelope and given time to consider participation.

8.2.3 Familiarisation and overnight polysomnography

Volunteers were invited in to Sheffield Hallam University to meet a study researcher and discuss the study in a similar process to that described in section 4.2.2 (background to the department, purpose and design of the study, techniques and equipment involved, medical history, questions and written informed consent; appendices 13 and 14).

Additionally, those participants recruited from the community underwent overnight dual-channel polysomnography using a portable ApnoeaLink device (ResMed, Oxfordshire, UK) validated for accurate measurement of AHI (Erman et al., 2007). The
first channel records nasal airflow (via a disposable nasal cannula and internal pressure transducer) and the second channel records capillary blood oxygen saturation (using a finger pulse oximeter and internal light source). This test was conducted on a convenient night in the participants' own home. During familiarisation participants were given instructions on how to set up the device and an opportunity to practice setting up in front of the researcher. The device was returned the following morning and data were assessed automatically using the manufacturer’s software (ApnoeaLink, ResMed, UK) and subsequently verified manually to ensure apnoeas and desaturations had been correctly identified. A minimum of four hours of data were required to determine the presence or absence of OSAS using standard criteria (AHI/ODI).

8.3 Inclusion criteria

Inclusion was determined on the basis of the following criteria:

- Aged 18-85 years of age
- Body mass index >30 kg·m⁻²
- No contraindications to exercise testing such as unstable angina, severe hypertension or joint/muscle problems
- Suitable OSAS/treatment status for one of the trial arms
  - Compliant with CPAP (>75% nights; >4 hours per night (OSA-HC)
  - Low compliance with CPAP (10% < nights < 60%; >4 hours per night (OSA-LC)
  - Confirmed OSA (AHI/ODI >10 events·h⁻¹) and never treated with CPAP (OSA-UN)
  - Absence of OSA confirmed with overnight dual-channel polysomnography (OSAS-free)
8.4 Exclusion criteria

Participants were excluded if they met any of the following criteria:

- Pregnant or post-menopausal females undergoing hormone replacement therapy or oral contraceptive therapy
- Males undergoing testosterone replacement therapy
- Body mass >180 kg (for safety reasons; 180 kg is the manufacturers maximum weight tolerance for a key piece of equipment)
- Volunteers with any mental impairment making consent unethical

8.5 Primary outcome measures

8.5.1 Vascular function

Vascular function assessments were performed under conditions previously described (Section 4.7.3). All participants were asked to complete an overnight fast (≥8 h) and refrain from exercise for >24 h, caffeine for >12 h and smoking for >12 h (Thijssen et al., 2011). OCP-free pre-menopausal females were tested between days 1 and 7 of their menstrual cycle (Hashimoto et al., 1995; Corretti et al., 2002; Harris et al., 2010). Three multifibre laser Doppler probes were used, each probe consisted of a 7-fibre integrating probe (PF 413, Perimed AB) housed in a separate thermostatic heating disc (PF 450, Perimed AB) as shown in Figure 8.1. Patients were allowed to take medication as normal; it has been shown previously that nitrate-free vasodilator therapy has no acute (Gokce et al., 2002) or chronic (Benjamin et al., 2004) effects on endothelium-dependent vasodilation.

Disposable double-sided adhesive rings (PF 105-1, Perimed AB) were used to position the probes securely. Each probe was connected to an independent laser unit (PF 5010) and heating unit (PF 5020) and placed on the volar aspect of the right forearm, between the wrist joint and the cuff on sites previously described (see section 4.7.3.) with a stable resting perfusion (usually 10-30 PUs) and total backscatter. Each heating disc was set to an initial temperature of 33 °C.
8.5.1.1 Post-occlusion reactive hyperaemia

A pneumatic cuff attached to a rapid inflation/deflation system (E20 rapid cuff inflator/AG101 cuff inflator air source, D.E. Hokanson Inc., Bellevue, WA, US) was placed around the forearm immediately distal to the elbow joint. Resting blood flow data were recorded for at least 5 min before initiating the occlusion protocol. Proximal arterial occlusion was performed by inflating the pneumatic cuff to 200 mmHg (or 50 mmHg suprasystolic, whichever was greater) for 5 min (Yamamoto-Suganuma & Aso, 2009). Post-deflation skin blood flow was recorded for 3 min or until skin blood flow had returned to normal (Yamamoto-Suganuma & Aso, 2009). These data were saved for future offline analysis. A recovery period sufficient to allow skin blood flow to return to baseline (at least 3 min) was allowed before initiating the local heating protocol.
Integrating probe

Figure 8.1 PF 450 integrating probe
To investigate the skin hyperaemia response to local heating, the temperature of the heating discs surrounding the LDF probes were increased at a rate of 1 °C·10s⁻¹ to a temperature of 42 °C (Tew et al., 2010). Thereafter, this site was continuously heated at 42 °C for 30 min, after which the temperature of the heating discs were increased to 44 °C for a further 10 min to elicit maximum vasodilation (Van Duijnhoven et al., 2009). Data were continuously recorded for subsequent offline analysis. Experimental set-up is depicted in Figure 8.2.
Figure 8.2 Experimental set-up (study 2)
8.5.1.3 Flow-mediated dilatation

FMD is a dilatation in response to increased arterial flow. It is most easily measured in the limbs (arms for adults, legs for children) in vessels 3-6 mm in diameter (Celermajer et al., 1994). Increased flow can be stimulated by a temporary distal occlusion causing ischaemia in the unperfused tissue. This period of ischaemia releases local neurotransmitters that dilate local arterioles. Upon deflation there is a large increase in brachial artery blood flow caused by a greater pressure gradient between the axillary artery and forearm arterioles (primarily mediated by the reduced resistance in the arterioles caused by dilation). Higher flow rates increase the shear stress stimulus on the endothelial cells lining the lumen of the brachial artery. Shear stress is a frictional force applied by the flow of blood on the luminal surface of blood vessels. Dilation is the normal physiological response to increased flow via the shear stress mechanism, which is triggered by an increase in eNOS expression and an increase in NO production (as outlined in section 4.7.3.3). NO stimulates vascular smooth muscle relaxation and subsequent arterial dilation. In the presence of endothelial dysfunction, FMD is impaired because of reduced expression of eNOS, interrupting the normal physiological response mid-cascade, which causes a higher overall shear stress on the arterial lumen. FMD was reported as an absolute change from baseline (mm) and as a percentage of the baseline diameter.

\[
\text{FMD (mm)} = \text{DIAM}_{\text{PEAK}} - \text{DIAM}_{\text{BASELINE}}
\]

\[
\text{FMD (\%)} = \left(\frac{\Delta \text{DIAM}}{\text{DIAM}_{\text{BASELINE}}} \times 100\right)
\]

Arterial blood flow was calculated using simultaneous arterial diameter and blood velocity measurements. It was assumed that the internal circumference of the brachial artery was circular, and hence flow rate was calculated as follows.

\[
\text{FLOW RATE (mL \cdot min}^{-1} = \pi \times (0.5 \times \text{DIAM})^2 \times \text{MEAN BLOOD VELOCITY}
\]
To address the covariate nature of baseline arterial diameter in FMD, changes in brachial artery diameter were scaled using allometric modelling (Atkinson et al., 2013). In brief, values for baseline diameter and peak diameter were transformed using $\log_{10}$, and the difference between them was used in a univariate ANCOVA with baseline diameter as a covariate.

Shear rate can be considered an estimate of the shear stress without accounting for blood viscosity. A number of equations to estimate the shear stimulus have been proposed. Pyke & Tschakovsky (2007) proposed shear rate to be equal to mean blood velocity-diameter$^{-1}$. Thijssen et al. (2008) included a constant of 4 in the same equation for shear rate (4-velocity-diameter$^{-1}$). Padilla et al. (2009) developed this formula to calculate shear stress by measuring blood viscosity using viscometry (4-viscosity-velocity-diameter$^{-1}$). Finally, Mitchell et al. (2004) used the same formula to calculate shear stress, but instead of measuring blood viscosity they applied an assumed value – this is weak because viscosity is variable between individuals, especially those found in a clinical setting. Methodological approaches to the assessment of FMD and its related statistics seem to be far less standardised than the experimental protocol, and hence comparing shear stimuli between studies should be done carefully, with particular attention to the calculation used by the investigators.

For the current study, shear rate was calculated as below.

$$\text{SHEAR RATE (s}^{-1}) = \frac{4 \times \text{MEAN BLOOD VELOCITY}}{\text{DIAM}}$$

Occlusions of 5 min in duration are most commonly used as a compromise between participant comfort and physiological response; occlusions of greater than 4.5 min are thought to induce maximal dilatation (Leeson et al., 1997). Furthermore, longer occlusions make fidgeting more likely – increasing the difficulty of performing a manual scan and probably decreasing the overall quality of the scan. Alternative methods of assessing endothelial function include intravenous acetylcholine administration coupled with quantitative angiography or strain-gauge plethysmography and harvesting endothelial cells. These alternatives are more invasive and within a research setting would likely impede recruitment of volunteers.
According to good clinical practice, it is unethical to put participants through more invasive procedures than is necessary.

Endothelial-dependent dilation of the brachial artery in response to ipsilateral forearm cuff occlusion was measured using a vascular ultrasound scanner (Terason t3000CV ultrasound system (V4.7.4), Terason, USA) and a 7-MHz linear array probe (7L3, Terason, USA). Data were recorded using screen-capture software (Camtasia, TechSmith, Okemos, MI, USA; this assessment was conducted using the cuff occlusion described in section 8.5.1.1). Using the ultrasound scanner, an optimised longitudinal view of the brachial artery was located on the medial aspect of the right upper-arm. The image was optimised by manipulating scan depth, focus depth, gain, compression, noise rejection and time-gain compensation.

The optimised longitudinal image was recorded for 1 min using screen capture software. After 1 min the forearm cuff was inflated to 200 mmHg (or 50 mmHg suprasystolic) and the recording paused. After 5 min the cuff was deflated and the recording resumed for a further 3 min. The recorded screen capture was saved for future offline analysis (see 8.5.3).

8.5.1.4 GTN-mediated dilatation

Glyceryl trinitrate (GTN) is a NO-donor and induces vasodilation independently of the vascular endothelium by activating guanylate cyclase in VSMCs directly. In a clinical setting it is used in the treatment of angina pectoris; patients are prescribed a GTN pump spray to carry with them to use during episodes of chest pain. GTN dilates the coronary vasculature and can alleviate symptomatic pain and reduce the risk of cardiac ischaemia. In a research setting, it is most commonly used to assess endothelium-independent vasodilation during vascular ultrasound assessments and has been since the inception of FMD. GTN has a short half-life (3 min) and is quickly metabolised by the liver, making it suitable for use within clinical trials. Because of the potential risk of nitrates toxicity and the unpleasant side-effects of headaches and light-headedness, participants' suitability for GTN administration was determined using medical history data and the following criteria:
- Not currently taking medication listed in the contraindications section of GTN in the British National Formulary 56
- Not currently taking medication that contain nitrates
- No clinical history of hypotension (<100 systolic blood pressure)
- No overt renal or liver disease

Endothelial-independent dilation of the brachial artery in response to a 400 μg dose of GTN (Coro-Nitro Pump Spray, Ayrton Saunders Ltd., Wirral, UK) delivered sublingually was measured using vascular ultrasound imaging. Using the ultrasound scanner, an optimised longitudinal view of the brachial artery was located on the medial aspect of the upper-right arm. Once in place, a 1-min baseline recording of the longitudinal view was recorded. After one min GTN was delivered sublingually by another researcher. The image of the artery was recorded for an additional six minutes. Blood pressure was monitored regularly on the contralateral arm using an automated blood pressure machine.

### 8.5.2 Offline laser Doppler flowmetry analysis

All LDF recordings were analysed once the final participant had completed the study. PWS 2.5 software was used to take mean average readings from areas of interest which included baseline flow, biological zero and peak flow (Figure 8.3). The duration of these areas was the longest consecutive period thought to represent each phase, and was typically between 5 s and 2 min. Furthermore, the time between reperfusion and peak flow, time to half recovery and the duration of the hyperaemic response. The area under the curve (AUC) of the hyperaemic response was calculated using the trapezoidal rule, accounting for differences in baseline skin blood flow between participants.

$$\sum_i (x_{i+1} - x_i) + 0.5(y_{i+1} - y_i)(x_{i+1} - x_i)$$
For the LTH data, areas of interest included the baseline flow, initial peak, nadir, plateau phase, and maximum flow were calculated over periods of at least one min. As with PORH, particular times were noted; time to initial peak and time to plateau phase. Mean values for each section were divided by the simultaneous MAP to convert into CVC. Although dispute exists regarding the best way to present skin blood flow data, this data was presented as raw CVC values; we have previously shown this method of data presentation to be reproducible (Tew et al., 2010).
Figure 8.3. Example analysis areas for PORH and LTH. A: PORH first area: baseline flow; second area: biological zero; third area: peak flow. B: Enlarged PORH showing times measured; top line: time to peak; middle line: time to half peak; bottom line: hyperaemia duration. Not shown: AUC, calculated as the integral product from cuff release to the end of the hyperaemia. C: LTH; first area: baseline flow; second area: initial peak; third area: nadir; fourth area: plateau; fifth area: maximal flow; top line: time to peak; second line: time to plateau.
8.5.3 Offline vascular ultrasound analysis

Recorded data were analysed at a later date. Raw screen capture data (.camrec) were compressed using Audio Video Interleave (.avi) format and transferred to VirtualDub (V1.9.11, FreeWare, Avery Lee) for compression. These files were then cropped according to the stage of the scan (FMD scans were separated into a baseline file (1 minute; ~900 frames) and a post-deflation file (3 minutes; ~2700 frames); GTN scans were not cropped and were kept as 7-min files (~6300 frames). Compressed files were then opened in Brachial Analyser for Research (v5.6.19, Medical Imaging Applications LLC, USA) for automated diameter and flow analysis.

Brachial artery diameter was tracked on a frame-by-frame basis by defining a region of interest (ROI) encompassing a good portion of the artery with clearly defined walls. Distance was calibrated using the depth scale on the image (Figure 8.4). Brachial artery mean blood velocity was measured by aligning the ROI over the Doppler flow data. Time and flow rate were calibrated using the x- and y-axis, respectively. Flow data were analysed on a frame-by-frame basis using an integral algorithm. Diameter and flow data, once analysis were complete, were checked visually for validity and exported into a Microsoft Excel template programmed to "smooth" data. As arterial ultrasound is a manual technique, the quality of recorded images is determined by the skill of the practitioner and the stillness of the participant. Therefore, small movements from either can result in some frames without discernible walls. Therefore we only included frames with a high level of confidence (i.e. 90%) and within a 1 SD trend of previous data points. To reduce the impact of erroneous measurements further, practitioners often smooth data by taking median values over a period of 2-5 seconds (30 to 75 frames on our system). We calculated the median value of 75 consecutive frames, with a 15-frame (20%) overlap from one median to the next for both variables in a similar way to Thijssen et al. (2008). Baseline diameter and flow were taken as the mean value of the smoothed baseline data.
Figure 8.4 Brachial analyser for research. Examples of how to calibrate the Brachial Analyser for Research automated analysis (top) and an example of analysis (bottom)
8.6 Secondary outcome measures

8.6.1 Anthropometry

Stature, body mass, and neck, waist and hip circumferences were measured using techniques previously described (section 4.7.1). Body fat percentage was assessed using bioelectrical impedance (InBody 720, BioSpace, South Korea). This system uses a tetrapolar 8-point tactile electrode system (2 per limb) and measures impedance (1, 5, 50, 250, 500 and 1000 kHz) and reactance (5, 50 and 250 kHz) at predetermined frequencies. The validity of InBody 720 in the assessment of body fat percentage is currently unclear. Some research groups have reported good agreement with either MRI or DXA, whereas some consider it to provide an underestimate (Sun et al., 2005).

8.6.2 Blood-borne biomarkers

To assess selected blood-borne biomarkers we used CholesTech reflectance photometry (CholesTech Systems, USA). We used a single-use analysis cassette capable of measuring glucose, cholesterol, high-density lipoprotein levels and triglycerides. Blood was drawn from the capillary beds in the fleshy part of the tip of the finger using a single-use lancet. Blood was collected into a capillary tube and inserted into a CholesTech device. All blood-drawing techniques were conducted using sterile procedures.

8.6.3 Questionnaires

Daytime tiredness was assessed using the ESS (appendix 15). This tool was administered in accordance with the published guidelines. The EuroQOL-5D-5L (EuroQol Executive Office, 3068 AV Rotterdam, Netherlands) questionnaire was used to characterise patients quality of life. This is an enhanced version of the EQ-5D-3L questionnaire used in study 1, providing 5 levels of response for each domain (no problems, slight problems, moderate problems, severe problems and unable to) (Appendix 16).

8.6.4 Exercise capacity

Exercise capacity was assessed using the ISWT as previously described (section 4.7.5.). Additionally, blood pressure was repeatedly measured for five minutes starting
immediately after test termination and getting the participant into a seated position.
Heart rate and recovery time post-exercise were also recorded simultaneously.

8.7 Statistical analyses

Data were assessed for normality, skew, kurtosis and homogeneity of variance using statistical tests (e.g. Shapiro-Wilk test and Levene’s test) and visual inspection of data. Parametric data were presented as mean ± SD and non-parametric data as median [interquartile range]. Groups were compared in pairs using independent samples t-tests or chi-squared tests. Effect sizes ($d$) were calculated by dividing the difference between comparison groups by the pooled standard deviation. Relationships between key variables were assessed using Spearman’s correlations. Statistical significance was set at $P<0.05$. Data were analysed using the SPSS 21.0 statistical package (SPSS, IBM corporation, Armonk, NY, USA).
Chapter 9 – Results

9.1 Baseline characteristics

In total, forty-five participants were recruited and populated the groups as follows: high-compliance (n=20); low-compliance (n=14); untreated OSA (n=6) and OSAS-free controls (n=5). Only the high-compliance group recruited to target and within the proposed timeframe. Baseline characteristics are described in Table 9.1. There were no differences in characteristics between the four groups. There was a higher prevalence of coronary artery disease in the OSA-HC group versus the OSA-UN and OSAS-free groups. The prevalence of other comorbidities were similar between the OSAS groups, but different to the non-OSAS group where no participants reported suffering from hypertension or diabetes. Approximately half of participants were ex-smokers and all groups except the OSA-HC group had one current smoker. Medication use was similar between OSAS groups. No listed medication was reported in the OSAS-free group. Due to under-recruitment in the OSA-UN and OSAS-free groups, all descriptions and discussions hereafter will concern the primary comparison between the OSA-HC and OSA-LC groups, unless otherwise stated. Some statistically significant comparisons may still be indicated within tables using superscript symbols, but not necessarily be discussed.
Table 9.1 Baseline characteristics (study 2)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
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</thead>
<tbody>
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<td>Participants (n)</td>
<td>20</td>
<td>14</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>51 ± 11</td>
<td>58 ± 7</td>
<td>53 ± 9</td>
</tr>
<tr>
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<td>11/3</td>
<td>5/1</td>
<td>4/1</td>
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<td>Body mass (kg)</td>
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<td>106 ± 15</td>
<td>108 ± 10</td>
<td>100 ± 5</td>
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<tr>
<td>Stature (cm)</td>
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<td>175 ± 9</td>
<td>177 ± 8</td>
<td>178 ± 12</td>
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<td>Body mass index (kg·m⁻²)</td>
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<td>35 ± 7</td>
<td>35 ± 3</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>Resting heart rate (beats·min⁻¹)</td>
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<td>69 ± 12</td>
<td>60 ± 5</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>138 ± 18</td>
<td>130 ± 10</td>
<td>124 ± 11</td>
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<td>85 ± 6</td>
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<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>6 (30)</td>
<td>2 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8 (40)</td>
<td>8 (57)</td>
<td>3 (50)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (20)</td>
<td>4 (29)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td>1 (17)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>12 (60)</td>
<td>7 (50)</td>
<td>3 (50)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>6 (30)</td>
<td>4 (29)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Calcium channel antagonist</td>
<td>2 (10)</td>
<td>2 (14)</td>
<td>2 (33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>4 (20)</td>
<td>2 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statin</td>
<td>7 (35)</td>
<td>3 (21)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Platelet inhibitor</td>
<td>6 (30)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS. ACE: angiotensin converting enzyme. Data are presented as mean ± SD, median [IQR], or frequency (%)
9.2 Macrovascular function

FMD was assessed in all participants; however post-study analysis was not possible for two patients because of the corruption of source data (OSA-HC: n=19; OSA-LC: n=13; Table 9.2). A further two patients in the OSA-LC group were contraindicated to GTN administration and there did not undertake GTN-mediated dilatation assessment (OSA-HC: n=19; OSA-LC: n=11) There were no between-group differences for baseline diameter between groups or between experiments. Allometric FMD was higher in the OSA-HC group compared with OSA-LC group (Figure 9.1), although this difference was not considered significant (4.6% vs. 3.3%). Absolute dilatation was approximately 0.2 mm in all groups and time to peak dilatation (FMD) was approximately one minute (except for the OSA-UN group, 38 ± 8 s). Allometrically-scaled GTN-mediated dilatation was similar in both groups at approximately 13%. GTN-mediated dilatation in these groups was greater than FMD percentage (P<0.001).
Table 9.2 Macrovascular function

<table>
<thead>
<tr>
<th></th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flow-mediated dilatation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>4.9 ± 0.8</td>
<td>4.8 ± 0.8</td>
<td>4.9 ± 0.4</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>5.1 ± 0.8</td>
<td>4.9 ± 0.8</td>
<td>5.2 ± 0.4</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td>Traditional FMD (%)</td>
<td>4.6 ± 4.1</td>
<td>3.3 ± 3.0</td>
<td>4.6 ± 2.1</td>
<td>4.8 ± 1.1</td>
</tr>
<tr>
<td>Allometric FMD (%)</td>
<td>4.6 ± 3.6</td>
<td>3.3 ± 3.6</td>
<td>4.6 ± 3.1</td>
<td>4.8 ± 2.8</td>
</tr>
<tr>
<td>Absolute FMD (mm)</td>
<td>0.2 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Time to peak diameter (s)</td>
<td>58 ± 40</td>
<td>65 ± 46</td>
<td>38 ± 8*†</td>
<td>55 ± 22</td>
</tr>
<tr>
<td>Time to peak flow (s)</td>
<td>14 ± 5</td>
<td>13 ± 4</td>
<td>13 ± 4</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Shear rate AUC</td>
<td>13744 ± 5535</td>
<td>16127 ± 7496</td>
<td>12800 ± 3399</td>
<td>17014 ± 5761</td>
</tr>
<tr>
<td>Baseline flow (mL-min⁻¹)</td>
<td>98 ± 38</td>
<td>114 ± 59</td>
<td>113 ± 77</td>
<td>95 ± 32</td>
</tr>
<tr>
<td>Peak flow (mL-min⁻¹)</td>
<td>687 ± 223</td>
<td>711 ± 198</td>
<td>855 ± 356</td>
<td>767 ± 310</td>
</tr>
<tr>
<td><strong>GTN-mediated dilatation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>4.9 ± 1.5</td>
<td>4.4 ± 1.2</td>
<td>5.1 ± 0.4</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>5.5 ± 1.7</td>
<td>4.8 ± 1.0</td>
<td>6.0 ± 0.6</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>GTN dilatation (%)</td>
<td>12.8 ± 3.4</td>
<td>10.3 ± 6.7</td>
<td>17.9 ± 10.6</td>
<td>13.2 ± 5.7</td>
</tr>
<tr>
<td>Allometric GTN dilatation (%)</td>
<td>13.4 ± 5.1</td>
<td>13.1 ± 5.7</td>
<td>18.1 ± 7.6</td>
<td>12.8 ± 7.5</td>
</tr>
<tr>
<td>GTN dilatation (mm)</td>
<td>0.7 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.9 ± 0.5</td>
<td>0.6 ± 0.3</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS; FMD: flow-mediated dilatation; UC: area under the curve (integral); GTN: glyceryl trinitrate; *: P<0.1 vs. OSA-HC; †: P<0.1 vs. OSA-LC. Data are presented as mean ± SD or mean [95% CI].
Figure 9.1 Macrovascular function in high- and low-compliance patients. Top: Allometric flow mediated-dilatation; middle: time to peak diameter; bottom: shear rate area under curve. OSA-HC: high compliance; OSA-LC: low compliance. Data are mean ± SD.
9.3 Microvascular function

All patients completed the microvascular assessments (Table 9.3). There were no differences in variables between the OSA-HC and OSA-LC groups in both LDF protocols. CVC in the peak PORH was lower in the untreated group compared with the high-compliance group (0.732 ± 0.22 vs. 0.959 ± 0.253 APU/mmHg; \( P=0.049 \)). Also, between the same two groups there was a trend for a longer time to peak in the untreated group (12 ± 11 vs. 8 ± 4 s; \( P=0.066 \)). These data combined suggest the hyperaemic profile was displaced rightwards and downwards. There were no noteworthy differences in the LTH response.

Table 9.3 Microvascular function

<table>
<thead>
<tr>
<th></th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Post-occlusion reactive hyperaemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline CVC (APU/mmHg)</td>
<td>0.201 ± 0.041</td>
<td>0.194 ± 0.050</td>
<td>0.205 ± 0.070</td>
<td>0.216 ± 0.070</td>
</tr>
<tr>
<td>Biological zero CVC (APU/mmHg)</td>
<td>0.072 ± 0.03</td>
<td>0.066 ± 0.022</td>
<td>0.066 ± 0.015</td>
<td>0.091 ± 0.050</td>
</tr>
<tr>
<td>Peak CVC (APU/mmHg)</td>
<td>0.959 ± 0.253</td>
<td>0.902 ± 0.281</td>
<td>0.732 ± 0.22*</td>
<td>0.827 ± 0.243</td>
</tr>
<tr>
<td>Time to peak CVC (s)</td>
<td>8 ± 4</td>
<td>8 ± 3</td>
<td>12 ± 11*</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Time to half peak CVC (s)</td>
<td>40 ± 21</td>
<td>32 ± 18</td>
<td>52 ± 39</td>
<td>57 ± 39</td>
</tr>
<tr>
<td>Hyperaemia duration (s)</td>
<td>130 ± 36</td>
<td>108 ± 48</td>
<td>110 ± 49</td>
<td>148 ± 56</td>
</tr>
<tr>
<td>Area under curve</td>
<td>6327 ± 2543</td>
<td>6569 ± 7368</td>
<td>5038 ± 3276</td>
<td>5973 ± 2692</td>
</tr>
<tr>
<td><strong>Local thermal hyperaemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline CVC (APU/mmHg)</td>
<td>0.206 ± 0.052</td>
<td>0.203 ± 0.062</td>
<td>0.211 ± 0.071</td>
<td>0.214 ± 0.073</td>
</tr>
<tr>
<td>Initial Peak CVC (APU/mmHg)</td>
<td>1.65 ± 0.477</td>
<td>1.604 ± 0.479</td>
<td>1.535 ± 0.407</td>
<td>1.39 ± 0.413</td>
</tr>
<tr>
<td>Nadir CVC (APU/mmHg)</td>
<td>1.493 ± 0.497</td>
<td>1.307 ± 0.532</td>
<td>1.425 ± 0.39</td>
<td>1.195 ± 0.295</td>
</tr>
<tr>
<td>Plateau CVC (APU/mmHg)</td>
<td>2.044 ± 0.602</td>
<td>1.982 ± 0.631</td>
<td>1.862 ± 0.459</td>
<td>1.838 ± 0.453</td>
</tr>
<tr>
<td>Maximal CVC (APU/mmHg)</td>
<td>2.17 ± 0.599</td>
<td>2.254 ± 0.64</td>
<td>2.06 ± 0.595</td>
<td>1.927 ± 0.398</td>
</tr>
<tr>
<td>Time to peak CVC (s)</td>
<td>214 ± 33</td>
<td>202 ± 23</td>
<td>220 ± 16</td>
<td>206 ± 26</td>
</tr>
<tr>
<td>Time to plateau CVC (s)</td>
<td>1127 ± 177</td>
<td>1092 ± 133</td>
<td>1060 ± 69</td>
<td>1067 ± 156</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS; CVC: cutaneous vascular conductance; APU: arbitrary perfusion units; *: \( P<0.05 \) vs. OSA-HC; †: \( P<0.1 \) vs. OSA-LC. Data are presented as mean ± SD.
9.4 Anthropometry

Anthropometric outcome measures are shown in Table 9.4. There was a lower mean waist circumference in the low-compliance group compared with the high-compliance group (116 ± 13 vs. 125 ± 15 cm; \( P=0.046 \)). The OSAS-free group was markedly different in several anthropometric variables compared with the high-compliance group. Body mass (100 ± 5 vs. 116 ± 22; \( P=0.008 \)), body mass index (32 ± 4 vs. 38 ± 7; \( P=0.019 \)), waist circumference (108 ± 6 vs. 125 ± 15; \( P=0.001 \)) and fat mass (33 ± 6 vs. 46 ± 17; \( P=0.007 \)) were all significantly lower.

### Table 9.4 Anthropometric outcome measures

<table>
<thead>
<tr>
<th>Manual anthropometry</th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>116 ± 22</td>
<td>106 ± 15</td>
<td>108 ± 10</td>
<td>100 ± 5†</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>38 ± 7</td>
<td>35 ± 7</td>
<td>35 ± 3</td>
<td>32 ± 4†</td>
</tr>
<tr>
<td>Neck (cm)</td>
<td>44 ± 3</td>
<td>44 ± 3</td>
<td>44 ± 4</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>125 ± 15</td>
<td>116 ± 13*</td>
<td>110 ± 24</td>
<td>108 ± 6†</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>121 ± 16</td>
<td>118 ± 13</td>
<td>121 ± 8</td>
<td>114 ± 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bioelectrical impedance</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle mass (kg)</td>
<td>40 ± 6</td>
<td>38 ± 6</td>
<td>38 ± 6</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>46 ± 17</td>
<td>39 ± 14</td>
<td>40 ± 9</td>
<td>33 ± 6†</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>39 ± 8</td>
<td>36 ± 10</td>
<td>37 ± 8</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>Right arm (kg)</td>
<td>4.3 ± 0.8</td>
<td>4.1 ± 0.7</td>
<td>4.1 ± 0.7</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Left arm (kg)</td>
<td>4.2 ± 0.8</td>
<td>3.9 ± 0.8</td>
<td>4.1 ± 0.6</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Trunk (kg)</td>
<td>31.9 ± 4.7</td>
<td>31.0 ± 4.2</td>
<td>31.0 ± 3.7</td>
<td>30.6 ± 3.8</td>
</tr>
<tr>
<td>Right leg (kg)</td>
<td>11.3 ± 4</td>
<td>9.9 ± 1.6</td>
<td>10.1 ± 1.6</td>
<td>10.4 ± 1.7</td>
</tr>
<tr>
<td>Left leg (kg)</td>
<td>10.6 ± 1.7</td>
<td>10 ± 1.6</td>
<td>10.2 ± 1.8</td>
<td>10.5 ± 1.8</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS; *: \( P<0.05 \) vs. OSA-HC; †: \( P<0.05 \) vs. OSA-HC
9.5 Cardiovascular risk

There were no differences in the cardiovascular risk profile between the OSA-HC and OSA-LC groups (Table 9.5). Fasting HDL and glucose measurements were higher in the untreated group compared with the high-compliance group. Daytime somnolence was higher in the low-compliance (10 ± 4 vs. 5 ± 4; P=0.001) and untreated (9 ± 3 vs. 5 ± 4; P=0.014) OSAS groups compared with the high compliance group.

Table 9.5 Cardiovascular risk and questionnaire outcomes

<table>
<thead>
<tr>
<th></th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol·L⁻¹)</td>
<td>4.3 ± 1.1</td>
<td>4.9 ± 1.6</td>
<td>5.4 ± 1</td>
<td>5.9 ± 1.4</td>
</tr>
<tr>
<td>HDL (mmol·L⁻¹)</td>
<td>0.8 ± 0.2</td>
<td>1 ± 0.2</td>
<td>1.1 ± 0.2*</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>non-HDL (mmol·L⁻¹)</td>
<td>3.5 ± 1.1</td>
<td>3.9 ± 1.6</td>
<td>4.3 ± 0.9</td>
<td>5 ± 1.4</td>
</tr>
<tr>
<td>Glucose (mmol·L⁻¹)</td>
<td>6 ± 0.9</td>
<td>5.6 ± 1</td>
<td>5.2 ± 0.5*</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132 ± 20</td>
<td>138 ± 18</td>
<td>130 ± 10</td>
<td>124 ± 11</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 9</td>
<td>80 ± 9</td>
<td>85 ± 6</td>
<td>77 ± 4</td>
</tr>
<tr>
<td><strong>Questionnaires</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EQVAS</td>
<td>72 ± 18</td>
<td>70 ± 13</td>
<td>60 ± 24</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>Epworth Sleepiness Scale</td>
<td>5 ± 4</td>
<td>10 ± 4†</td>
<td>9 ± 3†</td>
<td>8 ± 4</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS; HDL: high-density lipoprotein; EQVAS: EuroQol visual analogue scale; *: P<0.05 vs. OSA-HC; †: P<0.001 vs. OSA-HC.
9.6 Exercise capacity

Four participants in the OSA-HC group and one in each of the untreated and non-OSAS groups did not conduct the incremental shuttle walk test (Table 9.6). Reasons included high blood pressure (n=5) and injury (n=1). The OSAS groups were all similar with distance walked in the ISWT, post-exercise heart rate and post-exercise RPE. The OSAS-free group walked significantly further than each of the OSAS groups. This was matched with a higher post-exercise heart rate compared with the OSA-UN group only (151 ± 12 vs. 130 ± 12 beats-min⁻¹; P=0.040). Heart rate recovery at one minute was also similar between the OSA-HC and OSA-LC groups (-34 ± 14 vs. -28 ± 15 beats-min⁻¹; P=0.291).

Table 9.6 Exercise capacity

<table>
<thead>
<tr>
<th></th>
<th>OSA-HC</th>
<th>OSA-LC</th>
<th>OSA-UN</th>
<th>OSAS-free</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediately post-exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance walked (m)</td>
<td>562 ± 269</td>
<td>575 ± 288</td>
<td>564 ± 135</td>
<td>808 ± 158*†</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
<td>140 ± 22</td>
<td>139 ± 23</td>
<td>130 ± 12</td>
<td>151 ± 12*</td>
</tr>
<tr>
<td>RPE</td>
<td>15 ± 2</td>
<td>17 ± 3</td>
<td>16 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td><strong>One minute post-exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>176 ± 21</td>
<td>187 ± 25</td>
<td>178 ± 26</td>
<td>189 ± 33</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>92 ± 9</td>
<td>93 ± 12</td>
<td>92 ± 12</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
<td>106 ± 23</td>
<td>110 ± 23</td>
<td>95 ± 12†</td>
<td>112 ± 14‡</td>
</tr>
<tr>
<td><strong>Three minute post-exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>156 ± 20</td>
<td>166 ± 21</td>
<td>160 ± 34</td>
<td>159 ± 24</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83 ± 8</td>
<td>84 ± 11</td>
<td>90 ± 10</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
<td>81 ± 16</td>
<td>87 ± 16</td>
<td>82 ± 11</td>
<td>88 ± 7</td>
</tr>
<tr>
<td><strong>Five minute post-exercise</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>141 ± 16</td>
<td>146 ± 16</td>
<td>144 ± 17</td>
<td>141 ± 8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 11</td>
<td>80 ± 12</td>
<td>84 ± 9</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats-min⁻¹)</td>
<td>79 ± 14</td>
<td>85 ± 16</td>
<td>80 ± 14</td>
<td>81 ± 8</td>
</tr>
</tbody>
</table>

OSA-HC: high CPAP compliance; OSA-LC: low CPAP compliance; OSA-UN: untreated OSAS; OSAS-free: no OSAS; RPE: Rating of perceived exertion; *: P<0.05 vs. OSA-UN; †: P<0.1 vs. OSA-LC; ‡: P<0.1 vs. OSA-UN; †: P<0.05 vs. OSA-HC. Data presented are mean ± SD.
10.1 Overview

The aims of this study were to investigate the effects of OSAS and its treatment with CPAP on vascular function using a cross-sectional study design. We compared macro- and microvascular function between four groups: OSAS patients with high compliance to CPAP, OSAS patients with low compliance to CPAP, untreated OSAS patients and OSAS-free controls. Recruitment to this study was poor, particularly in the low-compliance, untreated OSAS and OSAS-free controls. In view of a limited sample size, the results of the study suggest low-compliance patients may have impaired macrovascular function compared with patients with high compliance. Patients with low compliance were also shown to have daytime somnolence, compared to high-compliance patients, and despite this both groups had similar self-perceived health-related quality of life. These findings are preliminary and must be verified in a larger study.

10.2 Vascular function

There were no statistically significant differences between the OSA-LC and OSA-HC for percentage FMD regardless of the method of data presentation, however, percentage FMD was 28% lower in the OSA-LC group compared with the OSA-HC group using the traditional method (4.6 ± 4.1% vs. 3.3 ± 3.0%; \( P=0.313 \)) and after allometric scaling (4.6 ± 3.1% vs. 3.3 ± 2.8%; \( P=0.331 \)). Although the large SDs overlapped considerably, the between-group differences were associated with a small effect size (\( d=0.35 \)). There is a physiological basis for the presence of a difference that could be masked by the large variability. Dissimilarly, the mean dilatation in response to sublingual GTN administration was similar in both group (13.4 ± 5.1% vs. 13.1 ± 5.7%; \( P=0.895; d=0.02 \)) which suggests that the difference in FMD was caused by impaired endothelial function or NO bioavailability, and not reduced vascular compliance or VSMC dilator capacity. There was a larger shear rate area under the curve to peak dilatation in the OSA-LC group compared with the OSA-HC, although again the large variability in
measurement limits interpretation. That aside, the effect size was small at $d=0.43$. These data suggest that endothelium-mediated arterial dilatation is impaired in patients not regularly undertaking CPAP therapy, evidenced by smaller dilatation in response to a larger eliciting stimulus. To our knowledge, this is the first study to assess FMD in a homogenous group of low-compliance OSAS patients, although a previous study has compared FMD between OSAS patients and matched controls and OSAS patients during a CPAP intervention (Jelic et al., 2008). That study did include low-compliance patients however they were allocated to a mixed group along with patients who refused to undergo CPAP therapy. The ratio was approximately 3:1 in favour of untreated patients, and this group will hereafter be referred to as non-responders. The study reported pre-CPAP mean baseline FMD percentage to be $3.1 \pm 4.2\%$ and $4.9 \pm 4.5\%$ for the responders and non-responders, respectively - the authors did not report baseline characteristics for the responders and non-responders, this makes it difficult to explain the difference between means. After the intervention the responders increased FMD to $6.7 \pm 4.2\%$ and the non-responders to $5.6 \pm 4.5\%$, which equated to effect sizes of $d=0.83$ and $d=0.15$. The Jelic et al. (2008) study did not report GTN-mediated dilatation so we are unsure how the current study compares to the samples previously reported for endothelium-independent vasodilatation. Jelic and colleagues also stratified their sample into three tertiles: OSAS-free (AHI< 5 events·h$^{-1}$); mild-to-moderate OSAS (5< AHI <20 events·h$^{-1}$); and, moderate-to-severe OSAS (AHI>20 events·h$^{-1}$). These tertiles had significant trends for important indicators of vascular health, which showed decreasing vascular health with increasing AHI, including eNOS ($P=0.001$), P-eNOS ($P=0.001$), COX-2 ($P=0.001$), endothelial progenitor cells (EPCs; $P=0.001$) and of course FMD ($P=0.001$); only eNOS was significantly different between the two OSAS tertiles ($P<0.05$). This study provided compelling evidence for the reduced repair capacity of the vascular endothelium in OSAS.

FMD has been previously reported to be $9.6 \pm 1.6\%$ (Ip et al., 2004) in patients having undertaken 4 weeks of CPAP therapy, which was almost twice their pre-CPAP FMD ($5.1 \pm 1.4\%$). Improvements in FMD have been reported after shorter CPAP durations; Ohike et al. (2005) demonstrated an improvement from $3.3 \pm 0.3\%$ to $5.8 \pm 0.4\%$ ($P<0.01$; mean ± SEM) after one week of CPAP therapy. The mechanism by which effective CPAP therapy improves endothelial function is thought to be clear;
elimination of apnoea reduces oxidative stress and sympathoexcitation-induced vasoconstriction (which increase luminal shear stress on the vascular endothelium), allowing the endothelium to repair itself and improve function.

The effects of different levels of CPAP therapy on microvascular function remain unclear. Contrary to expectation, there were no noteworthy differences between the OSA-HC and the OSA-LC groups for any of the variables investigated. These results can be interpreted in two ways: 1) low compliance to CPAP therapy does not reduce microvascular function in OSAS; or 2) the current study was insufficiently powered to detect any differences. Although evidence in the literature suggests that OSAS is associated with impaired microvascular function, which is reversed with effective CPAP therapy, the current study could not reproduce this finding. A major strength of this protocol was the use of three seven-fibre probes, which allows for the measurement of skin blood flow across up to 21 sites. This helps to reduce the impact of spatial variability in the cutaneous distribution of microvessels.

10.3 Secondary outcomes

10.3.1 Anthropometry

Body habitus was similar between groups although the low-compliance group had a smaller waist circumference. This difference could be attributed to the higher proportion of female participants in the OSA-LC versus the OSA-HC groups. A similar non-significant difference in mean waist circumference was identified in the Billings et al. study (Billings et al., 2013). The anthropometric characteristics of the OSA-HC group in particular closely match the intervention and control groups in study 1. CPAP alone has been shown to have no effect on obese middle-aged OSAS patients.

10.3.2 Cardiovascular risk

Blood-borne biomarkers representing cardiovascular risk were similar between the OSA-HC and OSA-LC groups. Surprisingly, the OSA-UN group had higher HDL and lower glucose than the OSA-HC group. The former is unlikely to be meaningful as the cholesterol to HDL ratio was less favourable in the OSA-LC group. The absence of a between-group difference for glucose is not surprising. Yang et al. (2012) performed a
meta-analysis on fasting blood glucose in OSAS. They pooled nine studies of mixed design and assessed the impact of regular CPAP on fasting glucose and found inconclusive evidence for any effect in non-diabetic or diabetic OSAS patients (although the latter conclusion was based on two studies only). Our groups contained a mixture of diabetic and non-diabetic patients and mean glucose concentrations were similar to the studies included in the Yang et al. (2012) meta-analysis.

10.3.3 Questionnaires

Self-perceived health-related quality of life was similar between all groups, however daytime tiredness was much higher in the OSA-LC and OSA-UN groups ($10 \pm 4$ and $9 \pm 3$ vs. $5 \pm 4$ in the OSA-HC group). The latter comparison is not a new finding, with numerous studies having reported reductions in daytime somnolence in response to CPAP therapy. This is a logical finding as these patients are probably having fragmented sleep due to frequent apnoea stimulating microarousals. There is little evidence in the literature that discusses tiredness in OSAS patients that are not CPAP-intolerant but are unable to achieve an acceptable level of compliance. This study used CPAP compliance for the three months prior to study entry to determine which group patients would be allocated to. The reasons for low compliance were not investigated in the current study and hence a number of phenotypes were probably included: patients unable to tolerate CPAP through chronic sinus blockage; patients unable to tolerate CPAP because of claustrophobia; patients not wanting to use CPAP because of a lack of daytime symptoms (and perhaps a misunderstanding of the role of CPAP in preventing chronic disease); and, patients refusing to wear the CPAP mask having just entered into a new relationship. Each of these reasons was mentioned anecdotally to the chief investigator during this study. The underlying causes of low compliance need to be conclusively identified and addressed, and obviously for patients unable to tolerate CPAP on a nightly basis it is important for researchers and health professionals to understand if occasional CPAP offers any benefit, or whether it is simply resource wastage. Douglas et al. (1998) conducted a systematic review of the effects of CPAP on daytime tiredness and daytime functioning and concluded that CPAP can effectively improve both. In the current study, the OSA-LC group showed no decrement in health-perceived quality of
life. There was no correlation between ESS and EQVAS, suggesting that although many patients were tired during normal daytime activities, participants did not consider this tiredness to negatively affect their health. This has been shown before (Sforza et al., 2013). Bollig et al. suggested that encouragement and support to improve CPAP compliance is everyone's job and that problems encountered with CPAP use should be resolved as soon as possible to reduce the amount of time patients spend without treatment and to improve the likelihood of successful adherence in the future.

**10.3.4 Exercise capacity**

Distance walked in the ISWT was similar in all three OSAS groups, which were each significantly lower than the OSAS-free group. The ISWDs reported in the current study are higher than those in the Billings et al. (2013) study, which is likely due to the differences in testing centre and the delivery of the test. Our ISWT protocol allows patients to jog during the test to increase speed, whereas the original Singh et al. (1992) protocol used by Billings et al. (2013) does not explicitly condone this. Both studies suggest there is no difference in exercise capacity between high- and low-compliance patients. The characteristics of the OSAS-free group suggest it was not matched for age, sex, BMI and comorbidity as was proposed at the outset. This group also under-recruited and with only four participants completing the ISWT, therefore only weak conclusions can be drawn. There were no differences in post-exercise heart rate or RPE, or in the rate of heart rate and blood pressure recovery in the high and low-compliance groups. Indeed, heart rate recovery after one min was similar to non-OSAS control groups in other studies (Chien et al., 2011; Kline et al., 2012).

**10.4 Limitations**

**10.4.1 Recruitment and sample size**

The proposed recruitment target and timescale was 80 participants in four months; this study recruited 45 participants in seven months. A provisional pre-study assessment of eligibility suggested 10 and 5 patients per week would be eligible to participate in the OSA-HC and OSA-LC groups. Assuming a 30% recruitment rate (higher than study 1 because of the personal in-clinic approach, the low-intensity
demands of the study, the minimal time commitment (2 visits) and the flexible nature of appointment setting) it was expected that recruitment of the primary groups would be complete within 4 months. Recruitment was much more problematic than anticipated. The high-compliance patients were recruited with relative ease, and although recruitment for this group lasted five months, this was because the researcher recognised the abundance of participants and the willingness of them to participate, and as a result reduced the proportion of total recruitment activities focussed on this group. Despite an informal pre-study assessment of eligibility rate, once recruitment started the number of eligible patients to recruit from was vastly reduced. This could be Lasagna’s Law in action, which anecdotally states that potential patient pool is often much smaller as soon as a study starts to recruit, and then increases again once recruitment is finished. Recruitment of low-compliance patients was limited by several reasons.

Firstly, the proportion of all patients attending sleep clinic that met the inclusion criteria for this group was relatively low. This was because many patients that were struggling with compliance were not obese, and they also tended to be younger with personal (e.g. childcare) and professional (e.g. employment) commitments that made taking a morning off to participate in voluntary research (with no overt benefit to the patient) difficult. Attitudes towards research in this patient group were also negative; this could reflect general attitude towards healthcare and CPAP compliance could predict willingness to participate in research. It was expected that interest in the study from low-compliance patients would closely match that of the high-compliance group because their sub-optimal compliance probably reflects discontent with CPAP as a treatment. If this study had provided strong evidence that vascular endothelial function was no different from high-compliance patients and significantly (and meaningfully) greater than untreated patients, then a follow-up study to assess a wider range of health outcomes in this sub-population would be merited and the evidence from those studies, could inform policy for the management of OSAS patients; in particular, how patients with sub-optimal compliance are treated. It could show that alternate nights of usage provide the majority of the benefits of nightly usage (in the absence of EDS). This of course is not supported by the current results. A final limiting factor in the recruitment of CPAP patients was clinic attendance.
Recruitment for this study was done on a face-to-face basis, which meant that the researcher attended regular clinics at Northern General Hospital. This is not a time-effective method of recruitment; however it was chosen to seamlessly integrate into the routine activities of the sleep clinic. It would be more effective in future to try and involve the hospital-based nurses, doctors and physiologists in the recruitment process. Also patients that missed appointments were often those with a history of borderline usage. Because of a major finding of study 1 (that previous CPAP compliance checks and self-reported compliance is not a reliable indicator of actual compliance), recruitment for study 2 took place during and immediately after CPAP compliance checks. As a result, patients who did not attend, did not bring their CPAP machine, or brought corrupted memory cards were not approached to take part. Finally, recruitment of control participants was difficult. Previous studies in our centre have not had difficulty recruiting control participants from local interest groups. It was expected that due to the short, low-intensity nature of the study commitment that recruitment of 20 control participants would not be difficult. The main barrier to participation was the overnight OSAS screening for covert presence of the disease. After attending familiarisation some patients were reluctant to participate after finding out that if OSAS was suspected that their general practitioner may refer them to a sleep specialist for confirmation. If confirmed and of at least moderate severity, CPAP therapy would be likely, and compliance with CPAP would be important otherwise their driving licence could be jeopardised. Despite iteration of the negative consequences of untreated OSAS to health, many participants declined the overnight PSG and hence to participate. Public awareness of OSAS is not high and discussing a condition that involves periods of no breathing and may require nightly use of a machine that blows high-pressure air into the airway can seem a daunting prospect. Altogether, this study did not recruit to target and hence all results should be interpreted with caution.

The FMD assessment as a surrogate marker of vascular endothelial health requires technical expertise. We followed the recommendation of Corretti et al. (2002) that practitioners perform more than 100 supervised scans to develop and assess competencies. Vascular imaging is a manual technique that requires a skilled practitioner to not only optimise an image of the brachial artery, but to keep it
optimised throughout the scan (this includes *surfing* movement in the patients arm if they cough, sneeze or flinch). As the scanned image is a two-dimensional cross-section of the brachial artery it is difficult to be certain that the imaged segment is actually the true diameter; only a few degrees of deviation would produce an image of a chord (a cross-sectional view of the same vessel that has a shorter width than the diameter that, for all intents and purposes would appear the same). To address these issues researchers often apply a rolling smoothing technique to raw data to try and remove outliers that could distort measurements. There is no universally accepted smoothing method and changing from 3 s, to 4 s to 5 s rolling median average will likely reduce the peak diameter reading.

### 10.5 Future research

Unfortunately the current study did not have appropriate statistical power to answer the principal research question. It is anticipated that with modifications to the recruitment strategies and revision of expected recruitment rates and goals that enough patients could be recruited to answer our research questions. Data from the current study can be used to estimate a sample size for a future study. This estimate (as it is based on internal preliminary data) can be more accurate than using data from other centres as it takes into account site-based factors such as outcome variance. Using an alpha level of 0.05, a beta (power) of 80% and a 2-tailed study design the current data suggest needing 83 participants per group to detect a significant difference. As there is no need to account for participant withdrawal, and including the current data with no changes to protocol (thereby making it an internal pilot) we would need to recruit and additional 64 high-compliance patients and 70 low-compliance patients. Modifications to the recruitment strategies would be needed to achieve this.

### 10.6 Summary

Vascular endothelial function was the primary outcome measure in this study. We identified potential evidence of impaired vascular endothelial function in OSAS
patients with low-compliance to CPAP therapy compare with high compliance patients. The reliability of these findings is unclear because the OSA-LC group did not recruit to target and may lack the scientific power to identify significant differences. This under-recruitment was because the prevalence of these patients was lower than expected, and their willingness to participate in research was also lower, compared with their high-compliance counterparts. Encouragement (or interventions) to improve CPAP usage in patients that are CPAP tolerant should be delivered by the primary care team.
Chapter 11 – Summary of findings

This research broadly aimed to investigate the physiological effects of different treatment strategies in OSAS. Both studies recruited patients that were undergoing CPAP therapy in the local NHS Foundation Trust. CPAP is currently the preferred treatment and is approved by the National Institute for Care and Health Excellence for its efficacy at eliminating apnoea and its cost-effectiveness. However two significant issues are associated with it. Firstly, CPAP is a management therapy and will not cure the condition, which means patients must use a machine that is often uncomfortable and embarrassing for the foreseeable future. Secondly, because of its arduous nature not all patients can tolerate CPAP on a nightly basis. Therefore it is important to investigate alternative treatment pathways for OSAS patients, and to develop understanding of the short-, medium- and long-term physiological effects of partially-treated OSAS.

Endothelial function has regularly been shown to be impaired in OSAS; a deficit that can be ameliorated with regular CPAP therapy (of even short durations). There were no changes in endothelium-dependent or -independent vasodilation identified in study one, which may be attributable to the use of a single fibre LDF probe (because microvascular function has previously been shown to suffer from high spatial variability). This issue was addressed in study two by using three seven-fibre probes to measure skin blood flow (which increases the fibre count from 1 to 21). Study two also used different provocation tests because of equipment-related issues. Comparisons between the OSA-HC and OSA-LC groups did not identify any differences any of the measured variables. Study two also investigated macrovascular function by assessing FMD. No significant differences were identified between the high- and low-compliance groups, although the OSA-LC group was slightly lower (Δ 1.3 [-1.3, 3.94]%) and was associated with a small effect size (d=0.35). As study two did not recruit to target and FMD was the primary outcome measure, it is possible that the comparison lacked the necessary power. Furthermore, this technique is associated with a large amount of inherent variability that may have contributed to the lack of statistical significance.
The participants in both studies were clinically obese. The lifestyle intervention had minimal effect on body mass, and the trivial changes observed did not result from a profound change in body composition (i.e. body mass will not change if reductions in fat mass coincide with increases in muscle mass). In study two the low-compliance group tended to have a lower body mass and a smaller waist circumference which could reflect the higher proportion of female participants.

The participants in study one had a number of raised cardiovascular risk factors. BMI was high (although we intentionally recruited only obese patients), serum insulin was high and serum CRP was high. Although not all participants were optimally compliant with CPAP, compliance did not correlate with any of the aforementioned factors. In study two, there was no difference in cardiovascular risk factors between the high- and low-compliance patients, although patients in both groups were borderline glycaemic and pre-hypertensive. These studies have demonstrated that despite CPAP therapy, OSAS patients may still have an increased cardiovascular risk profile; however the frequency of CPAP use did not seem to significantly affect risk, so even occasional CPAP therapy may have protective effects.

In study one the ISWD in the intervention group at end-point and follow-up was 724 ± 193 m and 737 ± 179 m, respectively, which are not dissimilar from the 808 ± 158 m observed in the OSAS-free group of study 2. This could be evidence of a reversal of OSAS-induced reductions in exercise capacity not achieved by CPAP therapy alone. With low cardiorespiratory fitness earmarked as a more important risk factor for cardiovascular disease than smoking or obesity, improved fitness is not to be underestimated. Future research is required to address whether these short-term changes in exercise capacity and behaviour translate into long-term increases in habitual physical activity. Although self-perceived quality of life was similar between groups in study one, there was no marked improvement in self-perceived health-related quality of life until follow-up, when the intervention group had increased 9 points from baseline. In study two, health-related quality of life reflected treatment status, with OSAS-free being greatest and the OSAS groups decreasing proportionately to treatment (i.e. OSA-HC>OSA-LC>OSA-UN). Unsurprisingly, the OSA-LC and OSA-UN
groups presented with a higher daytime somnolence that the OSA-HC group, which highlights the importance of regular CPAP use to reduce daytime tiredness.

This thesis has reported a number of findings not previously published in the medical literature.

1) Our pragmatic lifestyle intervention was feasible to deliver. Although previous studies have provided evidence for the feasibility of delivering similar lifestyle interventions in OSAS, this is the first to be conducted within the NHS and the U.K. and the first to assess the efficacy of basic dietary advice and behaviour change counselling delivered by an exercise physiologist. Recruitment for this study was good, exemplified by recruitment rates that closely matched those reported in studies conducted in Finland, Brazil and the United States of America. Compliance with study-related activities was excellent and in excess of the studies reported by Kline et al. (2011) and Tuomilehto et al. (2009), which suggests the method of delivery promoted adherence. Lastly, there were no adverse events emphasising the safety of the delivered intervention.

2) The intervention improved key health outcomes such as exercise capacity and serum C-reactive protein, which were maintained after 3 months of independence. The lifestyle intervention evoked improvements in some key health outcomes. Improvements in exercise capacity were identified using the ISWT as a metric. Maintenance of higher fitness levels at follow-up shows an absence of the detraining response to withdrawing the exercise stimulus – this suggests that patients may have continued regular exercise and maintained the improvement. Increased fitness and regular exercise has several benefits, including reduced age-related decline in lean muscle mass and bone density. Reductions in CRP indicate a reduced state of systemic inflammation and an overall lower risk, if sustained long term, of developing cardiovascular disease. These findings are important because they suggest that this pragmatic lifestyle intervention could improve the overall clinical picture of OSAS patients.
3) Self-reported CPAP compliance is an unreliable indicator of true compliance. This was an incidental finding from study one, which recruited patients that self-reported using CPAP therapy on a regular basis (defined as \( >4 \text{ h-night}^{-1} \) on \( >75\% \text{ nights} \)). Post-study compliance verification highlighted many patients (almost 25\%) had overestimated their usage beyond the inclusion criteria. This was unexpected and not discovered until the final participant had completed the study, and the reasons for the over-estimation are unclear. This could have been an innocent mistake, or an attempt to gain illegitimate entry to the study. Studies assessing CPAP compliance will usually use objective data from CPAP memory cards; however this was not feasible. Although recruitment took place from a local Trust, study activities took place in the University, which did not have the appropriate hardware or software to easily assess this. We could have collected cards, assessed them and returned them to patients’ days later; however this would have posed logistical and financial problems.

4) It is difficult to recruit low-compliance patients onto research trials, and recruiting newly diagnosed patients is also difficult without interrupting the patient pathway. There are a limited number of trials that have specifically sought to recruit low-compliance patients - usually a retrospective study design will allocate participants to one group or the other, depending on their treatment status. The accidental recruitment of low-compliance patients in study one informed the design of study two, as undertreated patients seemed to be more common in our OSAS population. We recruited different sub-sets of the OSAS population into a cross-sectional study design, and encountered problems recruiting low-compliance patients. It is likely that patients that have poor compliance with CPAP may be less interested in participating in OSAS research. The precise reasons influencing the participation of low-compliance patients in medical research is yet to be determined.

5) Vascular function seems impaired in low-compliance patients versus high-compliance patients, although further work is needed to confirm this. This finding is based on the difference in mean values and a small effect size. This
difference was not statistically significant suggesting the study may have been underpowered. There is currently no evidence in the literature regarding what effect occasional CPAP use has on reversing the OSAS-induced impairment in vascular endothelial function. These, and other, data about key health outcomes in low-compliance OSAS-patients are important to discern and could inform the way that this sub-population are treated. Moreover, the current data allow a more precise sample size estimation to be conducted to base a future study upon.

Future research should adapt the intervention and perhaps incorporate specialist support (e.g. nutritionist) to promote greater weight loss and evaluate the effects of the lifestyle intervention on indices of OSAS severity. We have also shown that the use of self-reported CPAP compliance is inadequate and an unreliable metric of true compliance, and recommend that future studies use only objective compliance from the CPAP machine to determine eligibility. This research has provided promising evidence to support the role of lifestyle intervention in a motivated subset of OSAS patients. Undoubtedly, such an intervention would not be indicated in all patients. Lifestyle intervention would probably be most efficacious when delivered in conjunction with nightly CPAP therapy. Furthermore, the effects of the intervention should be investigated in patients with sub-optimal CPAP compliance and CPAP intolerance. Finally, the effects of low compliance on vascular endothelial function should be reinvestigated by recruiting a larger number of patients. Such studies would provide evidence that could have implications for the way patients that struggle to reach optimal compliance are managed in the NHS.

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EFFECTS OF A LIFESTYLE INTERVENTION ON EXERCISE CAPACITY, QUALITY OF LIFE, BODY MASS/COMPOSITION AND CARDIOVASCULAR RISK IN PATIENTS BEING TREATED FOR OBSTRUCTIVE SLEEP APNOEA

Dear ..............................................

We are undertaking a research study to investigate the feasibility and effectiveness of a 12-week lifestyle intervention for patients being treated for obstructive sleep apnoea. The results of this research could ultimately prove to be of significant benefit to such patients by providing new insights into the effects of lifestyle modification on various aspects of health. You might have been selected as being a suitable patient from attending the hospital or because you have been involved in a previous study. Please find enclosed a participant information sheet, which describes the study in detail and answers the most frequently asked questions.
A member of the research team will be phoning you shortly to see if you would be interested in participating in this study and will gladly answer any questions you might have. It is important to note that there is no pressure to participate in this study and if you do not wish to be contacted about this study please phone James Moss on 0114 2252262 or 07738240670 before 16th October.

Yours sincerely,

Dr Stephen Bianchi
Respiratory Physician
Royal Hallamshire Hospital

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SLEEP APNOEA RESEARCH STUDY

☐ Yes. I am interested in taking part in the above named study. I understand that a member of staff will be contacting me, regarding this study.

☐ No, I am not interested in taking part in the study

Name: _________________________________
Telephone Number: ____________________

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PARTICIPANT INFORMATION SHEET
Version 3: 12th October 2009
EFFECTS OF A LIFESTYLE INTERVENTION ON EXERCISE CAPACITY,
QUALITY OF LIFE, BODY MASS/COMPOSITION AND CARDIOVASCULAR RISK
IN PATIENTS BEING TREATED FOR OBSTRUCTIVE SLEEP APNOEA

Part 1
We would like to invite you to take part in a research study. Before you decide you
need to understand why the research is being done and what it will involve for you.
Please take time to read the following information carefully. Talk to others about the
study if you wish. Ask us if there is anything that is not clear or if you would like more
information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
Obstructive sleep apnoea (OSA) is a common disorder characterised by interrupted
breathing while sleeping. It is associated with cardiovascular problems such as high
blood pressure, heart attack and stroke. Furthermore, many OSA patients are
overweight and have impaired quality of life. Lifestyle interventions incorporating
exercise training, dietary advice and behaviour change have been shown to elicit
favourable changes in exercise capacity, quality of life, body mass/composition and
cardiovascular risk in a range of patient groups. However, no study has investigated
the impact of lifestyle modification on such health outcomes in patients being treated
for OSA. This study will address this issue by investigating the effects of a lifestyle
intervention on exercise capacity, quality of life, body mass/composition and
cardiovascular risk in patients with OSA.

Why have I been invited to take part?
You have been invited to take part because you are being treated for OSA.

Do I have to take part?
It is up to you to decide. We will describe the study and go through this information
sheet, which we will then give to you. We will then ask you to sign a consent form to
show you have agreed to take part. If you do decide to take part, you will be free to
withdraw at any time, without giving a reason. This would not affect the standard of
care that you receive.
What will happen to me if I take part?
Your involvement in the study will last for about 26 weeks. During the first week you will undertake two assessment visits as detailed below. You will then be assigned to a lifestyle intervention group or a usual care control group. The group that you are assigned to will be decided by chance (randomly) and you will have an equal chance of being in each of the two groups. If you are in the lifestyle intervention group, you will receive supervised exercise training, dietary advice and behaviour change counselling for 12 weeks. If you are in the control group, you will receive an educational booklet detailing healthy eating and exercise guidelines but no supervised or structured intervention. At the end of the 12-week intervention period, you will redo the two assessment sessions. You will then be asked to complete the assessments for a third time, 12 weeks after the end of the intervention. After this, you might also be asked to attend a focus group meeting, in which we will ask for your feedback about the study.

What will the assessment visits involve?
There will be two assessment visits at the beginning of the study, at the end of the 12-week intervention period, and at 12 weeks after then end of the intervention. On each occasion it is essential that no food or caffeine is consumed for four hours before the assessment session and that you refrain from exercise and alcohol for 24 hours prior to the session.

Visit 1: Sheffield Hallam University
You will attend the research centre at Sheffield Hallam University for a medical examination to assess whether or not you are eligible to take part in the study. During this assessment, we will assess your blood pressure, height, weight, and body composition. We will also perform a resting electrocardiogram (ECG) to assess the rhythm and electrical activity of your heart. If you are deemed suitable for participating in this study, we will then take a 10 ml (two teaspoon) sample of blood from a vein which will be used to assess circulating glucose, insulin and lipoprotein concentrations. We will then ask you to complete a shuttle-walk test. During this test you will be asked to walk up and down a 10-m course. The walking speed will start slow, increase slightly each minute, and will be determined by an audio signal. The test will end when either you are too breathless to continue, you fail to complete two consecutive shuttles within the time allowed, or if you achieve 85% of your maximum heart rate. Overall, this visit will last around 1 hour.

Visit 2: Sheffield Hallam University
Your second assessment session will also take place at Sheffield Hallam University. On this visit, the blood flow in the small vessels of the skin will be assessed using a technique called iontophoresis. This technique involves the placement of electrodes onto the skin surface of your forearm. We will clean and shave the areas where
these electrodes are to be placed. Once the electrodes are in place skin blood flow will be stimulated using tiny amounts of drugs that will be delivered into the skin by applying a small electric current through the electrodes. The drugs used are acetylcholine and sodium nitroprusside. These drugs have been used in this way previously in many research volunteers over many years with no reported side effects. You may feel some 'tingling' of the test area during this test but this should not cause you any discomfort. Only the very small blood vessels in the skin that we are investigating will be affected by this technique. Blood pressure will be taken throughout this test session. During this session, we will also ask you to complete some quality of life questionnaires. Overall, this visit will last around 1 hour.

The lifestyle intervention programme
Once you have completed the two visits you will be assigned randomly (i.e. like tossing a coin) to one of two groups. One of the groups will undertake a lifestyle intervention programme and one will not. If you are in the lifestyle intervention group, you will receive exercise training sessions (60 minutes per session) three times a week, dietary advice, and behaviour change counselling for 12 weeks. For the first four weeks, all of the exercise sessions will be supervised at Sheffield Hallam University. Between weeks 4 to 12, the supervised sessions will gradually be replaced with home-based exercise. The exercise sessions will involve light-to-moderate intensity aerobic and resistance exercises. If you are assigned to the control group, you will receive an educational booklet detailing healthy eating and exercise guidelines but no supervised or structured intervention.

After the lifestyle intervention programme
You will repeat visits 1 and 2 above within 1 week of finishing, and then again about 12 weeks after finishing. All repeat visits will be completed at Sheffield Hallam University.

What are the disadvantages of taking part?
The procedures that we are using in this research are all well established techniques which have been used in other patient groups in numerous research studies without any significant side effects being reported. Therefore the only side effects that we would expect you to experience are the minor side effects associated with the test procedures described above.

What if I do not wish to take part?
This will in no way affect your treatment.

What if I change my mind during the study?
You are free to withdraw from the study at any time without affecting your treatment.

What are the possible benefits of taking part?
We cannot promise that the study will help you but the information we get from this study might help improve the treatment of people with obstructive sleep apnoea.
Will my involvement in the study be kept confidential?
Yes. We will follow legal and ethical practice and all information about you will be handled in strict confidence. Any information about you that leaves the research centres will have your name and address removed so that you cannot be recognised from it.

What will happen to the information from the study?
It is anticipated that the results of the study will be presented at scientific meetings and published in a scientific journal. These overall results will be available to you; however, it will not be possible to produce an individualised report of your performance.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might have suffered will be addressed. Detailed information on this will be given in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2
What if I have further questions or would like more information about the study?
If you would like more information about the study you are invited to contact:-
Mr Garry Tew Sheffield Hallam University Tel 0114 225 2358
Dr John Saxton Sheffield Hallam University Tel 0114 225 4414
Dr Stephen Bianchi Royal Hallamshire Hospital Tel 0114 271 1740

What happens if I have a complaint?
If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have complaints or concerns, please contact the project supervisor Dr John Saxton, Tel 0114 225 4414. Or alternately you can use the normal hospital complaints procedure and contact Professor Chris Welsh, Medical Director, Sheffield Teaching Hospitals NHS Foundation Trust, Tel 0114 271 2178 or you can use the normal University complaints procedure and contact the University Secretary and Registrar, Liz Winders, Tel 0114 225 2051.

What if I am harmed?
In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed as a result of someone’s negligence then you could have grounds for legal action for compensation, but you might have to pay your legal costs.
What will happen to the samples I give?
The blood samples taken at visit one will be stored at either the Royal Hallamshire Hospital or Sheffield Hallam University. Any excess not required for analysis will be discarded.

Who is organising and funding the research?
This study is being organised and lead by staff at The Centre for Sport and Exercise Science, Sheffield Hallam University and Sheffield Thoracic Institute, Royal Hallamshire Hospital. This study is being funded by Sheffield Hospitals Charitable Trust and the Sleep Apnoea Trust.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed by the South Yorkshire Research Ethics Committee.

Further information/independent advice
If you require any further information or independent advice about this study, please contact the Patient Advisory Liaison Service (PALS) on Tel 0114 271 2450 (Royal Hallamshire Hospital) or Tel 0114 271 5759 (Northern General Hospital).
Appendix 3

OSA STUDY
PARTICIPANT DETAILS AND MEDICAL SCREENING

STU STUDY NUMBER: 15488

PATIENT ID NUMBER:

Name:
DOB:

Address:

Telephone: h: m: w:
E-mail:

Consultant: Hospital:
GP:
Practice:

Current Medication:

BMI:

Waist/hip/neck circumferences:

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1. Medical History

a.

### Past History

<table>
<thead>
<tr>
<th>Have you ever had?</th>
<th>Y</th>
<th>N</th>
</tr>
</thead>
</table>

### Family History

<table>
<thead>
<tr>
<th>Have any immediate family had?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
</tr>
</tbody>
</table>

### Present Symptoms

<table>
<thead>
<tr>
<th>Have you recently had?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
</tr>
</tbody>
</table>

- **High blood pressure**
- **Any heart trouble**
- **Arterial disease**
- **Lung disease**
- **Asthma**
- **Diabetes**
- **Diabetes**
- **Diabetes**
- **Diabetes**
- **Epilepsy**
- **Arthritis**
- **Heart attacks**
- **High blood pressure**
- **High blood pressure**
- **Stroke**
- **Diabetes**
- **Early death**
- **Other family illness**
- **Orthopaedic problems**
- **Chest pain/discomfort**
- **Shortness of breath**
- **Heart palpitations**
- **Dizzy spells**
- **Frequent headaches**
- **Frequent colds**
- **Back pain**

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If you answered yes to any of the above, please give brief details

b. Have you had to consult your doctor in the last 6 months? Yes ☐ No ☐
If yes, please give details

c. Do you currently have any form of muscle or joint injury? Yes ☐ No ☐
If yes, please give details

d. Have you had to suspend normal activity in the last 2 weeks Yes ☐ No ☐
If yes, please give details

e. As far as you are aware, is there anything that might prevent you from successfully completing the assessments outlined to you? Yes ☐ No ☐

2. Lifestyle Habits

Smoking
a. Do you currently smoke? Yes ☐ No ☐
If yes, what do you smoke and how much per day
b. Are you a previous smoker? Yes ☐ No ☐
If yes, how long is it since you stopped and how much did you smoke?
**Drinking**

c. Do you drink any alcohol? Yes [ ] No [ ]  
If yes, do you have?  
- An occasional drink [ ]  
- A drink every day [ ]  
- More than one drink a day [ ]

d. Do you drink any caffeinated drinks? Yes [ ] No [ ]  
If yes, how many cups/glasses a day?  
- Coffee [ ]  
- Tea [ ]  
- Soft Drinks [ ]

**Physical Activity**

e. How would you describe your occupational activity level?  
- Sedentary [ ]  
- Light [ ]  
- Moderate [ ]  
- Heavy [ ]

f. Do you currently engage in any physical activity? Yes [ ] No [ ]  
If yes, what type?  
On average: How often? [ ] times/week For How long? [ ] time/session

**If blood samples are to be taken, please answer the following questions:**

a) Are you suffering from any known serious infection? Yes [ ] No [ ]
b) Have you had jaundice within the previous year? Yes [ ] No [ ]
c) Have you ever had any form of hepatitis? Yes [ ] No [ ]
d) Are you HIV positive? Yes [ ] No [ ]
e) Have you had unprotected sexual intercourse with any person from an HIV high risk population? Yes [ ] No [ ]
f) Have you ever been involved in intravenous drug use? Yes [ ] No [ ]
g) Are you haemophiliac? Yes [ ] No [ ]

*If the answer to any of the above is Yes then please discuss the nature of the problem.*
3. Future Goals

a. How important is your health to you?

Not at all □ Slightly □ Moderately □ Quite a bit □ Extremely □

b. What else is important?

Social Life □ Family □ Work □ Other - please specify □

Other - please specify □

c. What are your main reasons for attending the session?

Feel healthier □ Fitness □ Weight loss □

Look better □ Manage my illness □ Other - please specify □

Other - please specify □

d. What personal health and wellness goals would you like to achieve within the next 6 months?

______________________________________________________________

e. What do you feel will affect your ability to achieve this?

______________________________________________________________

______________________________________________________________

Signature: ___________________________ Date: __________________

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CONSENT FORM
Version 3: 12th October 2009

Effects of a lifestyle intervention on exercise capacity, quality of life, body mass/composition
and cardiovascular risk in patients being treated for obstructive sleep apnoea

Please initial box

1. I confirm that I have read and understand the information sheet dated ......................... for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I give permission for my GP to be informed of my participation in this research.

5. I agree to take part in the above study.

_________________________  ______________________  ___________________
Name of Patient            Date                    Signature

_________________________  ______________________  ___________________
Name of Person taking consent Date                    Signature
(if different from researcher)

_________________________  ______________________  ___________________
Researcher                        Date                    Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes
### OSA STUDY - HOME EXERCISE SESSION(S)
#### DATA COLLECTION SHEET (WEEKS 5-12)

<table>
<thead>
<tr>
<th>Activity (walking, swimming etc)</th>
<th>Suggested Exercise</th>
<th>Achieved Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity (RPE Scale)</td>
<td></td>
<td></td>
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</tbody>
</table>

1. HOME SESSION(S)

<table>
<thead>
<tr>
<th>DATE .............</th>
<th>TIME .............</th>
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</table>

**Intensity - Borg's RPE Scale**

6
7 Very, very light
8
9 Very light
10
11 Fairly Light
12
13 Somewhat hard
14
<table>
<thead>
<tr>
<th></th>
<th>Activity</th>
<th>Type of Exercise:</th>
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<tbody>
<tr>
<td></td>
<td>Monday</td>
<td>Duration:</td>
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<td></td>
<td></td>
<td>Intensity (RPE):</td>
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<td></td>
<td>Tuesday</td>
<td>Type of Exercise:</td>
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<td></td>
<td></td>
<td>Duration:</td>
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<td></td>
<td></td>
<td>Intensity (RPE):</td>
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<td>Wednesday</td>
<td>Type of Exercise:</td>
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<td>Duration:</td>
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<td></td>
<td>Intensity (RPE):</td>
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<td></td>
<td>Thursday</td>
<td>Type of Exercise:</td>
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<td>Duration:</td>
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<td></td>
<td></td>
<td>Intensity (RPE):</td>
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<td></td>
<td>Friday</td>
<td>Type of Exercise:</td>
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<td></td>
<td></td>
<td>Duration:</td>
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<td></td>
<td></td>
<td>Intensity (RPE):</td>
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<tr>
<td></td>
<td>Saturday</td>
<td>Type of Exercise:</td>
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<td>Duration:</td>
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<td>Intensity (RPE):</td>
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<td>Sunday</td>
<td>Type of Exercise:</td>
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<td>Duration:</td>
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<tr>
<td></td>
<td></td>
<td>Intensity (RPE):</td>
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</table>
Three Day Diet Diary

It is important that you note down everything that you eat and drink in the three days covered by this diary, including things consumed away from home, e.g. snacks or alcohol. This is important because if items are missed out the resulting analysis will not produce a true picture of your diet and we will not be able to compare it to the other diaries you will complete over the course of this study. Try to be as honest as possible, your responses will not be shown to the group.

When recording items in the diary you should enter the type of food or drink consumed and approximate quantity, e.g. two slices of toast or a can of coke. Where possible try to list the different food items separately and in as much detail as possible, e.g. two slices of toast with a thin layer of butter and two teaspoonfuls of jam. Quantities can be reported as common measures where applicable, such as a cup/mug of tea or a spoonful of jam, otherwise please try and estimate the weight, e.g. 8oz steak. For ready meals, please note the pack weight and proportion consumed, e.g. half an 800g lasagne.

Also, please record the approximate time you consumed the item and whether it was a snack or part of a meal. This will help us build a picture of your daily eating pattern.

To save you from having to note down how you take your tea and coffee every time please complete the following two statements:

I usually drink my tea with milk / no milk and ___ teaspoonfuls of sugar
I usually drink my coffee with milk / no milk and ___ teaspoonfuls of sugar
<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Meal or Food Consumed</th>
<th>Quantity</th>
</tr>
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<tr>
<td>Weeks</td>
<td>Process of change</td>
<td>Exercise/diet counselling framework: Examples of skills and techniques to be used</td>
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<td>---------------</td>
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<td>----------------------------------------------------------------------------------</td>
<td></td>
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<tr>
<td>1-2 Cognitive</td>
<td>Consciousness raising</td>
<td><strong>a. Review first session:</strong></td>
<td></td>
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<tr>
<td></td>
<td>Dramatic relief</td>
<td>• How did it feel? Was it difficult/easy?</td>
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<tr>
<td></td>
<td>Decisional balance</td>
<td>• Did you enjoy it?</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Importance of exercise, why do we need to warm-up and cool-down</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Heart rate monitoring, what to wear, what and when to drink</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• What to expect in coming weeks</td>
<td></td>
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<td></td>
<td></td>
<td>• Any questions</td>
<td></td>
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<td></td>
<td><strong>b. Healthy eating</strong></td>
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<td></td>
<td></td>
<td>• What is it? (refer to The Eatwell Plate)</td>
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<td></td>
<td></td>
<td>• When should I eat? (breakfast, lunch and dinner + mid-morning/afternoon</td>
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<td></td>
<td></td>
<td>drinks/snacks)</td>
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<td></td>
<td></td>
<td>• What types of foods are good/not so good? (refer to The Eatwell Plate)</td>
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<td></td>
<td></td>
<td>• Hand out standard dietary information leaflets (BHF So you want to lose</td>
<td></td>
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<td></td>
<td></td>
<td>weight... for good; 5-a-day; FSA EatWell)</td>
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<td></td>
<td><strong>c. Benefits of exercise</strong></td>
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<td></td>
<td></td>
<td>• How often?</td>
<td></td>
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<td></td>
<td>• How hard?</td>
<td></td>
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<td></td>
<td>• Where and when?</td>
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<td></td>
<td></td>
<td>• Hand out BHF '30 mins a day any way' leaflet</td>
<td></td>
</tr>
</tbody>
</table>

218
<table>
<thead>
<tr>
<th>Weeks</th>
<th>Process of change</th>
<th>Exercise/diet counselling framework: Examples of skills and techniques to be used</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4 Cognitive</td>
<td>Self re-evaluation</td>
<td>d. Which physical exercise do I prefer?</td>
</tr>
<tr>
<td></td>
<td>Decisional balance</td>
<td>- Previous exercise experiences, why this worked/failed</td>
</tr>
<tr>
<td></td>
<td>Consciousness raising</td>
<td>- What other exercises might you like to try?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. Do you know?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Benefits of exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Importance of healthy eating</td>
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<tr>
<td></td>
<td></td>
<td>f. Are you enjoying the sessions?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- What do you like?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- What do you dislike?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- What would you change?</td>
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<tr>
<td></td>
<td></td>
<td>- Is it what you had expected?</td>
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<tr>
<td></td>
<td></td>
<td>g. Active and healthy living</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Food groups, choices, portion sizes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Importance of breakfast</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Review The Eatwell Plate</td>
</tr>
</tbody>
</table>

219
<table>
<thead>
<tr>
<th>Weeks</th>
<th>Process of change</th>
<th>Exercise/diet counselling framework: Examples of skills and techniques to be used</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-6 Cognitive and Behavioural</td>
<td>Self re-evaluation</td>
<td>h. Evaluate sessions so far</td>
</tr>
<tr>
<td></td>
<td>Goal setting/self-regulation</td>
<td>• How do you feel about exercise now?</td>
</tr>
<tr>
<td></td>
<td>Social support</td>
<td>• How comfortable do you feel exercising?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• What exercises do you enjoy the most?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i. Introduce goal setting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Set one SMART exercise goal and one SMART healthy eating goal each week -</td>
</tr>
<tr>
<td></td>
<td></td>
<td>record these in BHF weight loss leaflet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Process versus outcome goals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Agree a non-food based reward for week 8 if goals are achieved or if measurable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>progress has been made</td>
</tr>
<tr>
<td></td>
<td></td>
<td>j. Finding support for exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thinking of others who might encourage participation in exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Finding someone to talk to when exercising is difficult</td>
</tr>
<tr>
<td>Weeks</td>
<td>Process of change</td>
<td>Exercise/diet counselling framework: Examples of skills and techniques to be used</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7-8 Behavioural</td>
<td>Goal setting/self-regulation</td>
<td>k. Review goals</td>
</tr>
<tr>
<td></td>
<td>Stimulus control</td>
<td>• Did you achieve them?</td>
</tr>
<tr>
<td></td>
<td>Reinforcement management and self-liberation</td>
<td>• If yes then well done!</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If not then why not? What can we do to help change this?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>l. Cues for action</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thinking of tasks that might prompt participation in exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>m. Thinking about moving on from the programme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Home-programme goals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Future exercise options</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n. Looking and planning ahead. SWOT analysis</td>
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<tr>
<td></td>
<td></td>
<td>• What will help me to exercise in the future?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• What will stop me?</td>
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<tr>
<td></td>
<td></td>
<td>o. What have I achieved so far?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Review exercise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Review healthy eating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• What do I want to achieve from here?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thinking positively and taking positive action</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• What has been learned?</td>
</tr>
</tbody>
</table>

221
Health Questionnaire

*English version for the UK*

*(validated for Ireland)*
By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

**Mobility**
- I have no problems in walking about
- I have some problems in walking about
- I am confined to bed

**Self-Care**
- I have no problems with self-care
- I have some problems washing or dressing myself
- I am unable to wash or dress myself

**Usual Activities** *(e.g. work, study, housework, family or leisure activities)*
- I have no problems with performing my usual activities
- I have some problems with performing my usual activities
- I am unable to perform my usual activities

**Pain/Discomfort**
- I have no pain or discomfort
- I have moderate pain or discomfort
- I have extreme pain or discomfort

**Anxiety/Depression**
- I am not anxious or depressed
- I am moderately anxious or depressed
- I am extremely anxious or depressed
To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.
Effects of obstructive sleep apnoea and its treatment on macrovascular and microvascular function

Dear ........................................

We are undertaking a research project to investigate the effects of obstructive sleep apnoea and its treatment on blood vessel function. Obstructive sleep apnoea is a medical condition causing people to stop breathing at regular intervals during sleep. This puts significant strain on the heart and the blood vessels. Please find enclosed a participant information sheet which describes the study in detail and answers the most frequently asked questions.

Your participation in the study would involve being part of a sleep apnoea group, which we will compare to two other groups of patients with sleep apnoea and a control group. The study involves two visits to our department, at times to suit you. Parking permits can be arranged if necessary. It is important to note that there is no pressure to participate in this study. Please contact the research team to let them know if you are interested in taking part, or do not wish to be contacted again. Please telephone James Moss on 0114 225 2262 or 07738 240670 or return the reply slip below in the enclosed freepost envelope before ........................................

Yours sincerely,

Dr Stephen Bianchi
Consultant Physician in Respiratory Medicine
Northern General Hospital

SLEEP APNOEA RESEARCH STUDY

☐ Yes. I am interested in taking part in the above named study. I understand that a member of staff will be contacting me, regarding this study.

☐ No, I am not interested in taking part in the study

Name: .................................................................

Telephone Number: ....................................................
EFFECTS OF OBSTRUCTIVE SLEEP APNOEA AND ITS TREATMENT ON MACROVASCULAR AND MICROVASCULAR FUNCTION

Invitation
We would like to invite you to take part in a research study. Before you decide, we need you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

What is the purpose of the project?
Obstructive sleep apnoea (OSA) is a common breathing disorder characterised by repeated episodes of upper airway collapse during sleep, which results in elevated blood pressure, sleep disturbance and increased risk of heart attack and stroke. To reduce risk and improve sleep quality, patients are usually treated with continuous positive airway pressure (CPAP), which involves wearing a special face mask during the night to help keep the airways open. CPAP should be used every night for sleeping, however, not all patients use their mask on a daily basis. Therefore, the purpose of this study is to investigate how well occasional CPAP use protects the cardiovascular system of OSA patients compared to daily use and to no use.

Why have I been invited?
You have been invited either because you have previously shown an interest in sleep apnoea research at Sheffield Hallam University or because you have been identified as potentially suitable from a recent OSA annual check.

Do I have to take part?
It is up to you to decide whether or not to be involved with this research. If you do decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you
decide to take part you are still free to withdraw at any time without giving a reason. Your future care will not be affected if you choose not to take part and you will not be contacted again by our research office.

**What will happen to me if I agree to take part?**
Your involvement in this study will involve one or two visits to the research facility at Sheffield Hallam University (Centre for Sport and Exercise Science, Collegiate Hall, Collegiate Crescent Campus, just off Ecclesall Road). These visits can be scheduled at a time to suit you.

**Are there any expenses or payments involved?**
Unfortunately we are not able to offer any payment or financial support for visits, however we can arrange a free permit for on-site parking should you choose to drive.

**What will the visit to Sheffield Hallam University involve?**
During your first visit to Sheffield Hallam University, a study researcher will take a medical history to assess your eligibility for this study. After this we will demonstrate or explain all of the main testing procedures that you will be required to complete as part of this study. If you are deemed provisionally eligible, and you are still interested in participating, then you will be asked to sign a consent form. Final eligibility will be determined by a consultant respiratory physician. For this session, you will need to bring a list of all the medications that you taking.

This session will be performed in the morning, after you have completed an overnight fast (no food) and avoided products containing tobacco and caffeine for at least 6 hours. At the beginning of this session, we will use simple non-invasive techniques to measure your height, weight, body fat percentage, and neck, waist and hip circumferences.

After this, we will assess your large blood vessel function using ultrasound. This test involves measuring the change in size of the largest blood vessel in your upper arm in response to an increase in arm blood flow. The increase in arm blood flow is produced by placing a blood pressure cuff on your upper forearm and inflating it to a high pressure for 5 minutes. You may experience some pins and needles in your hand and fingers during this procedure, but this will go away when the pressure is removed. We will also attach two non-invasive probes to the surface of the forearm using double-sided tape. These probes will non-invasively measure skin blood flow before, during, and after the cuff inflation. This assessment will last approximately 15 minutes.

After a recovery period, we will heat the probes to 42 °C (the temperature of bath-water) and measure changes in skin blood flow. This may be uncomfortable, but not painful, and after a couple of minutes the sensation will feel much milder. This assessment will last approximately 45 minutes.
After another recovery period, the response of the blood vessel to glyceryl trinitrate (GTN) will be measured. GTN is taken as a spray under the tongue and occasionally causes short-term (less than 10 minutes) feelings of light-headedness.

After a suitable recovery period we will then take a couple of drops of blood from the fleshy part of the fingertip. This procedure may involve a moment of discomfort when the surface of the skin is broken.

Finally, we will ask you to complete a shuttle-walk test. During this test you will be asked to walk up and down a 10 metre (11 yards) course. The walking speed will start slow and increase slightly each minute. The test will end when either you are too breathless to continue, you fail to complete two consecutive shuttles within the time allowed, or if you achieve 85% of your maximum calculated heart rate.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence, except if there is an issue of safety, in which case we will let your GP know, as well as yourself. Some parts of your medical records and the data collected for the study will be looked at by only authorised persons from the research team.

**What will happen if I don't want to carry on with the study?**

You can withdraw from the study at any time and, if necessary, let us know if you no longer want to be contacted. If you do wish to drop out, you do not have to give a reason. Information collected may still be used.

**What if there is a problem?**

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and you are not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns, please contact the study manager, James Moss on (0114) 225 2262

OR

You can use the normal University complaints procedure and contact the following person:

Dr. Liz Winders, ‘Registrar and Secretary’ of Sheffield Hallam University, telephone (0114 225 2051)

OR
You can use the normal hospital complaints procedure and contact Professor Chris Welsh, Medical Director, Sheffield Teaching Hospitals NHS Foundation Trust, Tel 0114 271 2178

**Why do you want to tell my GP that I am taking part in the study?**
If you take part in any clinical trial then the research team is required to inform your GP, this will also make sure your GP is aware of anything you might do in the study that could potentially influence your medical care. However, your GP will not be given any of the information we collect from you.

**What are the possible risks and benefits of taking part in this research?**
The tests in which you have a blood pressure cuff inflated on your arm will probably cause some slight discomfort and feelings of pins and needles in your hand and fingers. These feelings are usually mild and they soon go away once the cuff is deflated. Taking GTN can sometimes make people feel light headed or nauseous for around 10 minutes afterwards. The risk of this procedure is low. You will be lying down and closely monitored during this procedure.

**What if I am harmed?**
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action, but you may have to pay for it.

**What will happen to the results of the research study?**
This research will take place over the next year, after which the results will be presented at academic conferences and published as academic reports in scientific journals. You will not be identified in any presentation, report or publication. You will be provided with an overview of the main study findings.

**Who is organising and funding the research?**
The research is organised by Sheffield Hallam University in collaboration with Sheffield Teaching Hospitals NHS Foundation Trust.

**Who has reviewed the study?**
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favourable opinion by Sheffield Research Ethics Committee.
Contact for further information:
For study specific information, please contact James Moss, Centre for Sport and Exercise Science, Sheffield Hallam University, Sheffield, S10 2BP, Tel: 0114 2252262
For general enquiries about taking part in research at Sheffield Hallam University, please contact Stephen Gilbert, Centre for Sport and Exercise Science, Sheffield Hallam University, Sheffield, S10 2BP, Tel: 0114 2255413

THIS INFORMATION SHEET IS FOR YOU TO KEEP. THANK YOU FOR YOUR TIME AND HELP.
Effects of obstructive sleep apnoea and its treatment on macrovascular and microvascular function

Dear ........................................

We are undertaking a research project to investigate the effects of obstructive sleep apnoea and its treatment on blood vessel function. Obstructive sleep apnoea is a medical condition causing people to stop breathing at regular intervals during sleep. This puts significant strain on the heart and the blood vessels. Please find enclosed a participant information sheet which describes the study in detail and answers the most frequently asked questions.

Your participation in the study would involve being part of the control group, which we will compare to three groups of patients with sleep apnoea. The study involves two visits to our department, at times to suit you. Parking permits can be arranged if necessary. It is important to note that there is no pressure to participate in this study. Please contact the research team to let them know if you are interested in taking part, or do not wish to be contacted again. Please telephone James Moss on 0114 225 2262 or 07738 240670 or return the reply slip below in the enclosed freepost envelope before ........................................

Yours sincerely,

Dr Stephen Bianchi
Consultant Physician in Respiratory Medicine
Northern General Hospital

SLEEP APNOEA research study

☐ Yes. I am interested in taking part in the above named study. I understand that a member of staff will be contacting me, regarding this study.

☐ No, I am not interested in taking part in the study

Name: .............................................................................................................

Telephone Number: ..................................................................................
EFFECTS OF OBSTRUCTIVE SLEEP APNOEA AND ITS TREATMENT ON MACROVASCULAR AND MICROVASCULAR FUNCTION

Invitation
We would like to invite you to take part in a research study. Before you decide, we need you to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

What is the purpose of the project?
Obstructive sleep apnoea (OSA) is a common breathing disorder characterised by repeated episodes of upper airway collapse during sleep, which results in elevated blood pressure, sleep disturbance and increased risk of heart attack and stroke. To reduce risk and improve sleep quality, patients are usually treated with continuous positive airway pressure (CPAP), which involves wearing a special face mask during the night to help keep the airways open. CPAP should be used every night for sleeping, however, not all patients use their mask on a daily basis. Therefore, the purpose of this study is to investigate the how well occasional CPAP use protects the cardiovascular system of OSA patients compared to regular use and to no use.

Why have I been invited?
You may have been invited because you have shown an interest in this research by contacting a member of the research team or because you have shown an interest or participated in our research in the past.

Do I have to take part?
It is up to you to decide whether or not to be involved with this research. If you do decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you
decide to take part you are still free to withdraw at any time without giving a reason. Your future care will not be affected if you choose not to take part and you will not be contacted again by our research office.

**What will happen to me if I agree to take part?**

Your involvement in this study will involve two visits to the research facility at Sheffield Hallam University (Centre for Sport and Exercise Science, Collegiate Hall, Collegiate Crescent Campus, just off Ecclesall Road). These visits can be scheduled at a time to suit you.

**Are there any expenses or payments involved?**

Unfortunately we are not able to offer any payment or financial support for visits, however we can arrange a free permit for on-site parking should you choose to drive.

**What will the visit to Sheffield Hallam University involve?**

*Visit 1: Medical screening, habituation and preparation for home sleep study (45 minutes)*

During your first visit to Sheffield Hallam University, you will undergo a brief medical assessment to assess your eligibility for this study. After this we will demonstrate or explain all of the main testing procedures that you will be required to complete as part of this study. If you are deemed eligible, and you are still interested in participating, then you will be asked to sign a consent form. For this session, you will need to bring a list of all the medications that you taking.

You will be required to undergo a home-based, overnight sleep test. This device will record your breathing patterns and measure fingertip oxygen levels during sleep. You will be shown how to set this up.

If the sleep test suggests the presence of an undiagnosed sleep disorder we will, with your permission, notify you GP and allow them to decide the best course of action. You will be reminded of your responsibility to drive in accordance with the Road Traffic Act (1988) which stipulates that drivers should not drive when they are excessively tired.

This first visit to our facility should last around 45 minutes.

*Visit 2: Blood vessel function tests (~2.5 hours)*

This session will be performed in the morning, after you have completed an overnight fast (no food for 12 hours) and avoided products containing tobacco and caffeine for at least 6 hours. At the beginning of this session, we will use simple non-invasive techniques to measure your height, weight, body fat percentage, and neck, waist and hip circumferences.

After this, we will assess your large blood vessel function using ultrasound. This test involves measuring the change in size of the largest blood vessel in your upper arm in response to an increase in arm blood flow. The increase in arm blood flow is produced by placing a cuff on your
upper forearm and inflating it to a high pressure for 5 minutes. You may experience some pins and needles in your hand and fingers during this procedure, but this will go away when the pressure is removed. We will also attach two non invasive probes to the surface of the forearm using double-sided tape. These probes will measure skin blood flow before, during, and after the cuff inflation. This assessment will last approximately 15 minutes.

After a recovery period, we will heat the probes to 42 °C (the temperature of bath-water) and measure changes in skin blood flow. This may be uncomfortable, but not painful, and after a couple of minutes the sensation will feel much milder. This assessment will last approximately 45 minutes.

After another recovery period, the response of the blood vessel to glyceryl trinitrate (GTN) will be measured. GTN is taken as a spray under the tongue and occasionally causes short-term (less than 10 minutes) feelings of light-headedness.

After a suitable recovery period we will then take a couple of drops of blood from the fleshy part of the fingertip. This procedure may involve a moment of discomfort when the surface of the skin is broken.

Finally, we will ask you to complete a shuttle-walk test. During this test you will be asked to walk up and down a 10 metre (11 yards) course. The walking speed will start slow and increase slightly each minute. The test will end when you are too breathless to continue, you fail to complete two consecutive shuttles within the time allowed, or if you achieve 85% of your maximum calculated heart rate.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence, except if there is an issue of safety, in which case we will let your GP know, as well as yourself. Some parts of your medical records and the data collected for the study will be looked at by only authorised persons from the research team.

What will happen if I don’t want to carry on with the study?
You can withdraw from the study at any time and, if necessary, let us know if you no longer want to be contacted. If you do wish to drop out, you do not have to give a reason. Information collected may still be used.

What if there is a problem?
If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and you are not compromised in any way because you have taken part in a research study.
If you have any complaints or concerns, please contact the study manager, James Moss on (0114) 225 2262

OR

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OR

You can use the normal hospital complaints procedure and contact Professor Chris Welsh, Medical Director, Sheffield Teaching Hospitals NHS Foundation Trust, Tel 0114 271 2178

**Why do you want to tell my GP that I am taking part in the study?**

If you take part in any clinical trial then the research team is required to inform your GP, this will also make sure your GP is aware of anything you might do in the study that could potentially influence your medical care. However, your GP will not be given any of the information we collect from you.

**What are the possible risks and benefits of taking part in this research?**

The tests in which you have a blood pressure cuff inflated on your arm will probably cause some slight discomfort and feelings of pins and needles in your hand and fingers. These feelings are usually mild and they soon go away once the cuff is deflated. Taking GTN can sometimes make people feel light headed or nauseous for around 10 minutes afterwards. The risk of this procedure is low. You will be lying down and closely monitored during this procedure.

Part of the screening procedure involves overnight testing for sleep apnoea. This is a common but underdiagnosed medical condition which can put you at higher risk for cardiovascular disease. Routine screening is uncommon without GP referral - if we diagnose this condition we may refer you to a specialist at Northern General Hospital.

**What if I am harmed?**

If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for legal action, but you may have to pay for it.

**What will happen to the results of the research study?**

This research will take place over the next year, after which the results will be presented at academic conferences and published as academic reports in scientific journals. You will not be identified in any presentation, report or publication. You will be provided with an overview of the main study findings.
Who is organising and funding the research?
The research is organised by Sheffield Hallam University in collaboration with Sheffield Teaching Hospitals NHS Foundation Trust.

Who has reviewed the study?
All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given a favourable opinion by Sheffield Research Ethics Committee.

Contact for further information:
For study specific information, please contact James Moss, Centre for Sport and Exercise Science, Sheffield Hallam University, Sheffield, S10 2BP, Tel: 0114 2252262
For general enquiries about taking part in research at Sheffield Hallam University, please contact Stephen Gilbert, Centre for Sport and Exercise Science, Sheffield Hallam University, Sheffield, S10 2BP, Tel: 0114 2255413

THIS INFORMATION SHEET IS FOR YOU TO KEEP. THANK YOU FOR YOUR TIME AND HELP.
Appendix 13

Sheffield Teaching Hospitals
NHS Foundation Trust
Sheffield Hallam University

Consent form
VERSION 1: 14th April 2011

Effects of obstructive sleep apnoea and its treatment on macrovascular and microvascular function

Please initial box

1. I confirm that I have read and understood the information sheet dated .................. for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I give permission for my GP to be informed of my participation in this research.

5. I agree to take part in the above study.

Name of Patient Date Signature

Name of Person taking consent Date Signature (if different from researcher)

Researcher Date Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

237
Consent form

Effects of obstructive sleep apnoea and its treatment on macrovascular and microvascular function

Please initial box

1. I confirm that I have read and understood the information sheet dated ........................................ for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I give permission for my GP to be informed of my participation in this research.

5. I give permission for an overnight sleep study to be performed to investigate the possibility of existing undiagnosed sleep disordered breathing/obstructive sleep apnoea.

6. I give permission for the research team to notify my GP of the result of the sleep study if it suggests that further investigations may be required.

7. I am aware that should a positive diagnosis of sleep-disordered breathing/obstructive sleep apnoea be made that it is my responsibility to ensure I drive in accordance with the Road Traffic Act (1988) and that further investigations may be required.

8. I agree to take part in the above study.

Name of Patient __________________________ Date __________ Signature __________________________

Researcher __________________________ Date __________ Signature __________________________
## Epworth Sleepiness Scale

The Epworth Sleepiness Scale is used to determine the level of daytime sleepiness. Use the following scale to choose the most appropriate number for each situation over the past two weeks. Even if you don’t usually do this activity, please give your best estimate:

0 = would *never* doze or sleep.
1 = slight chance of dozing or sleeping
2 = *moderate* chance of dozing or sleeping
3 = *high* chance of dozing or sleeping

Name: ____________________________ Date: ____________________

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of Dozing or Sleeping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting inactive in a public place</td>
<td></td>
</tr>
<tr>
<td>Being a passenger in a motor vehicle for an hour or more</td>
<td></td>
</tr>
<tr>
<td>Lying down in the afternoon</td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after lunch (no alcohol)</td>
<td></td>
</tr>
<tr>
<td>Stopped for a few minutes in traffic</td>
<td></td>
</tr>
</tbody>
</table>

**Total score**

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239
Health Questionnaire

*English version for the UK*

*(validated for Ireland)*
By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

**Mobility**
I have no problems in walking about □
I have slight problems in walking about □
I have moderate problems in walking about □
I have severe problems in walking about □
I am unable to walk about □

**Self-Care**
I have no problems with self-care □
I have slight problems washing or dressing myself □
I have some problems washing or dressing myself □
I have severe problems washing or dressing myself □
I am unable to wash or dress myself □

**Usual Activities** (e.g. work, study, housework, family or leisure activities)
I have no problems with performing my usual activities □
I have slight problems with performing my usual activities □
I have some problems with performing my usual activities □
I have severe problems with performing my usual activities □
I am unable to perform my usual activities □

**Pain/Discomfort**
I have no pain or discomfort □
I have slight pain or discomfort □
I have moderate pain or discomfort □
I have severe pain or discomfort □
I have extreme pain or discomfort □

**Anxiety/Depression**
I am not anxious or depressed □
I am slightly anxious or depressed □
I am moderately anxious or depressed □
I am severely anxious or depressed □
I am extremely anxious or depressed □
To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.