DOCTOR OF PHILOSOPHY

Factors in Obstructive Sleep Apnoea: Chemosensitivity and Perception of Effort before and after loading of respiratory and locomotor systems

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FACTORS IN OBSTRUCTIVE SLEEP APNOEA:

CHEMOSENSITIVITY AND PERCEPTION OF EFFORT BEFORE AND AFTER LOADING OF RESPIRATORY AND LOCOMOTOR SYSTEMS

By

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DECLARATION

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

SUMMARY

Obstructive Sleep Apnoea (OSA) is a breathing disorder characterised by repeated episodes of narrowing or complete collapse of the upper respiratory airways during sleep. The condition is associated with chronic intermittent hypoxia and overloading of the inspiratory muscles during sleep. Consequently, there is a need to better understand the impact OSA has upon the chemosensitivity and perceptual response to muscle loading.

The central and peripheral chemoreceptors (i.e. ventilatory response to hypoxia and hypercapnia) was examined during wakefulness in OSA patients and endurance-trained (VO_{2max} >55 ml·kg·min^{-1}) and untrained individuals (VO_{2max} <40 ml·kg·min^{-1}). Central and peripheral chemosensitivity was re-assessed in OSA patients after receiving Continuous Positive Airway Pressure (CPAP) treatment. The perception of effort and activation of the locomotor muscles was examined before and after loading in Trained, Untrained and OSA groups. The perception of effort and activation of the inspiratory muscles was also examined before and after loading Trained, Untrained and OSA groups. Follow-up assessments were conducted to examine the impact of CPAP treatment on perception of effort before and after inspiratory loading in OSA patients.

In chapter 4 patients with newly diagnosed OSA were shown to have an attenuated ventilatory response to hypoxia and hypercapnia (reduced central and peripheral chemosensitivity) compared with Trained and Untrained individuals. Body characteristics and cardiovascular fitness were found to explain the differential alterations in central and peripheral chemosensitivity. The ventilatory response to hypoxia and hypercapnia was unchanged following 3-months of CPAP treatment in OSA patients.

In chapter 5 patients with newly diagnosed OSA were shown to generate higher relative forces before loading compared with healthy individuals. The relative Vastus Medialis and Lateralis Electromyography (EMG) amplitudes were increased during loading in healthy and OSA groups. Repeated loading of the knee extensor muscles at 50% of Maximal Voluntary Contraction (MVC) resulted in lower relative forces being produced at a rating of perceived exertion (RPE) of 14 in OSA patients. The relative Vastus Medialis and Lateralis EMG amplitudes were increased at RPE14 following loading in OSA patients.

In chapter 6 patients with newly diagnosed OSA were shown to generate similar relative inspiratory pressures to healthy individuals before loading. The relative Intercostal EMG amplitude was increased during loading in healthy and OSA participants. Repeated bouts of inspiratory loading at 50% of Maximal Inspiratory Pressure (PI_{max}) resulted in higher relative pressures being generated at RPE14 in OSA patients. The relative Trapezius EMG amplitude was increased at RPE14 following loading in OSA patients compared with Trained individuals. The relative pressures generated at the reference point were increased after 3-months of CPAP therapy in OSA patients.

Central and peripheral chemosensitivity was attenuated in OSA patients and unchanged following 3 months of CPAP treatment. The perception of effort and muscle recruitment were impaired following loading of the inspiratory and skeletal muscles in OSA patients. CPAP treatment modified the perception of inspiratory effort at rest. These outcomes indicate OSA patients demonstrate a generalised neuromuscular impairment that is observed across a number of respiratory muscles and modified to some extent following CPAP treatment.

Key words: **Obstructive Sleep Apnoea, Chemosensitivity, Perception of effort, Electromyography, Continuous Positive Airway Pressure.**

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PUBLICATIONS

I was involved in all aspects of protocol design, data collection, data analyses and preparation of the following thesis chapters. I gratefully acknowledge input from the other named authors for the publication. The following is a list of publications arising from the material presented in this thesis.

Full papers

Earing, C.M.N., Owen, J., Griffith-Mcgeever, C., McKeon, D., Engeli, S., Moore, J. and Kubis, H-P. (2019). An act of balance: Interaction of central and peripheral chemosensitivity with inflammatory and anti-inflammatory factors in obstructive sleep apnoea. *Respiratory Physiology and Neurobiology*, 266, 73-81.

Conference communications

Griffith-Mcgeever, C., Earing, C.M.N., Owen, J., McKeon, D., Engeli, S., Moore, J. and Kubis, H-P. (2019). Determinants of apnoea–hypopnoea-index (AHI) levels in newly diagnosed obstructive sleep apnoea patients (Poster presentation). *Europhysiology 2018, London, 14-16th September.*

The publication and conference communication reported above included the physical characteristics and chemosensitivity data of healthy individuals that were recruited for the present thesis (Chapter 4). Whilst, the chemosensitivity data, physical characteristics, and markers of inflammation were previously measured in OSA patients as part of Dr Christopher Earing's thesis.

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ABBREVIATIONS

AHI	Apnoea Hypopnea Index
ANOVA	Analysis of variance
APAP	Automatic-titrating Positive Airway Pressure
BF	Body Fat
BiPAP	Bilevel Positive Airway Pressure
bpm	Beats per minute
BMI	Body Mass Index
BSA	Body Surface Area
CCHS	Congenital Central Hypoventilation Syndrome
cm	Centimetre
cmH_2O	Centimetre of water
CPG	Central Pattern Generator
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CO_2	Carbon Dioxide
CPAP	Continuous Positive Airway Pressure
DRN	Dorsal Raphe Nuclei
EMG	Electromyogram
EPAP	Expiratory Positive Airway Pressure
ESS	Epworth Sleepiness Scale
FEV_1	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity

Hz	Hertz
ICC	Intraclass Correlation Coefficient
$IF_{14reference}$	Isometric Force at RPE 14 (reference)
$IF_{14loading}$	Isometric Force at RPE 14 (loading)
$IP_{14reference}$	Inspiratory Pressure at RPE 14 (reference)
$IP_{14loading}$	Inspiratory Pressure at RPE 14 (loading)
IPAP	Inspiratory Positive Airway Pressure
INT	Intercostal
kHz	kiloHertz
kg	Kilogram
m	Metre
ms	Milliseconds
MVC	Maximal Voluntary Contraction
NTS	Nucleus Tract Solitarius
OSA	Obstructive Sleep Apnoea
O_2	Oxygen
PaO_2	Partial pressure of Oxygen
$PaCO_2$	Partial pressure of Carbon Dioxide
P_{crit}	Closing critical pressure
P_{ds}	Pressure downstream
pH	Power of hydrogen
PI_{max}	Maximal Inspiratory Pressure
PFT	Pulmonary Function Test

PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
P_{us}	Upstream pressure
RCCP	Registration Council for Clinical Physiologists
RDI	Respiratory Disturbance Index
RER	Respiratory Exchange Ratio
RERA	Respiratory Effort Related Arousal
RMS	Root Mean Square
RPE	Ratings of perceived exertion
RPM	Revolutions per minute
RTS	Retrotrapezoid nucleus
SD	Standard Deviation
SEM	Standard Error of the Mean
SpO_2	Oxygen saturation
TRA	Trapezius
W	Wattage
VAS	Visual Analogue Scale
VL	Vastus Lateralis
VM	Vastus Medialis
°C	Degrees Celsius
% PI_{max}	Percentage of Maximal Inspiratory Pressure
% MVC	Percentage of Maximal Voluntary Capacity
% RMS	Percentage of Root Mean Square

GLOSSARY OF TERMS

Obstructive Sleep Apnoea:	Condition characterised by repeated partial (hypopnoea) or complete obstruction (apnoea) of the upper airway during sleep.
Continuous Positive Airway Pressure:	Treatment used to restore / reopen the upper airway during sleep. Reduces frequency of obstructive and central events.
Chemosensitivity:	Sensitivity of the central and peripheral chemoreceptors to hypoxia (O_2) and hypercapnia (CO_2). *Alternative terms used: Ventilatory control/stability and Controller gain.*
Perception of Effort:	The subjective intensity of effort, strain, discomfort, and/or fatigue that is experienced during physical exercise. *Alternative terms used: Effort perception, Exertion, and Effort sensation.*
Muscle Fatigue:	Reduced capacity of the neuromuscular system to generate force or perform work during exercise.
Electromyography:	Technique used to measure and evaluate the electrical activity of the working muscles.

THESIS FORMAT

The general introduction highlights the research investigating the ventilatory response to hypoxia and hypercapnia along with the impact of Continuous Positive Airway Pressure (CPAP) that is prescribed as the main treatment of Obstructive Sleep Apnoea (OSA). The research underpinning the perceptual and muscular response to loading in healthy and clinical populations will also be discussed **(Chapter 1)**. The literature review provides an overview of the research for each experimental chapter and states the research aims **(Chapter 2)**. A general methods chapter provides in-depth details of all procedures and analyses performed in the following experimental chapters **(Chapter 3)**. The first study is separated into two parts. The first part examined the ventilatory response to hypoxia and hypercapnia in Trained, Untrained, and OSA groups. The second part examined the impact of CPAP therapy on the ventilatory response to hypoxia and hypercapnia in OSA patients **(Chapter 4)**. The second study examined the perception of effort and activation of the locomotor muscles before and after loading in Trained, Untrained, and OSA groups **(Chapter 5)**. The third study is separated into two parts. The first part examined the perception of effort and activation of the inspiratory muscles before and after loading in Trained, Untrained, and OSA groups. The second part examined the impact of CPAP therapy on perception of effort before and after loading in OSA patients **(Chapter 6)**. The final chapter of the thesis provides a general discussion of the main findings with relevance to existing literature, applied implications, and future directions for the research **(Chapter 7)**.

CHAPTER ONE

General Introduction

Obstructive sleep apnoea (OSA) is a breathing disorder characterised by repeated episodes of narrowing (hypopnea) or collapse (apnoea) of the upper respiratory airways, that occur intermittently, and at regular intervals during sleep (Carter & Watenpaugh, 2008). Recurrent cessations of airflow result in periodic asphyxia, transient periods of hypoxemia, and hypercapnia (Halliwill & Minson, 2002; Mullington et al., 2009). Futile inspiratory efforts against the obstructed upper airway produce changes in intrathoracic pressure and dysregulates blood flow to the respiratory muscles (Remmers et al., 1978; Wiegand & Zwillich, 1994; Drager, Polotsky, & Lorenzi-Filho, 2011). The ventilatory disturbance and intermittent hypoxia result in sleep fragmentation and respiratory effort-related arousals (RERA) that attempt to re-establish the airway patency and normalise blood gases (Guilleminault, Tilkian, & Dement, 1976; Somers et al., 1995).

Central and peripheral chemosensitivity (i.e. ventilatory response to CO_2 and O_2) is thought to be decisive for the ventilatory instability observed in OSA. The chronic exposure to intermittent hypoxia during episodic sleep has been shown to influence the sensitivity of central and peripheral chemoreceptors during wakefulness in OSA patients (Littner et al., 1984; Mateika & Narwani, 2009; Narkiewicz et al., 1999; Osanai et al., 1999; Verbraecken et al., 1995). It has been proposed that intermittent exposure to hypoxia and hypercapnia during episodic sleep leads to a depression or progressive resetting of the receptors to a lower sensitivity threshold (Forster & Dempsey, 1981; Guilleminault & Cummiskey, 1982). However, the ventilatory response to hypoxia and hypercapnia has yet to be fully elucidated in OSA patients compared with Trained (VO_{2max} >55 ml·kg·min^{-1}) and Untrained individuals VO_{2max} <40 ml·kg·min^{-1}) (Astorino et al., 2012; Hassmén, 1990). Furthermore, the contribution of body characteristics and cardiovascular fitness to the hypoxic and hypercapnic ventilatory response is not fully understood. In addition, there is some evidence to suggest that Continuous Positive Airway Pressure (CPAP) treatment has the ability to modify the central and peripheral chemosensitivity during wakefulness in OSA patients (Lin, 1994; Tun et al., 2000). However, the effect of short-term CPAP treatment on the ventilatory response to hypoxia and hypercapnia in OSA patients is not yet confirmed.

Another important feature of the chronic condition is the ventilatory response to the occluded airway and subsequent obstructive event (Wiegand & Zwillich, 1994). Repeated inspiratory efforts against the occluded airway and the obstruction potentially leads to a chronic

overloading of the inspiratory muscles during sleep (Wilcox et al., 1990). The chronic overloading of inspiratory muscles during sleep is thought to lead to an increased risk of fatigue during rest and low-intensity exercise (Chien et al., 2013). The inspiratory activity could therefore be alternated between the diaphragm and inspiratory/accessory muscles in order to balance the work of breathing and minimise the development of fatigue (Fitting et al., 1988; Sharp et al., 1964; Yokoba et al., 2003). In contrast, the peripheral (lower extremity) muscles are considered to be underloaded as a consequence of progressive deconditioning observed within this population. The disuse of locomotor muscles is thought to result in changes to the muscle characteristics that promote muscle atrophy, weakness, and general dysfunction (Barreiro et al., 2007; Chien et al., 2010; Sauleda et al., 2003; Larsson et al., 2008). However, relatively few studies have addressed the perceptual alterations that accompany fatigue and/or dysfunction of the muscles in OSA patients. There is some evidence to suggest that OSA patients demonstrate an altered perceptual response to inspiratory loading (Earing, 2014; Tun et al., 2000). However, no study to date has examined the perceptual and muscular response to inspiratory and skeletal muscle loading in OSA patients compared with healthy individuals. Furthermore, the effect of CPAP therapy on the perceptual response to loading is relatively unknown in OSA patients.

The results of this thesis provide an opportunity for further exploration into the pathophysiological mechanisms of OSA with future outlooks of better treatment options for patients. Data will enable us to understand whether OSA is associated with peripheral and/or central alterations that are unique to the respiratory system or represent a more generalised impairment of the neuromuscular system.

CHAPTER TWO

Literature Review

2.1. Definition of Obstructive Sleep Apnoea (OSA)

Obstructive sleep apnoea (OSA) is characterised by episodes of narrowing (hypopnea) or collapse (apnoea) of the upper respiratory airways, that occur intermittently, and at regular intervals during sleep (Carter & Watenpaugh, 2008). Recurrent cessations of airflow result in periodic asphyxia, transient periods of hypoxemia, and hypercapnia (Halliwill & Minson, 2002; Mullington et al., 2009). Futile inspiratory efforts against the obstructed upper airway produce changes in intrathoracic pressure and dysregulates blood flow to the overloaded respiratory muscles (Remmers et al., 1978; Wiegand & Zwillich, 1994; Drager, Polotsky, & Lorenzi-Filho, 2011). The ventilatory disturbance and intermittent hypoxia result in sleep fragmentation and respiratory effort-related arousals (RERA) that attempt to re-establish the airway patency and normalise blood gases (Guilleminault, Tilkian, & Dement, 1976; Somers et al., 1995).

2.1.1 Prevalence of OSA

OSA is the most prevalent form of sleeping disorder in the UK with approximately 1.5 million adults or 3-7% of the general population known to be living with this chronic condition (Punjabi, 2008; Rejon-Parrilla, Garau, & Sussex, 2014). The population-based epidemiological study conducted in 1994 was the first to highlight what we know today, which is, OSA is widely unrecognised in the UK and consumption of healthcare resources is higher in this specific population. However, there are still conflicting reports regarding the prevalence of OSA (Lopez et al., 2008; Young, Peppard, & Gottlieb, 2002). The most recent health economics report by the British Lung Foundation have estimated 330,000 adults are currently diagnosed with and receive treatment for OSA in the UK (Rejon-Parrilla et al., 2014). However, the official figures are thought to be underestimated due to the general population's lack of understanding regarding the condition and its symptoms. Furthermore, many patients will often account the clinical features of OSA as part of other illnesses they may have or a consequence of ageing. Undiagnosed OSA patients are also generally reluctant to report symptoms for social and / or work-related reasons (Engleman et al., 1997). These recent reports and the lack of clarity behind the prevalence figures provide an even greater dilemma given that many individuals are unaware they are living with a chronic condition that poses such a risk for overall health.

2.1.2 Epidemiology of OSA

A subgroup of the general population is known to possess factors that place them at an advanced risk of developing OSA including: ageing, male sex, ethnicity, and genetics (Dempsey et al., 2010; Punjabi, 2008). However, obesity has long been recognised as the major contributor to OSA due to the increased deposition of adipose tissue situated within the pharyngeal walls resulting in external compression of the upper airways by surrounding fat mass (Horner et al., 1989; Koenig & Thach, 1988; Remmers et al., 1980). Anatomical abnormalities such as retrognathia, micrognathia, and tonsillar hypertrophy also play a key role in predisposing individuals to develop OSA by altering the position of the tongue and obstructing the pharyngeal airways (Deegan & McNicholas, 1995; Ryan & Bradley, 2005). The side effects of many health-related conditions such as hypothyroidism, menopause, oedema, and polycystic ovary syndrome further heighten the risk of individuals developing OSA due to effect of obesity (Vgontzas et al., 2001; Young et al., 1993; Zwillich et al., 1975). There is also a contributory role for health-related behaviours such as smoking, excessive alcohol consumption, and use of sedatives, hypnotics, and opioids (Krol, Knuth, & Bartlett, 1984; Leiter et al., 1985; Webster et al., 2008). Taken together, OSA poses a substantial risk to overall health in the Western society as evidence has shown that OSA is linked with many undesirable health consequences such as cardiovascular disease, hypertension, type II diabetes, cerebrovascular disease, insomnia, and depression (Jean-Louis et al., 2008). The diagnosis of OSA is also an expensive, time consuming, and labour intensive process that adds to the ever growing problem of many individuals being left undiagnosed and untreated for an extended period of time (Epstein et al., 2009).

2.1.3 Diagnosis and clinical features of OSA

The diagnosis of OSA requires the combined assessment of patient's clinical features and an objective measure of abnormal breathing during sleep (McNicholas, 2008). The 'gold standard' protocol for diagnosing OSA is a laboratory-based sleep study, commonly referred to as polysomnography. Polysomnography typically includes, simultaneous measures of electromyogram of the genioglossus and submental muscle, electroencephalogram (C3-A2), epiglottis pressure, oronasal airflow, snore, chest wall effort, body position, pulse rate, and oxygen saturation (Iber et al., 2007). Overnight monitoring determines the frequency of abnormal respiratory events (apnoeas, hypopneas, and RERA) patients experience during

sleep (Berry et al., 2012). In recent times there has been a move to conduct unattended home-based sleep studies to counter the ever-growing number of patients with suspected OSA being referred for assessment. The cost of performing laboratory-based sleep studies and the limited resources available is considered when selecting the most appropriate method.

The American Academy of Sleep Medicine (AASM) criteria for scoring an apnoea is the reduction in peak thermal sensor excursion by ≥ 90% of pre-event baseline values that lasts a minimum of 10 seconds recorded with an oronasal thermal sensor. The detection of a hypopnea is defined by a reduction in peak signal excursion by ≥ 30% of pre-event baseline value with a ≥ 3% arterial oxygen desaturation or an arousal that lasts a minimum of 10 seconds recorded with an oronasal sensor. The grading of OSA severity is calculated based on the frequency of apnoeas and hypopneas a patient will experience per hour of sleep (apnoea hypopnea index; AHI), and is classified according to the following scale; mild AHI, between 5 and 15 events per hour; moderate AHI, between 15 to 30 events per hour; and severe AHI, greater than 30 events per hour (Berry et al., 2012; Epstein et al., 2009). An alternative method for classifying OSA is the Respiratory Disturbance Index that accounts for the frequency of apnoeas, hypopneas, and RERA during sleep. Based on this classification system, mild RDI is categorised as ≥ 5 to < 15 events per hour, moderate RDI, ≥ 15 to < 30 events per hour, and severe RDI, ≥ 30 events per hour. Many sleep clinics decide to measure the AHI instead of the RDI given the technical requirement of calculating RERA.

The subjective symptoms of OSA are also considered to provide a holistic assessment of the impact disturbed sleep is having for each patient. Patients with suspected OSA often report poor sleep quality causing excessive daytime sleepiness, impaired quality of life, social functioning, work performance, and participation in activities of daily living (Engleman & Douglas, 2004). Neuropsychological deficits such as impaired cognitive function, working memory, attention, and poor executive control are likely to present in OSA patients than (Naegele et al., 1995; Roehrs et al., 1995; Salorio et al., 2002). Neuropsychological dysfunction is more commonly reported in OSA patients than healthy age matched individuals due to the periodical sleep fragmentation and / or intermittent hypoxia (Greenberg, Watson, & Deptula, 1987). Nocturnal symptoms such as habitual snoring, observed choking and/or gasping are frequently identified in OSA patients as they reflect a breathing abnormality occurring during sleep (Myers et al., 2013; Rowley, Aboussouan, &

Badr, 2000). The most appropriate choice of treatment is selected based on the diagnosed severity of OSA and the range and severity of symptoms habitually experienced.

2.1.4 Treatment and alternative options

Sleep clinicians take an individualistic approach when selecting the most appropriate treatment option for patients. Positive Airway Pressure is typically considered the first-choice treatment for patients across the severity spectrum. There are many different forms of ventilatory support available to patients, these include; Continuous Positive Airway Pressure (CPAP), Bilevel Positive Airway Pressure (BiPAP), and Automatic Positive Airway Pressure (APAP). CPAP works by delivering a predetermined intraluminal pressure (~ 4-10 cmH_2O or 2.9-7.3 mmHg) that is positive in reference to the atmospheric pressure into the pharyngeal airway of a patient during sleep. The splinting of the upper airway improves airflow by increasing the lateral pharyngeal volume and decreasing airway resistance (Abbey et al., 1989). The device is the most commonly used treatment option for OSA as it is generally well tolerated by the majority of patients and has been proven to improve cardiovascular health, daytime function, and OSA-related symptoms (Sanders, Gruendl, & Rogers, 1986; Montserrat et al., 2001). Evidence has also shown CPAP treatment is associated with an attenuated cardiovascular risk, incidence of fatal and nonfatal events, risk of road traffic accidents, and improved mental health (Drager et al., 2017; Barbé et al., 2007; Siccoli et al., 2008).

A dose-response relationship exists between the adherence/compliance of CPAP and improvement in health outcomes (Weaver et al., 2007; Zimmerman et al., 2006). The overall CPAP non-adherent rate is found to persistently low (~ 30 %) and has been shown to deteriorate after 1 year of use (Rotenberg, Murariu, & Pang, 2016; Weaver & Sawyer, 2010; Weaver & Grunstein, 2008). There are many reasons for this problem including discomfort, inconvenience, claustrophobia, and social reasons (Aurora et al., 2010). This is despite significant advancements being made with the machine dynamics, improved comfort of masks, and long-term support from clinics. The estimated cost of treating OSA with CPAP therapy is approximately £5,000 which becomes a further problem for clinics when patients decide not use the device (Guest et al., 2008). A number of randomised controlled trials have also shown that CPAP therapy leads to a modest weight gain which complicates matters further (Drager et al., 2015; Quan et al., 2013).

The BiPAP device is an alternative treatment when patients show poor tolerance or no response to CPAP. It works by automatically adjusting expiratory positive airway pressure (EPAP) and inspiratory positive airway pressure (IPAP) levels to meet the patient's requirements during sleep. The non-invasive device can detect apnoeas and snoring and responds by increasing the EPAP levels. The presence of a hypopnea or flow limitation will lead to an increased IPAP level (Carlucci et al., 2015). In contrast, the APAP device is an automatic titrating continuous positive airway pressure (CPAP) that delivers fluctuating levels of low and high pressure. This particular device is responsive to changes in body position, sleep stages, weight loss, and type / frequency of respiratory events (Morgenthaler et al., 2008). However, the APAP device is contraindicated in patients with chronic heart failure and obesity hypoventilation syndrome. The most appropriate device for each patient is typically based upon preference, costs, suitability according to the condition, and the reasons for non-adherence.

Oral appliances are another form of device used to treat mild to moderate OSA (Hoffstein, 2007). Mandibular repositioning devices prevent the upper airway from collapsing by holding the mandible in an advanced position (Menn et al., 1996). However, patients express difficulties using mandibular repositioning devices and report mild side effects (e.g. mandibular joint discomfort, dry mouth, and problems with swallowing) (Cistulli et al., 2004; Fritsch et al., 2001). Alternatives to CPAP treatment also include positional therapy, pharmacologic therapies, pharyngeal and maxillomandibular surgery (Morgenthaler et al., 2006). Behavioural weight-loss therapies have also shown promise in improving the severity of OSA (AHI) (Kuna et al., 2013; Peppard, Young, Palta, Dempsey, & Skatrud, 2000). However, several of these therapies have shown conflicting results and are limited by the lack of randomized controlled trials, excessive costs, and long-term effectiveness. Therefore, lifestyle changes are primarily offered as an adjunct to CPAP therapy with the aim of achieving weight loss via smoking cessation, reducing alcohol intake, diet modification, and becoming more physically active. While CPAP therapy works well for reducing the severity of OSA, at present there is no cure, therefore an important step forward for future research is the identification of effective treatments that target the underlying pathophysiology.

2.2 Pathophysiology of OSA

At the onset of sleep, the activity of upper airway dilator muscles is markedly reduced in patients with OSA (Eckert & Malhotra, 2008; Edwards & White, 2011). A withdrawal of tonic input of the dilator muscles coincides with an increased airway resistance that renders the upper airway vulnerable to collapse. As a result, the partial pressure of oxygen (pO_2) begins to fall and the partial pressure of carbon dioxide (pCO_2) rapidly increases along with increases in breathing effort due to hyperventilation. These responses stimulate ventilatory drive but are deemed ineffective for reversing the obstructive event during sleep. Therefore, an arousal is required to bring about a full recovery of the airway patency and blood gases. The sequence of events is yet again repeated once sleep is resumed (**Figure 2.1)**. The above explanation has long been used to describe the cycle of events that occur as part of OSA, however, the factors involved are complex, interlinked, and most are yet to be fully defined given the multifaceted nature of the condition (Schwartz et al., 2010).

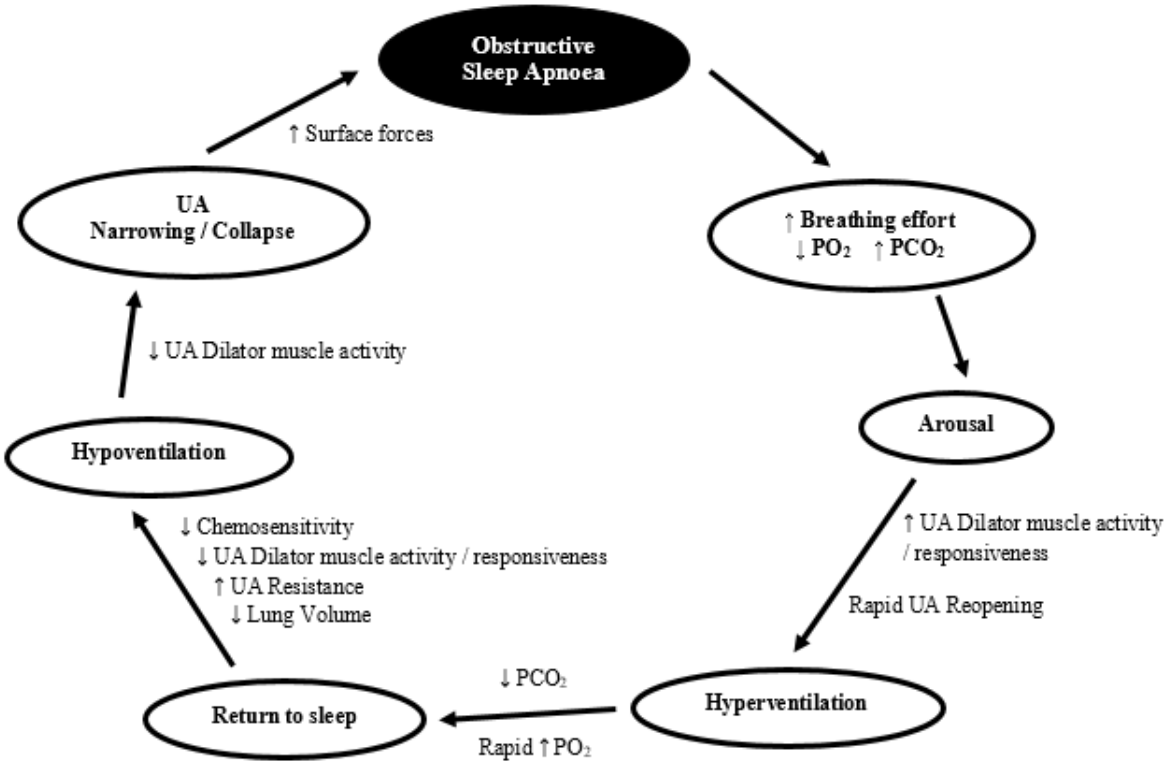


Figure 2.1. Schematic diagram demonstrating the pathophysiology of Obstructive Sleep Apnoea (UA = Upper Airway) (Eckert & Malhotra, 2008).

2.2.1 Upper airway control

The patency of the upper airway is known to be influenced by the activity of pharyngeal dilator muscles during wakefulness and sleep (van Lunteren & Strohl, 1986). There is increasing evidence that shows OSA patients have an augmented activity of the genioglossus muscles during wakefulness (Jordan et al., 2007). However, the muscle activity of upper airway dilators is found to be reduced at the onset of sleep in these patients suggesting a possible loss of wakefulness stimuli (Fogel et al., 2005). The pharyngeal airway dilators in particular the genioglossal muscles respond to increases in negative intraluminal pressures and mechanical loading by facilitating a neuromuscular compensatory reflex that aims to restore the airway patency and normalise blood gases during sleep (Mezzanotte, Tangel, & White, 1991). The responsiveness of the dilator muscles to breathing abnormalities during sleep is referred as the 'negative pressure reflex'. A reduction or complete loss of negative pressure reflex has also been reported in OSA patients which has led investigators to suggest that this neuromuscular defect may contribute to the collapsibility of the anatomically compromised airway during sleep (Eckert et al., 2007; Horner et al., 1994; Wheatley et al., 1993). Other factors presumed to influence the pharyngeal dilator muscle activity in OSA include, sleep-wake state, obesity-related hormones and cytokines (e.g. leptin, interleukin 1 alpha, and transforming growth factor beta), increased blood pressure, temperature, reduced lung volume, pharyngeal airflow, and intrapharyngeal negative pressure (Fogel et al., 2001; Kimoff et al., 2011). The negative pressure reflex and drive to the upper airway dilator muscles is also strongly influenced by chemical (pO_2 and pCO_2) and proprioceptive feedback (Loewen et al., 2011). Therefore, the combined influence of these factors may contribute to the alteration of upper airway anatomy and neuromuscular control in OSA. Another factor that may predispose individuals to OSA is the pressure within the pharyngeal airway.

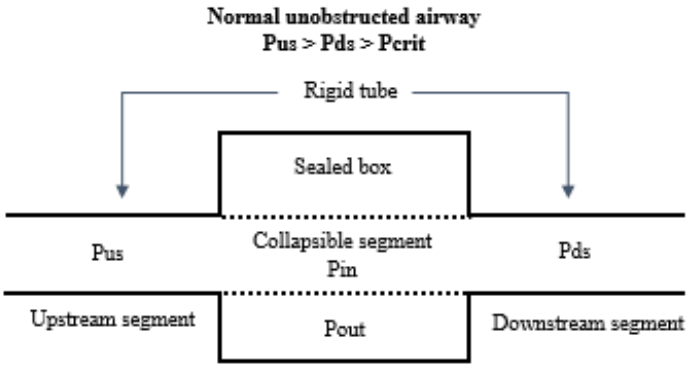
2.2.2 Pharyngeal critical pressure

The pharyngeal upper airway has been closely modelled as a Starling resistor model or collapsible conduit to explain the collapsibility of the airways in OSA (Schwartz et al., 1988; Smith et al., 1988). The model consists of a collapsible segment inside a sealed box with upstream (nasal) and downstream (tracheal / hypopharyngeal) segments fixed at either end **(Figure 2.2)**. The critical closing pressure (P_{crit}) is the pressure inside the airways (P_{in}) at the point of collapse. The upstream and downstream (P_{us} and P_{ds}) segments also possess

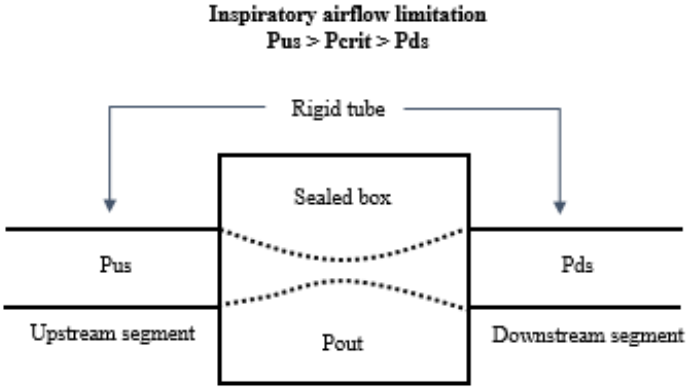
corresponding pressures and resistances that produce a fixed airflow. Normal airflow occurs throughout the upper airways when the P_{crit} is lower than upstream (P_{us}) and downstream (P_{ds}) pressures. Inspiratory airflow limitation (hypopnoea) or a repeated oscillation between collapse and reopening of the upper airway occurs when the P_{ds} decreases below the P_{crit}. A complete obstruction of the upper airway (apnoea) occurs when the Pus falls below that of P_{crit} (Schwartz et al., 2010). Elevations in P_{crit} is also associated with anatomical changes and / or disturbances of neuromuscular control which are commonly observed in OSA patients (Schwartz et al., 2008). P_{crit} is known to be heavily influenced by mechanical and neural factors such as dilator muscle hypotonia, sedation, anaesthesia, submucosal fat, and oedema (Dempsey et al., 2010; Hillman et al., 2003). However, adopting the Starling resistor model to explain upper airway collapsibility has recently come under significant criticism. The inspiratory flow through the upper airway tends to decrease with increasing effort instead of remaining fixed as previously suggested (Wellman et al., 2014). The inspiratory flow limitation (i.e. constant flow independent of downstream pressure) has also shown considerable variability amongst OSA patients. Therefore, these findings suggest the classic Starling resistor model does not accurately explain the intricate and complex mechanisms that contribute to the pharyngeal collapse in OSA.

Investigators have further examined the pharyngeal passive and active pressures in order to determine the relative contribution of mechanical loads and dynamic neuromuscular response to pharyngeal airway collapse during sleep (Isono et al., 1998; Mezzanotte et al., 1991). The critical pressures are separated into the passive 'mechanical' properties (passive critical pressure) and active 'dynamic' responses to upper airway obstructions (active critical pressure). These measures are commonly assessed in order to analyse pressure-flow relationships when the upper airway is obstructed via changes in nasal pressures (Patil et al., 2004). Mechanical loads have been shown to be significantly elevated (i.e. higher passive critical pressure) in OSA patients compared with age-matched controls (Patil et al., 2007). Whilst the dynamic response to upper airway obstructions was shown to be depressed as indicated by the higher active critical pressure in OSA patients. Overall, these findings suggest the upper airway mechanics of OSA patients are poorly compensated against obstructive events as indicated by the increased mechanical loading and disturbance of neuromuscular control during sleep. Instabilities of the ventilatory control system may further contribute to the pathogenesis of OSA.

A



B



C

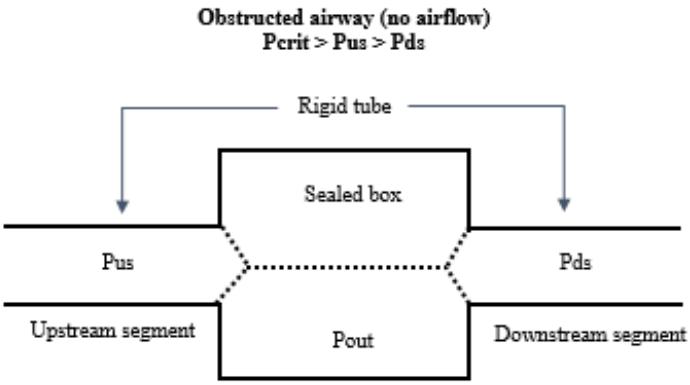


Figure 2.2. Illustration of the Starling resistor model demonstrating a normal unobstructed airway (**A**), airflow limitation (hypopnea) (**B**), and complete collapse of the upper airway (apnoea) (**C**) (P_{us} = Pressure upstream; P_{in} = Pressure inside collapsible segment; P_{crit} = Critical closing pressure; P_{ds} = Pressure downstream; P_{out} = Pressure within sealed box) (Gold & Schwartz, 1996).

2.2.3 Loop gain

An elevated 'loop gain' has also been suggested to contribute to the ventilatory instability of the pharyngeal airways during sleep (Khoo et al., 1982; White, 2005). Loop gain is an engineering term used to describe the overall "gain" of a system that is controlled by negative feedback loops that act to maintain pO_2, pCO_2 and pH levels (Younes et al., 2001). A high loop gain system responds to a breathing disturbance with a waxing and waning ventilatory response leading to increased breathing efforts and further airway instability during sleep (Edwards & White, 2011). The controller and plant gain are the major determinants of loop gain. Controller gain is synonymous with the ventilatory response to hypoxia and hypercapnia (chemosensitivity) that is described in full in **Chapter 2.3.1**. Whilst plant gain refers to the efficiency in which pulmonary ventilation eliminates CO_2 from the system. It is also known to be influenced by low functional residual capacity (FRC), dead space, cardiac output, metabolic rate, and unstable blood gases (high pCO_2), and is defined by the magnitude of change in pCO_2 versus the change in ventilation (ΔpCO_2 / ΔVE). An elevated loop gain is defined by the following conditions: 1) phase delay is observed between the system's effector (the lungs) and sensor (detection of CO_2 in the carotid body and brainstem); and 2) the loop gain must be greater than 1 when calculated according to the following equation:

$$\textbf{Loop gain} = \frac{\text{(response to disturbance)}}{\text{(the disturbance itself)}}$$

(White, 2005)

The equation is defined as the response to a respiratory disturbance (i.e. hyperpnoea) divided by the presence of disturbance itself (i.e. obstructive event) (White, 2005). Ventilation will show a small but sufficiently stable response to a respiratory disturbance when the loop gain is calculated to be less than 1. However, when the loop gain is greater than 1 a ventilatory overshoot and subsequent undershoot will be observed **(Figure 2.3)**. This generates significant ventilatory instability that is observed following repeated obstructive events during sleep. The waxing and waning ventilatory pattern observed during episodic sleep also has the potential to disrupt the muscle activity of pharyngeal airway dilators and respiratory drive (Sunderram & Androulakis, 2012; Younes et al., 2001). Therefore, an arousal (brief awakening from sleep) is crucial for the recovery process from an obstructive event.

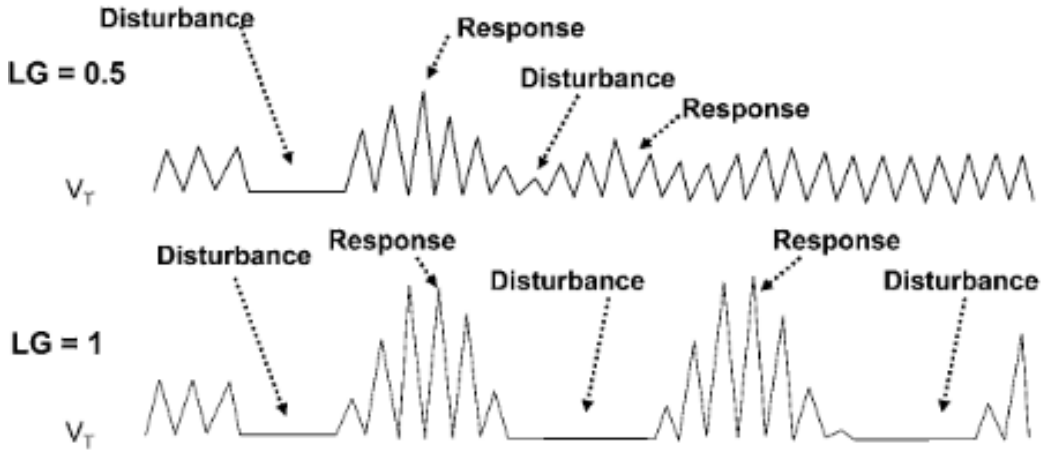


Figure 2.3. Ventilatory response to an apnoea demonstrated in an individual with a loop gain of 0.5 and 1 (LG = Loop Gain; V_T = Tidal Volume) (White, 2005).

2.2.4 Respiratory arousal threshold

The arousal threshold provides information about the degree of respiratory effort required to trigger an arousal from sleep and is viewed as a contributing factor in the pathogenesis of OSA (Edwards et al., 2014). An arousal is defined by a heightened state of brain stem activity that is responsive to both chemical and mechanical stimuli. Cortical arousals serve as a protective mechanism to reinstate ventilation and improve blood gas tensions (Eckert & Younes, 2014) via inputs from the central respiratory drive and upper airway mechanoreceptors (Ayas, Brown, & Shea, 2000; Younes et al., 1990). The respiratory effort measured via epiglottis or oesophageal pressures during the polysomnography is used to quantify the arousal threshold. Patients with a high respiratory arousal threshold (more negative than -15cmH_2O) will require larger intrathoracic pressure swings to initiate an arousal (Berry & Gleeson, 1997). The resulting event will be prolonged due to the greater stimulus required and poor responsiveness of the upper airway dilators. In contrast, patients who possess a low respiratory arousal threshold (less negative than -15 cmH_2O), or demonstrate premature awakening to a mild narrowing of the airway, will require smaller intrathoracic pressure swings to initiate an arousal in order to terminate the respiratory event (Edwards et al., 2014). The consequence of having a lower arousal threshold is sleep fragmentation and repeated apnoea or hypopneas that promote further ventilatory instability (Younes, 2004). However, the physiological changes associated with the arousal threshold is likely to differ between individuals due to the impact of various factors (e.g. use of sedative

agents, sleep stage, and fragmentation) (Berry & Gleeson, 1997). The mechanical and neural properties of obesity is also proposed to influence the function of the upper airway and waking mechanisms.

2.2.5 Obesity and control of breathing

Obesity is a major risk factor for OSA due to the mechanical loading of the pharyngeal structures and respiratory system (Sharp et al., 1964; Koenig & Thach, 1988; Xiao et al., 2016). Excessive abdominal fat desposition is the key pathophysiological feature that increases the collapsibiltiy of the upper respiratory airways (retropalatal and retroglossal region of the pharynx) during sleep (Isono, 2012). Adipose deposition within the soft tissues of the upper airway also encroach the pharyngeal lumen and increase tissue pressures. This results in a loss of neuromuscular tone and temporary desynchronization of the upper airway and inspiratory muscles at the onset of sleep (Hudgel, Harasick, & Hudgel, 1990; Eckert et al., 2007; Steier et al., 2010). The magnitude of adipose tissue that lies adjacent to the pharyngeal airway and within the intraperitoneal space is associated with the severity of OSA (Shelton et al., 1993). Another factor to consider is the mechanical impact of posture on the pharyngeal airway patency at the onset of sleep and periodically throughout the night. There is evidence to show a postural effect occurs during the shifting of body mass from an upright to supine position which reflects the gravitational forces acting on the narrowed airway (Fouke & Strohl, 1987). Patients with OSA often demonstrate reduced lung volumes (lower functional residual capacity) due to the greater abdominal fat mass and supine position adopted during sleep (Hoffstein, Zamel, & Phillipson, 1984). These alterations reduce the caudal traction on the trachea and larynx and attenuate tissue pressures, all of which can lead to an alteration of the lateral pharyngeal airway (Ballard et al., 1990; Van de Graaff, 1988, 1991). The transition from wakefulness to sleep results in an increased nasal resistance that is presumed to reflect a fall in tonic muscle activity during sleep (De Vito et al., 2001; Hudgel, Hendricks, & Hamilton, 1988). The chemical control of breathing is another pathophysiological feature of OSA that is influenced.

2.3 Chemical control of breathing

Ventilation is a tightly regulated process that aims to maintain the homeostasis of arterial blood gases in the face of different challenges experienced during wakefulness and sleep (Krimsky & Leiter, 2005). Higher centres of the brain (Cerebral Cortex, Limbic System, and Hypothalamus) exert a conscious control over a range of complex functions such as; voluntary control of breathing, emotions, speech, and motor behaviour via action potentials projected downstream to the respiratory muscles (Phillipson, 1978). The respiratory control centre (Medulla Oblongata and Pons) automatically modifies respiration in an unconscious manner via interconnecting neuronal groups that are located along the border of the brainstem (also known as the Central Pattern Generator) (Bianchi, Denavit-Saubié, & Champagnat, 1995). The Medulla Oblongata is commonly viewed as the 'rhythmicity centre' due to its ability to control rate and depth of breathing pattern by means of several neuronal groups focused within the ventrolateral and dorsal regions of the medulla (Richter, 1982). The brainstem receives afferent feedback from pulmonary stretch receptors and multiple mechanoreceptors that are located within the airway, thorax, and lungs in order to regulate ventilation (O'Donnell et al., 2007). The ventilatory control system is also reliant upon the synergistic performance of both central and peripheral chemoreceptors for the maintenance of arterial blood gases (pCO_2 and pO_2) and pH level (Guyenet & Bayliss, 2015) (**Figure 2.4**).

2.3.1 Central and Peripheral Chemoreceptors

Central chemoreceptors located in the rostral ventrolateral medulla are sensitive to changes in arterial pCO_2 and pH (H^+ ion concentration). The central chemoreceptors provide a homeostatic control of the acid-base balance in response to ventilatory disturbances and provides a source of excitatory/inhibitory afferent information to the respiratory control system (Guyenet, Stornetta & Bayliss, 2010; Nattie, Shi, & Li, 2001). Central chemoreception provides a slow time course (~ 50 seconds) for the equilibrium of brain extracellular pH. Carbon dioxide stimulates the central (and peripheral) chemoreceptors in order to maintain the level of pCO_2 within the normal range of ~40 mmHg during rest, exercise, and sleep (Berthon-Jones & Sullivan, 1984; Kuwaki, 2008). These chemoreceptors are increasingly activated during periods of stress such as experimentally induced hypercapnia and obstructive events. The major central chemoreceptor sites are distributed throughout the lower brainstem (Nattie & Li, 2012). Several intrinsic neurons have emerged

as key contributors to the sensing of CO_2 / H^+ (Putnam, Filosa, & Ritucci, 2004). The retrotrapezoid nucleus (RTN) is viewed as the key site of central CO_2 chemosensitivity that is characterised by glutamatergic interneurons (known to express Phox2b), and are altered by inputs from carotid chemoreceptors, vagal afferents, and the hypothalamus (Stornetta et al., 2006). Glia cells located within the RTN region are proposed to maintain a normal extracellular pH and possess chemosensitive properties (Nattie & Li, 2012). Chemoreceptors sites are further located within the rostral aspect of the medullary raphe, midbrain raphe, locus coeruleus, nucleus tractus solitarius, caudal ventrolateral medulla, pre-Bötzinger region, and fastigial nucleus of the cerebellum; all of which are thought to be implicated in regulating ventilation and the homeostatic control of cardiorespiratory function (Putnam, 2001).

Serotonergic neurons are thought to possess chemosensitive properties responsive to changes in CO_2 / H^+ and are crucial for the plasticity of the respiratory network (Brust et al., 2014; Richerson, 2004). In particular, the midbrain raphe serotonergic neurons are thought to be involved in the maintenance of pH by inducing arousal, anxiety, and alterations of cerebrovascular tone in response to metabolic acidosis (Severson et al., 2003). The activity of hypothalamic orexin / hypocretin neurons has previously been overlooked as playing a key role in regulating ventilation and arousal (Williams et al., 2007). However, orexin cells are known to stimulate breathing by innervating the brain stem-spinal cord respiratory related network (Young et al., 2005). Whilst, the underlying mechanism/s of orexin are currently unknown, there is evidence to suggest that the destruction orexin cells and / or disruption of the hypocretin (orexin) receptor 2 gene is linked with the pathophysiology of sleep-related respiratory disorders such as narcolepsy and OSA (Lin et al., 1999; Mignot, 2004; Nishijima, Sakurai, Arihara, & Takahashi, 2003).

Peripheral chemoreceptors are located at the bifurcation of the internal and external carotid bodies and to a lesser extent at the aortic arch (Krimsky & Leiter, 2005). The peripheral chemoreceptors rapidly respond to changes in pO_2 and to some extent pCO_2 of the blood flowing throughout the small carotid body artery that branches to either internal or external carotid artery (Duffin, 2011; Prabhakar & Peng, 2004). Neuronal messages are sent via afferent fibres to different regions of the brain (pons and medulla oblongata) responsible for controlling the rate and depth of breathing in order to maintain homeostasis during hypoxaemia. The carotid body is composed of glomus (type 1) cells and glial-like stem cells

that are sensitive to the hypoxia during wakefulness and sleep (Peers, Wyatt, & Evans, 2010; Prabhakar & Semenza, 2012). The glomus cells are primarily responsible for oxygen sensing, however, polymodal receptors of the carotid body are also known to respond to changes in potassium, noradrenaline, temperature, glucose, and insulin (Ding & Li, 2011; Kumar & Bin-Jaliah, 2007). It has generally been assumed that the central and peripheral chemoreceptors work in isolation at sensing changes in pCO_2 and pO_2. However, there is some evidence to suggest that peripheral oxygen-sensitive chemoreceptors interact or work in a hyper-additive manner with central chemoreceptors in order to maintain arterial gases at a constant level during hypercapnic breathing (Smith, Rodman, Chenuel, Henderson, & Dempsey, 2006; Nattie, 1999). Additionally, chemoreceptor interdependence is proposed to occur in order to maintain normal breathing during eupnoea and acute hypoxia (Dempsey et al., 2012). The ventilatory response to hypoxia and hypercapnia is frequently assessed in healthy and clinical populations using a variety of protocols to determine the sensitivity of the chemical drives. The sensitivity of the central and peripheral chemoreceptors is known to be affected in OSA patients during sleep and wakefulness.

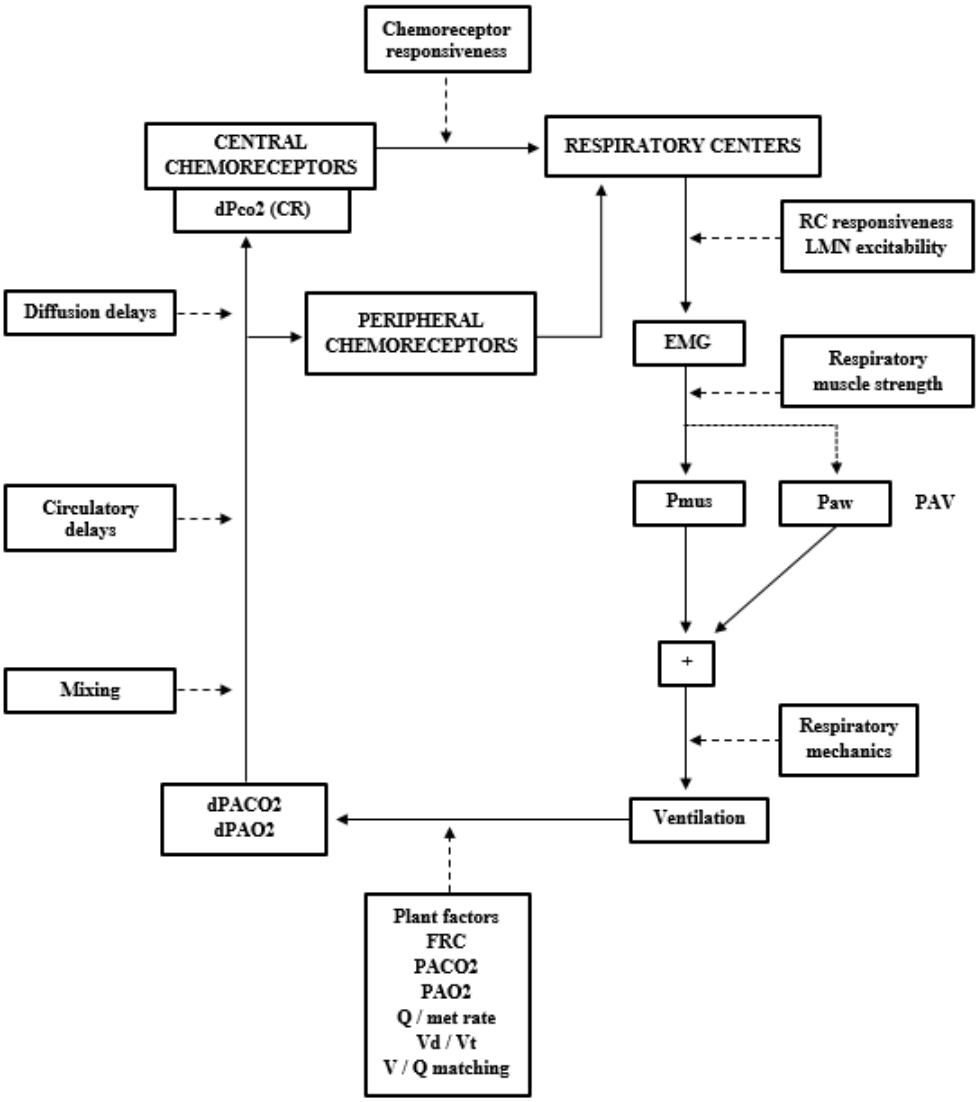


Figure 2.4. Schematic diagram of the determinants influencing ventilatory stability (RC = Respiratory centre; LMN = Lower motor neurons; EMG = Electromyography; Pmus = Respiratory muscle pressure; Paw = Airway pressure; PAV = Proportional Assist Ventilation; FRC = Functional Residual Capacity; $PaCO_2$ = Alveolar carbon dioxide tension; PaO_2 = Alveolar oxygen tension; Q / met rate = Cardiac output / Metabolic rate; Vd / Vt = Ventilatory drive / Tidal volume; V / Q ratio = Ventilation / Perfusion ratio; d = Diffusion; CR = Chemoreceptors) (Younes et al., 2001).

2.3.2 Methodological approach to assess ventilatory control

Traditionally, the clinical assessment of ventilatory abnormalities have been limited to measurements of arterial pCO_2 and pO_2. However, the current approach is to perform clinically applicable, simple, and quick assessments of the ventilatory drives in healthy and clinical populations. Central chemoreflex sensitivity is most commonly assessed using rebreathing or steady-state methods which increase carbon dioxide whilst maintaining hyperoxia constant in order to reduce the influence of peripheral chemoreflexes (Cunningham, 1987). The rebreathing technique was first developed by Read (1966) in order to determine the ventilatory response to carbon dioxide in patient populations. It has become the most commonly chosen method due to its simple and quick protocol as opposed to the classical, steady-state method that requires several measures to be performed with different CO_2 concentrations (10-20 minutes per gas mixture).

The protocol consists of an individual rebreathing the content of a small bag (7 % of CO_2, 50% O_2, and 43% N_2) for ~ 4 minutes. End-tidal pCO_2 increases at a constant rate of 4-6 mmHg per minute after the first 15 seconds of the protocol. This 'open-loop' condition generates a slope (Δ VE/ Δ pCO_2) between the changes in ventilation and end-tidal CO_2 and is presumed to reflect the sensitivity of the respiratory centre. The initial high O_2 concentration is designed to prevent the hypoxic drive from influencing ventilation during rebreathing. Consequently, pO_2 is maintained at approximately 200 mmHg during rebreathing. Whereas, the pCO_2 equilibrium rapidly develops between mixed venous blood, arterial blood, and end-expiratory gases (lung and rebreathing bag) under these conditions (Fowle & Campbell, 1964). As a result, the changes observed in end-tidal pCO_2 reflect those of arterial pCO_2. The Duffin rebreathing test (also known as modified rebreathing technique) is also frequently performed given that it permits a simultaneous measurement of central and peripheral chemosensitivity. It consists of a 5-minute period of hyperventilation (reaching the target $PETCO_2$ of ~ 20-25 Torr) followed by rebreathing the contents of a 5-litre bag (7 % CO_2, 93% O_2, and 43% N2; end $PETCO_2$ ~ 55 Torr) for 4-6 minutes (Jensen, Mask, & Tschakovsky, 2010; Mohan & Duffin, 1997).

Alternative methods of the 'classical' rebreathing protocol are utilised for the ventilatory assessment of CO_2. The steady-state CO_2 inhalation technique is an exception to the modalities reported above given that no rebreathing takes place. The hypercapnic ventilatory

response is assessed with the use of a 120-litre Douglas bag containing pre-mixed gas concentrations (Bittencourt et al., 1998). The steady-state method requires simultaneous measures of minute ventilation and pCO_2 to be recorded during a 5-minute period of inspiring the fixed concentrated of CO_2 (e.g. 6-9 % CO_2). Dynamic end-tidal forcing is yet another steady-state technique that has been increasingly utilised to assess the ventilatory response to CO_2 (Robbins et al., 1982). The protocol has the advantage of utilising specifically designed software to produce an accurate control of inspired fractional concentrations of CO_2, O_2, and N_2 whilst the end-tidal gases are held constant. The protocol is comprised of a 10-minute period of breathing isocapnic euoxia ($PETCO_2$ = +1.0 Torr above resting values; $PETCO_2$ = +88.0 Torr), followed by a rapid increase of the inspired gas for a duration of 20 minutes ($PETCO_2$ of +9 Torr). $PETO_2$ is held at a constant rate of 88.0 Torr throughout the protocol while $PETCO_2$ returns to +1.0 Torr above resting values (Ainslie & Poulin, 2004; Poulin et al., 1996). This technique is limited given the sophisticated equipment and large volume of gases required to conduct laboratory-based assessments. Previous investigations have compared the sensitivity of the central chemoreceptors using either re-breathing or steady-state methods and have found no differences between the two techniques (Clark, 1968; Linton et al., 1973; Mohan et al., 1999; Soto Campos et al., 1996).

Similar methodologies have also been adopted in the assessment of peripheral chemoreflexes during wakefulness (Duffin, 2007). The most commonly selected technique introduced by Weil et al., (1970) requires subjects to breathe a gas mixture of 40% O_2 until pO_2 gradually decreases from 120 to 40 mmHg over the course of 15-20 minutes. This protocol utilises a larger bag and does not produce 'open loop' conditions. An alternative method was established by Rebuck & Campbell (1974) to create progressive isocapnic hypoxic conditions with a protocol resembling that of Read (1967). The technique requires subjects to breathe a gas mixture containing 24% O_2, 7% CO_2, and balance of N_2 in an 'open loop' fashion. End tidal pCO_2 is held constant throughout the protocol whilst pO_2 decreases to 30-40 mmHg. Several factors are known to influence the ventilatory response to CO_2 and O_2, these include long-term residence at altitude, drug use, ageing, athleticism, obesity, genetic traits, and pathological conditions (congenital central hypoventilation syndrome, sudden infant death syndrome, Fibrosis, Asthma, and OSA) (Guyenet, Stornetta, & Bayliss, 2010; Hirschman, McCullough, & Weil, 1975; Zwillich et al., 1975; Lane & Howell, 1970; Patton & Freedman, 1972; Rebuck & Read, 1971).

2.3.3 Ventilatory response to hypoxia and hypercapnia in health and OSA

Previous research investigating the ventilatory response to hypoxia and hypercapnia in endurance-trained and untrained individuals have produced conflicting results potentially due to the methodology adopted and/or training status of the populations studied (**Table 2.1**). Cross-sectional studies have examined the ventilatory response to hypoxia and / or hypercapnia in endurance-trained athletes (elite, world-class and collegiate level) and non-athletic individuals using a range of techniques during wakefulness. The ventilatory response to hypoxia in endurance trained athletes was reported to be lower (Byrne-Quinn et al., 1971; Scoggin et al., 1978) or similar to non-athletic individuals (Godfrey et al., 1971; Mahler et al., 1982; Sheel et al., 2006). The ventilatory response to hypercapnia in endurance trained athletes was also shown to be lower (Byrne-Quinn et al., 1971; Miyamura, Yamashina, & Honda, 1976; Scoggin et al., 1978) or similar to non-athletic individuals (Godfrey et al., 1971; Mahler et al., 1982).

Further investigations have examined potential associations between the maximal oxygen uptake (VO_{2max}) and ventilatory response to hypoxia and hypercapnia. Byrne-Quinn et al., (1971) revealed the VO_{2max} was inversely related to the attenuated ventilatory response to hypoxia and hypercapnia observed in the group of athletes and non-athletes. In contrast, Sheel et al., (2006) found the hypoxic ventilatory response was unrelated to VO_{2max} in three groups that included untrained, moderately untrained, and highly trained individuals. Hirschman et al., (1975) also reported no such relationship in a non-athletic group. These observations suggest the lower ventilatory response to hypoxia and hypercapnia appears to reflect some element of athleticism rather than simply possessing a higher VO_{2max}. It is unknown whether genetic predisposition and/or prolonged physical training leads to endurance-trained athletes showing an attenuated hypoxic and hypercapnic ventilatory response.

The underlying mechanism/s of the attenuated hypoxic and hypercapnic ventilatory response observed in endurance-trained individuals is currently unknown. There is evidence to suggest the blunted ventilatory response to hypoxia and hypercapnia is attributed to changes in neural drive to the respiratory muscles and / or diminished sensitivity of the peripheral chemoreceptors in response to the physical training (Miyamura et al., 1988; Peterson et al., 1981). However, several methodological approaches were conducted in the studies above

which potentially account for the disparity found within the results, particularly the state of oxygenation during hypercapnic breathing (normoxia vs. hyperoxia), and the technique chosen to induce hypercapnia (rebreathing vs. steady state). These studies have largely focused on the central and peripheral chemosensitivity of world-class or collegiate endurance-trained athletes which may greatly differ to that observed in club level athletes. Studying the ventilatory response to mixed hyperoxic/hypercapnic and hypoxic/hypercapnic gases would provide a better differentiation of the central and peripheral chemosensitivity in endurance-trained and untrained individuals.

Previous studies have also investigated the ventilatory response to hypoxia and hypercapnia in OSA patients and non-obese controls, yet again producing conflicting results due to the methodology adopted (**Table 2.2**). The prevailing view held by the majority of studies investigating central chemosensitivity indicate that patients with OSA have a reduced ventilatory response to carbon dioxide compared with non-obese controls (Garay et al., 1981; Gold, Schwartz, Wise, & Smith, 1993; Javaheri et al., 1994; Littner et al., 1984; Lopata & Onal, 1982; Osanai et al., 1999). However, many other studies have reported that OSA patients have a similar (Benlloch et al., 1995; Radwan et al., 1995; Rajagopal, Abbrecht, & Tellis, 1984; Bittencourt et al., 1998; Sin, Jones, & Man, 2000; Narkiewicz et al., 1999), or higher ventilatory response to hypercapnia than non-obese controls (Verbraecken et al., 1998). Likewise, the prevailing view reported by the majority of studies investigating peripheral chemosensitivity indicate that OSA patients have a lower ventilatory response to hypoxia than non-obese controls (Garay et al., 1981; Javaheri et al., 1994; Osanai et al., 1999). However, other studies have found OSA patients have a similar (Gold et al., 1993; Littner et al., 1984) or higher ventilatory response to hypoxia than non-obese controls (Narkiewicz et al., 1999).

The mechanism/s underlying the altered ventilatory response to hypoxia and hypercapnia in OSA patients is unclear. The large body of evidence have postulated the diminished hypoxic ventilatory response is attributed to a genetic predisposition or an acquired defect in response to the prolonged exposure to hypoxia during sleep (Collins et al., 1978; Hirschman et al., 1975). Chronic exposure to intermittent hypoxia has been shown to alter the ventilatory response to hypoxia in humans and animal models (Mateika & Narwani, 2009; Mitchell et al., 2001; Rey et al., 2004). Many studies have also suggested the altered ventilatory response

to hypercapnia is attributed to a defective central respiratory drive in OSA patients (Gold et al., 1993; Lopata & Onal, 1982). Further investigations have hypothesised that frequent exposure to hypoxia and hypercapnia during episodic sleep could potentially depress or progressively 'reset' the sensitivity of the carotid body to a lower sensitivity threshold (Forster & Dempsey, 1981; Guilleminault & Cummiskey, 1982). Other authors have indicated that mechanical changes such as reduced airway patency during apnoeic events may be responsible for the alterations in chemical drive (Dempsey, Xie, Patz, & Wang, 2014).

The disparity between the results above is potentially the consequence of adopting different techniques to induce hypercapnia (rebreathing vs. steady state) and the heterogeneous patients recruited (eucapnic vs. hypercapnic OSA). During steady-state techniques slow increases in CO_2 are observed which potentially results in lower sensitivities being observed compared with the rebreathing technique. Furthermore, hypercapnic OSA patients have been shown to possess lower hypoxic and hypercapnic ventilatory drives compared with eucapnic OSA patients. Therefore, further research is required to determine whether alterations in the chemosensitivity is an outcome of pathological alterations that are unique to the condition or are linked to lifestyle dependent outcomes such as sedentary behaviour, physical fitness, and body characteristics (i.e. possessing a higher BMI and reduced lung volumes). Furthermore, it is yet unknown whether CPAP treatment has the ability to modify the central and peripheral chemosensitivity in OSA patients.

Table 2.1. Summary of studies investigating the ventilatory response to hypoxia and hypercapnia in endurance-trained athletes and controls.

Study	Participants	Age (mean ± SD) / Gender	Rebreathing or Steady-state protocol	Results	Summary
Godfrey et al., (1971)	7 Endurance athletes 7 Controls	27 years / M 29 years / M	Read (1967) Air-O_2 mixture 25-30%	2.05 vs. 2.36 (l/min/mmHg) 6.32 vs. 7.69 (l/min/mmHg)	No difference between HCVR and HVR slopes of controls and athletes.
Byrne-Quinn et al., (1971)	13 Endurance athletes (60.1 ± 2.0 ml/kg/min) 10 Sedentary Controls (40.2 ± 2.4 ml/kg/min)	22 ± 1 years / M 29 ± 2 years / M	Read (1967) Weil et al. (1970)	0.94 vs. 2.02 (l/min/mmHg)** 62.4 vs. 180.3 (VE - P_ACO_2 curves)**	↓ HCVR and HVR in Athletes vs. Controls.
Miyamura et al., (1976)	10 Marathon runners 14 Controls	22 ± .9 years / M 20 ± .5 years / M	Read (1967)	1.12 vs. 1.86 (l/min/mmHg)*	↓ Slope of HCVR in Runners vs. Non-Athletic controls.
Scoggin et al., (1978)	5 Long-distance runners 34 Controls	19 years / M N/A	Read (1967) Weil et al. (1970)	1.92 vs. 2.53 (l/min/mmHg) NS 74 vs. 128 (VE - P_ACO_2 curves)*	↓ Slope of HCVR and HVR during wakefulness in Runners vs. Non-Athletic controls.
Mahler, Moritz & Loke (1982)	20 Marathon runners 20 Controls	27.8 years / M 27.4 years / M	Modified version of Read rebreathing method Rebuck and Campbell method	2.23 vs. 2.61 (l/min/Torr) 0.57 vs. 0.88 (l/min/1%)	No difference between HCVR and HVR of controls and athletes.
Sheel et al., (2006)	24 Untrained (40.7 ± 5.8 ml/kg/min) 18 Moderately Trained (54.9 ± 2.9 ml/kg/min) 13 Highly Trained (65.8 ± 4.2 ml/kg/min)	24 ± 3 years / M 28 ± 6 years / M 24 ± 6 years / M	Kochle et al. (2005)	0.28 - 1.61 (l/min/%SaO_2^{-1}) 0.23 - 2.39 (l/min/%SaO_2^{-1}) 0.08 - 1.73 (l/min/%SaO_2^{-1})	No difference in the HVR of the groups.

HVR = Hypoxic ventilatory response; HCVR = Hypercapnic ventilatory response; VE = Ventilation; M = Male; F = Female; ↓ = decrease; * = $p < .05$; ** = $p < .01$.

Table 2.2. Summary of studies investigating the ventilatory response to hypoxia and hypercapnia in OSA patients and controls.

Study	Participants	Age (mean ± SD) / Gender	Rebreathing or Steady-state protocol	Results	Summary
Garay et al., (1981)	6 Eucapnic OSA (BSA: 2.34 ± 0.21 m^2) 7 Hypercapnic OSA (BSA: 2.40 ± 0.27 m^2)	48 ± 9 years (M/F) 55 ± 7 years / M	Modified version of Read (1967) Rebuck and Campbell method	2.41 vs. 0.79 (l/min/mmHg)** 0.53 vs. 0.38 ($\Delta VE/\Delta O_2$ Sat) NS	↓ HCVR and HVR in Hypercapnic OSA patients.
Lopata and Onal (1982)	34 Non-Obese controls 7 Obese controls; 7 Obese & OSA 8 OHS patients	N/A 41 ± 5 years / M 45 ± 4 years / M 50 ± 3 years / M	Read (1967)	3.65 (l/min/mmHg) 2.03 (l/min/mmHg)* 1.68 (l/min/mmHg)* 1.00 (l/min/mmHg)*	↓ HCVR in OHS and Obese / OSA patients vs. non-Obese Controls.
Rajagopal, Abbrecht, & Tellis (1984)	8 OSA patients (BM: 104.2 ± 7.0 kg) 8 Controls (BM: 100.2 ± 6.7 kg)	45 ± 4 years / M 45 ± 5 years / M	Read (1967)	2.17 vs. 2.17 (l/min/mmHg)	No difference in HCVR of OSA patients and Controls.
Littner et al., (1984)	16 SRDB (BM: 84 ± 3.2 kg) 10 Controls (BM: 83 ± 2.7 kg)	65 ± 1 years / M 64 ± 1 years / M	Read (1967) Isocapnic rebreathing method	1.92 vs. 3.58 (l/min/mmHg)** -0.50 vs -0.84 ($l \cdot min \cdot \%SaO_2^{-1}$) NS	↓ HCVR in SRDB patients vs. Controls. No difference in HVR of SRDB patients and Controls.
Gold et al., (1993)	35 Normocapnic OSA (BM: 103.6 ± 17.7 kg) 17 Controls (BM: 101.9 ± 13.8 kg)	51 ± 11 years (M/F) 52 ± 12 years (M/F)	Read (1967)	2.05 vs. 3.02 (l/min/mmHg)** 0.76 vs 0.91 ($l \cdot min \cdot \%SaO_2^{-1}$) NS	↓ HCVR in OSA patients vs. Controls. No difference in HVR of Controls and OSA patients.
Javaheri, et al., (1994)	23 Hypercapnic OSA (BM: 128 ± 34 kg) 32 Eucapnic OSA (BM: 112 ± 22 kg)	55 ± 8 years / M 56 ± 10 years / M	Read (1967) Rebuck & Slutsky (1981)	0.96 vs. 2.42 (l/min/mmHg)** -0.26 vs -1.67 ($l \cdot min \cdot \%SaO_2^{-1}$)**	↓ Slope of HCVR and HVR in Hypercapnic vs. Eucapnic OSA patients.

BSA = Body Surface Area; BM: Body Mass; HVR = Hypoxic ventilatory response; HCVR = Hypercapnic ventilatory response; VE = Ventilation; SRDB = Sleep-related disordered breathing; OHS = Obesity Hypoventilation Syndrome; M = Male; F = Female; ↓ = decrease; * = $p < .05$; ** = $p < .01$.

Continued

Study	Participants	Age (mean ± SD) / Gender	Rebreathing or Steady-state protocol	Results	Summary
Radwan et al., (1995)	20 Obese OSA (BMI: 34.4 ± 6.3 kg/m^2) 12 Controls (BMI: 22.3 ± 3.0 kg/m^2)	45 ± 6 years / M 34 ± 7 years / M	Read (1967)	2.7 (l/min/mmHg) 2.1 (l/min/mmHg)	No difference in HCVR of Obese OSA patients vs. controls.
Benlloch et al., (1995)	27 Obese OSA (BMI: 32.6 ± 4.8 kg/m^2) 27 Controls (BMI: 33.5 ± 8.4 kg/m^2)	55 ± 10 years (M/F) 52 ± 10 years (M/F)	Read (1967)	2.49 vs. 2.35 (l/min/mmHg)	No difference in HCVR of Obese OSA patients vs. Controls.
Verbraecken et al., (1998)	14 Controls (BMI: 30 ± 1 kg/m^2) 17 Normocapnic OSA (BMI: 30 ± 1 kg/m^2)	45 ± 3 years (M/F) 48 ± 2 years (M/F)	Read (1967)	1.65 (l/min/mmHg) 2.55 (l/min/mmHg)*	Slope of HCVR ↑ Hypercapnic OSA patients vs. Controls.
Bittencourt et al., (1998)	22 Obese Normocapnic OSA (BMI range: 25.5 - 39.4 kg/m^2)	32 - 63 years (M/F)	Steady-state CO_2 inhalation test (8% CO_2 and 42% O_2)	12.2 (5.4 - 18.2 L/min) 12.5 (8.9 - 23.12 L/min)	Normal ventilatory response to CO_2 in normocapnic OSA patients.
Narkiewicz et al., (1999)	12 Controls (BMI: 33 ± 2 kg/m^2) 16 Newly-diagnosed OSA (BMI: 33 ± 2 kg/m^2)	40 ± 6 years (M/F) 42 ± 6 years (M/F)	Read (1967) 10% O_2; 90% N_2	14.5 vs. 13.7 (L/min) NS 11.1 vs. 14.1 (L/min)*	No difference in HCVR of OSA patients and Controls. ↑ HVR in OSA patients vs. Controls.
Osanai et al., (1999)	9 Controls (BMI: 27.2 ± 2.4 kg/m^2) 16 OSA (BMI: 27.8 ± 1.4 kg/m^2)	43 ± 4 years (M/F) 46 ± 3 years (M/F)	Read (1967) Isocapnic progressive hypoxia method	0.96 vs. 0.71 ($\Delta V'I/\Delta PETCO_2$/ BSA/l/min/mmHg/m)* 0.55 vs. 0.34 ($\Delta V'I/\Delta SaO_2$/ BSA/l/min/mmHg/m)*	↓ HCVR and ↓ HVR in OSA patients vs. Controls.
Sin, Jones, & Man (2000)	104 OSA (BMI: 31.4 ± 6.2 kg/m^2) 115 Non-OSA (BMI: 29.6 ± 5.9 kg/m^2)	47 ± 10 years (M/F) 45 ± 11 years (M/F)	Read (1967)	1.93 vs. 1.79 (l/min/mmHg) NS	No difference in slope of HCVR for OSA & Non-OSA patients.

BSA = Body Surface Area; BM: Body Mass; HVR = Hypoxic ventilatory response; HCVR = Hypercapnic ventilatory response; VE = Ventilation; SRDB = Sleep-related disordered breathing; OHS = Obesity Hypoventilation Syndrome; M = Male; F = Female; ↓ = decrease; ↑ = increase; * = $p < .05$; ** = $p < .01$.

2.3.4 Ventilatory response to hypoxia and hypercapnia after CPAP treatment

The ventilatory response to hypercapnia was shown to be increased (Lin, 1994; Tun et al., 2000) or not different in OSA patients after receiving CPAP treatment (Berthon-Jones & Sullivan, 1987; Foster et al., 2009; Greenberg & Scharf, 1993; Spicuzza et al., 2006; Verbraecken et al., 1995) **(Table 2.3)**. Whilst the ventilatory response to hypoxia was shown to be increased (Lin, 1994) or reduced in OSA patients after receiving CPAP treatment (Radovanovic et al., 2019; Spicuzza et al., 2006; Tun et al., 2000). The mechanism/s underlying the impact of CPAP therapy on the hypoxic and hypercapnic ventilatory drives is currently unknown in OSA patients. Long term CPAP treatment is postulated to improve the hypercapnic ventilatory response by resetting and downregulating the hypercapnic drive (Berthon-Jones & Sullivan, 1987). However, some authors have postulated that CPAP therapy may improve the peripheral chemoreceptor sensitivity indirectly by increasing the baroreflex sensitivity and reducing sympathetic nerve activity (Narkiewicz et al., 1999; Somers et al., 1995; Spicuzza et al., 2003).

The conflicting results shown in Table 2.3 are potentially explained by the duration of CPAP treatment that ranges from ~ 2 weeks to 12 months and the heterogeneous patients recruited for the studies (eucapnic vs. hypercapnic OSA). Previous studies have suggested that a minimum of 1-month of CPAP treatment is required in order to improve the ventilatory responses to hypoxia and hypercapnia in OSA patients (Foster et al., 2009; Spicuzza et al., 2006). However, other studies have employed CPAP therapy (> 3 months) and shown no differences in the hypoxic and hypercapnic ventilatory responses of OSA patients. Therefore, inter-individual differences such as age, gender, presence of co-morbidities, and $PaCO_2$ level (normocapnic vs. hypercapnic) may explain the differences observed between the studies. However, further research is required to determine whether central and peripheral chemosensitivity can be modified following CPAP treatment in OSA patients with no co-morbidities.

Table 2.3. Summary of studies investigating the ventilatory response to hypoxia and hypercapnia in OSA patients after CPAP treatment.

Study	Participants	Age (mean ± SD) / Gender	Rebreathing or Steady-state protocol	CPAP therapy	Results	Summary
Berthon-Jones & Sullivan (1987)	9 Normocapnic OSA 10 OSA Hypercapnic	47 ± 3 years / M 53 ± 3 years / M	Read (1967)	2 weeks - 3 months of nCPAP	Pre: 2.6 vs. Post: 2.3 l/min/mmHg Pre: 2.5 vs. Post: 2.5 l/min/mmHg	No difference in Slope of HCVR after CPAP in normocapnic OSA.
Greenberg & Scharf (1993)	16 Newly-diagnosed OSA 6 Controls	43 ± 7 years (M/F) 34 ± 3 years (M/F)	Steady-state CO_2 stimulation	4 weeks of nCPAP	1.93 vs. 1.38 l/min/mmHg Pre: .71 vs. Post: .49 l/min/mmHg	No difference in HCVR between Controls and OSA patients. No change in HCVR after CPAP.
Lin (1994)	6 Hypercapnic OSA 24 Moderate to Severe Eucapnic OSA	48 ± 7 years (M/F) 47 ± 10 years (M/F)	Read (1967) Rebuck and Campbell method	4 weeks of nCPAP	0.46 vs. 2.59 l/min/kPa* Pre: 0.46 vs. Post: 2.5 l/min/kPa* Pre: 2.59 vs. Post: 2.74 l/min/kPa Pre BMI: 39.1 ± 2.4 kg/m^2 vs Post BMI: 38.1 ± 2.5 kg/m^2 -1.49 vs -2.97 (l·min·%SaO_2^{-1})* Pre: -1.49 vs. Post: -2.89* Pre: -2.97 vs. Post: -3.02 Pre BMI: 34.1 ± 2.1 kg/m^2 vs Post BMI: 33.6 ± 2.2 kg/m^2	↓ HCVR and HVR in Hypercapnic vs. Eucapnic OSA patients. ↑ HCVR and HVR after 4 weeks of CPAP in Hypercapnic OSA patients only. No difference in Eucapnic OSA.
Verbraecken et al., (1995)	14 Controls 14 Heavy Snorers 14 Normocapnic OSA 11 Hypercapnic OSA 11 Overlap	45 ± 3 years (M/F) 45 ± 2 years (M/F) 47 ± 2 years (M/F) 59 ± 3 years (M/F) 54 ± 4 years (M/F)	Read (1967)	4 weeks of nCPAP	1.66 l/min/mmHg 1.26 l/min/mmHg NS 2.41 l/min/mmHg* 0.93 l/min/mmHg* 2.93 l/min/mmHg*	↑ Slope of HCVR in Normocapnic & Overlap OSA patients vs. Controls. ↓ Slope of HCVR in Hypercapnic OSA vs. Controls.
	15 OSA follow-up	45 ± 2 years / M			Pre: 1.93 vs. Post: 1.97 l/min/mmHg	No change in HCVR after CPAP.

HVR = Hypoxic ventilatory response; HCVR = Hypercapnic ventilatory response; VE = Ventilation; SRDB = Sleep-related disordered breathing; OHS = Obesity Hypoventilation Syndrome; M = Male; F = Female; nCPAP = nasal Continuous Positive Airway Pressure.; ↓ = decrease; ↑ = increase; * = $p < .05$; ** = $p < .01$.

Continued

Study	Participants	Age (mean ± SD) / Gender	Rebreathing or Steady-state protocol	CPAP therapy	Results	Summary
Tun et al., (2000)	28 Newly-diagnosed OSA	48 years (M/F)	Read (1967) Rebuck and Campbell method	2 weeks of nCPAP 3-6 months follow-up (n = 10)	Pre: 1.47 vs. Post 2-weeks: 1.80 l/min/mmHg* Pre: .80 vs. Post 2-weeks: .61 l·min·%SaO_2* Pre BMI: 30.5 ± 5.8 kg/m^2 vs Post BMI: 29.8 ± 5.4 kg/m^2)*	↑ Slope of HCVR and ↓ Slope of HVR after 2 weeks of CPAP. No further improvement after 3-6 months of CPAP.
Spicuzza et al., (2006)	25 Moderate to Severe OSA: 15 nCPAP group, 10 Sham nCPAP group	55 ± 9 years (M/F) 55 ± 9 years (M/F)	Read (1967) Rebuck and Campbell method	1 month of nCPAP 1 month of Sham nCPAP	CPAP: 1.22 vs. Post: 1.22 l/min/mmHg Sham: 1.00 vs. Post: 1.20 l/min/mmHg CPAP: 1.08 vs. Post: 0.53 l/min/·%SaO_2** Sham: 0.83 vs. Post: 0.85 l/min/·%SaO_2 NS	No difference in Slope of HCVR after CPAP & Sham CPAP. ↓ Slope of HVR after 1 month of CPAP. No change with Sham nCPAP.
Foster et al., (2009)	8 OSA patients 10 Controls	41 ± 2 years (M) 37 ± 2 years (M)	Dynamic end-tidal forcing (Poulin et al., 1996; Ainslie & Poulin, 2004)	4 - 6 weeks of CPAP	Baseline: 47.5 vs 53.0 (L/min) Follow-up: 47.5 vs. Post: 48.5 (L/min) NS	No difference in HCVR of OSA patients and Controls. No change with CPAP.
Radovanovic et al., (2019)	62 OSA patients 48 Controls	50 ± 12 years (M/F) 51 ± 11 years (M/F)	Modified version of Read rebreathing method Benlloch et al., 1995	1, 3, and 12 months of CPAP		Normocapnic HVR ↑ in OSA patients vs. Controls. Significantly HVR ↓ after CPAP. No difference in Normoxic HCVR between groups. No change with CPAP.

HVR = Hypoxic ventilatory response; HCVR = Hypercapnic ventilatory response; VE = Ventilation; BMI = Bod Mass Index; SRDB = Sleep-related disordered breathing; OHS = Obesity Hypoventilation Syndrome; CSA = Central Sleep Apnoea; M = Male; F = Female; CPAP = Continuous Positive Airway Pressure.; ↓ = decrease; ↑ = increase; * = $p < .05$; ** = $p < .01$.

2.4 Perception of Effort

Overview of section

As will have become clear from the previous section, OSA patients experience chronic intermittent hypoxia and a chronic overloading of the respiratory muscles during sleep that has the potential to alter the central and peripheral chemosensitivity in response to hypoxia and hypercapnia. However, to our knowledge, there are very few studies that have examined the impact of loading upon the perceptual and activation of the respiratory and skeletal muscles in OSA. Therefore, the following section will integrate research underpinning the perceptual and muscular response to loading in healthy and clinical populations.

2.4.1 Definition of perception of effort

Perception of effort, also known as sense of effort or perceived exertion, has traditionally been defined as the feeling of how heavy, strenuous and laborious exercise is (Borg, 1962). The original definition was later extended to include the sensations of discomfort and / or fatigue and interpreted as the subjective intensity of effort, strain, discomfort, and / or fatigue that is experienced during physical exercise (Robertson & Noble, 1997). However, more recently, Marcora (2010) referred to perception of effort as the conscious sensation of how hard, heavy, and strenuous a physical task is, with a greater emphasis placed upon the perceived effort within the working limbs and sensation of heavy breathing. The sensation is known to play an important role in activities of daily living (Julius et al., 2012), exercise performance (Pageaux et al., 2015), and rehabilitation in both health and disease (Noble & Robertson, 1996). Several pathological conditions have been reported to possess abnormal perceptions of effort and muscle fatigue, these include; chronic fatigue syndrome, fibromyalgia, stroke, spinal cord injury, and multiple sclerosis (Gandevia, 2001; Lloyd et al., 1991; Miller et al., 1996). As a result, effort perception is frequently assessed during fatiguing protocols to tease apart the underlying neurophysiology of many conditions.

2.4.2 Traditional measurement of perception of effort

Perception of effort is most frequently measured using two psychophysical scales; 15-point Rating of Perceived Exertion (RPE) scale and Borg category ratio (CR10) scale. Both scales have been validated according to measurements of blood lactate and heart rate during

exercise (Day et al., 2004; Noble et al., 1983). The 15-point rating of perceived exertion (RPE) scale ranges from 6 “no exertion at all” to 20 “maximal exertion”. It was originally constructed to monitor exercise intensity during steady-state aerobic exercise (Borg, 1998). The Borg category ratio (CR10) scale is also used to rate perception of effort and has been further modified to assess sensations such as pain and breathlessness. The 10-point Likert scale runs from 0 “no effort at all” to 10 “maximal effort” with the possibly to rate effort above the numerical value of 10 **(Figure 2.5)**. These scales have been further used as a gauge of effort perception during isometric contractions and inspiratory pressures in both healthy and stroke subjects (Hampton et al., 2014) and OSA patients (Earing, 2014). Other scales are available to quantify perception of effort, these include; Visual Analogue Scale (Crichton, 2001), Numeric Rating Scale (Williamson & Hoggart, 2005), Likert scales (Grant et al., 1999), Verbal Rating Scales (Lund et al., 2005). However, the Borg scale remains the most widely used in experimental research given its multi-purpose function and numerical values that are equidistant of each other.

A

6	No Exertion at all
7	Extremely Light
8	
9	Very Light
10	
11	Light
12	
13	Somewhat Hard
14	
15	Hard (Heavy)
16	
17	Very Hard
18	
19	Extremely Hard
20	Maximal exertion

B

0	No effort at all
0.5	Very, very light
1	Very light
2	Light
3	Moderate
4	Somewhat hard
5	Heard (heavy)
6	
7	Very hard
8	
9	Very, very hard
10	Maximal effort
•	

Figure 2.5. Psychophysical scales used to quantify perception of effort. Panel A illustrates the Borg 6-20 RPE scale and panel B the modified version of the CR10 scale (Borg, 1998, 2007).

2.4.2 Neurophysiology of perception of effort

The neurophysiology underlying muscle sensations (i.e. effort, force, and heaviness) has received significant debate over the last century. However, the question remains whether effort perception is entirely generated by central and peripheral mechanisms or is attributed to a combined central-peripheral input. The prevailing view held by Helmholtz (1867) proposed that all muscular activity and movement was generated within the central nervous system. In other words, a descending motor command is perceived and consequently an "effort of will" or "sense of effort" is generated (McCloskey, Ebeling, & Goodwin, 1974). This concept was later referred as the "sensation of innervation". Sperry (1950) later introduced the concept of corollary discharge which refers to a copy of motor discharge sent to the sensory cortex of the brain in order to generate a perceived effort. Centrally mediated sensations are presumed to arise from internal neural correlates (i.e. corollary discharges) of the descending motor command (McCloskey, 1981). The corollary discharges function to provide an internal adjustment of the sensory centres and are presumed to reflect the magnitude of the voluntary motor command. This efference copy of motor drive is shaped by its previous experiences and external environment (Proske, 2005).

Sherrington (1900) opposed this viewpoint and suggested that peripheral receptors located within the muscle spindles, tendon organs, and pressure-sensitive skin receptors were responsible for producing muscle sensations (e.g. effort, force, and heaviness) (Matthews, 1982). Von Holst & Mittelstaedt (1950) studied the concept further by introducing the term "reafference" which refers to the afferent information generated by the body's own actions / movements (Proske & Gandevia, 2012). Afferent feedback generated within the muscles is key to providing and updating the central motor patterns with information regarding the force and effort required to complete a given task. The complete afferent signal that terminates at the brain has both exafferent and reafferent components (Proske & Allen, 2019). The term exafference refers to additional afferent signals that are imposed by external influences (McCloskey, 2011).

Bastian (1888) suggested that both central and peripheral contributions to muscular sensations were a possibility. The combined model postulates that effort perception is derived from not only corollary discharge but also the afferent feedback that is sensed within the working muscles (Amann et al., 2010; Bergstrom et al., 2015). Feedforward (efference

outflow) and feedback mechanisms (reafference signal) are compared to produce a continuous adjustment of force or pressure production (Cafarelli, 1982). Any misalignment between the two signals is unconsciously adjusted with the assistance of proprioceptive receptors / afferents (Cafarelli, 1988; Carson, Riek, & Shahbazpour, 2002; Gandevia & Burke, 1992). However, the afferent feedback is not thought to be the direct sensory signal that generates the perception of effort. It remains uncertain whether this theory holds true due to the lack of studies that have investigated the model. However, this viewpoint still forms our current understanding underlying perceptions of effort (Proske & Allen, 2019) (**Figure 2.6)**. Collectively, these studies suggest that central sensory areas receive input from the voluntary motor command with the guidance of incoming signals from the periphery in order form a perception of effort.

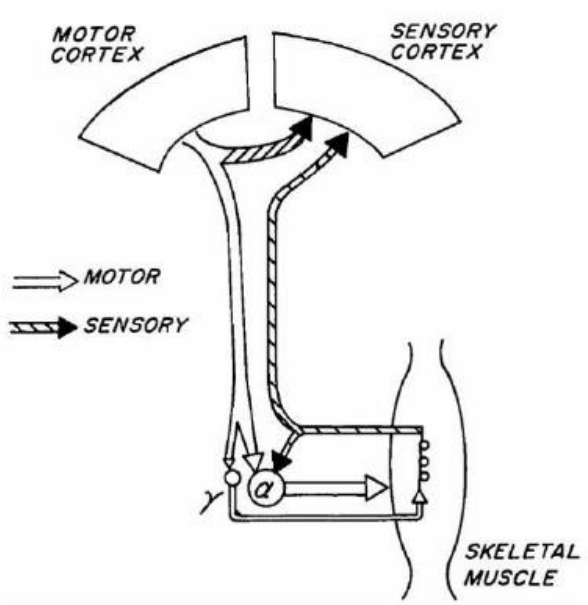


Figure 2.6. Pathways for corollary discharge and afferent feedback mechanisms of effort perception (α = Alpha; γ = Gamma) (Cafarelli, 1982) .

2.4.3 Perception of effort and force estimation

Perception of effort is studied using a number of different methods such as magnitude estimation which involves assigning a numerical value to a force produced based upon the perceived magnitude of a tension or load (Cain & Stevens, 1971; Eisler, 1965). Weight

discrimination involves discriminating between a set of weights based upon the perceived heaviness of the weight lifted (Gandevia & Mccloskey, 1977; Mccloskey & Gandevia, 1978). Contralateral force-matching tasks are used to examine the forces exerted by a muscle group in one reference limb (with use of external feedback) to the magnitude of forces generated by the corresponding muscle group of the contralateral limb (without assistance of feedback) (McCloskey et al., 1974). Subjects are asked to re-produce the force or tension with the opposite limb after the reference limb is disturbed via muscle fatigue, high-frequency vibration, or electrical stimulation (Jones & Hunter, 1983). These techniques are utilised to determine whether force sensation is based upon centrally derived sensations or sensory feedback arising from the working muscles (Cafarelli, 1982; Jones, 1995).

Several studies have shown that effort perception in neurologically intact subjects is influenced by muscle fatigue as shown by the significant overestimation of forces produced with the contralateral limb when participants are asked to match the reference force (Gandevia & Mccloskey, 1978; Jones & Hunter, 1983; Walsh, Taylor, & Gandevia, 2011). These results suggested an attenuated central motor drive was responsible for the force overestimation (Mccloskey, 1980; Gandevia et al., 2006). These studies further reported that healthy subjects based their judgement of force on the signal that is related to the size of the motor command (or the effort required) and not the peripheral signal that relates to the tension produced (pressure or absolute force). However, neural systems underlying force estimation is thought to be continuously calibrated via afferent input according to the capacity of the respiratory/locomotor muscles. Afferent feedback from fatigued muscles is crucial given that it increases the conscious awareness of ‘bodily discomforts’ experienced during loading (Craig, 2009). In particular, the muscle spindles and golgi tendon organ receptors located within the contracting muscles are responsible for providing information regarding changes to body movement, position, and muscle force/tension (Proske & Gandevia, 2012). Whilst, the sensory information responsible for detecting changes in breathing effort is reliant upon the input from a multitude of proprioceptive receptors located throughout the respiratory system (upper/lower airways, thorax, lungs, and muscles) that feeds back to the brainstem (Gandevia, 1988).

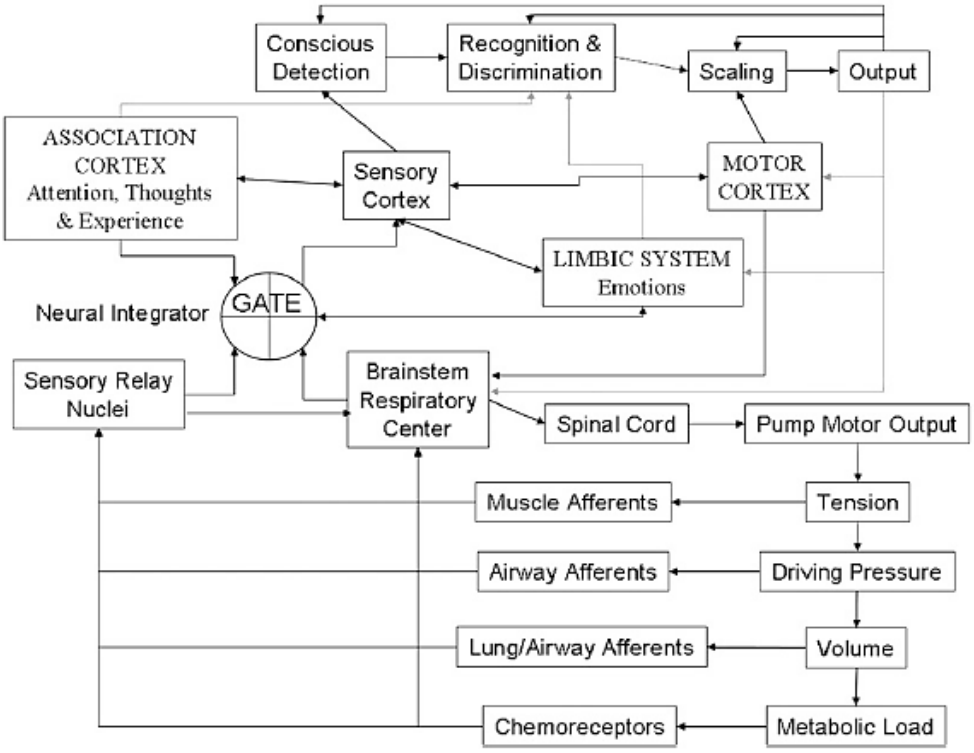


Figure 2.7. The respiratory gating system model (O'Donnell et al., 2007).

Muscle fatigue can be broadly defined as a loss of capacity to develop force and/or velocity in response to a load, which is reversible by rest (NHLBI, 1990). However, the definition of muscle fatigue may also include an impaired motor performance, gradual reduction of force production, increased electromyography (EMG) activity, and a shift in the power spectrum to lower frequencies (Roussos & Zakynthinos, 1996). Central fatigue is seen as the failure to produce force due to a reduction of the central motor output that affects the activation of the motorneuron pools and is measured by producing maximal voluntary and maximal electrically stimulated contractions following loading. Peripheral fatigue is viewed as the reduction of force in response to the failure of impulse propagation along the neuromuscular junction and/or membrane, failure of the contractile properties (e.g. excitation-contractile coupling), and biomechanical alterations occurring within the muscles (e.g. metabolic processes) (Amann et al., 2015; Enoka & Duchateau, 2008). Whilst, respiratory muscle fatigue is defined as the inability to maintain the pressure required for adequate alveolar ventilation which leads to the development of hypercapnic respiratory failure (Roussos & Macklem, 1986).

Inspiratory loading protocols are frequently performed to study the underlying mechanisms involved in effort perception (Cafarelli & Bigland-Ritchie, 1979). Supinski et al., (1987) examined the relationship between inspiratory muscle fatigue and effort perception in response to threshold loading (60-120 cmH_2O). Loaded breathing was shown to produce inspiratory muscle fatigue and a progressive increase in effort perception (CR10 Borg scale) in healthy subjects. These findings led the authors to suggest the increased effort perception and reduction in the power spectrum of the diaphragm (marker of muscle fatigue) were indicative of the conscious awareness of central motor drive that is consistent with previous findings in the respiratory and skeletal muscles (Gandevia, Killian, & Campbell, 1981; McCloskey et al., 1974). Further studies have reported patients with neuromuscular conditions (e.g. Muscular Dystrophy and Myasthenia Gravis) exhibit heightened neuromuscular output which is perceived as an increased respiratory effort before completing the fatiguing loading protocol (maximal voluntary ventilation) (Bégin et al., 1982; Spinelli et al., 1992). The heightened sense of effort was attributed to weakness and fatigability of the respiratory muscles as reflected by the marked reduction in the maximal inspiratory and expiratory pressures.

Therefore, in keeping with this concept, patients with OSA possess mechanical and neuromuscular constraints on the respiratory system that could potentially lead to an altered perceptual and muscular response when loaded during wakefulness. Unfortunately, there has been very little research undertaken that has examined the perceptual and muscular response to loading in OSA patients. Tun et al., (2000) was the first study to examine the inspiratory effort sensation following resistive loading in OSA patients and healthy subjects. The study revealed that OSA patients have an impaired inspiratory effort sensation (measured via Borg scale) to added inspiratory resistive loading that is reversed following 2 weeks of nasal CPAP therapy. These findings suggested that OSA patients demonstrate an altered effort sensation in response to loading that is modified by CPAP therapy. However, the study used the modified Borg scale to assess effort sensation and used fixed breathing resistances as a method of loading the respiratory system that were not modified on an inter-individual basis. Thereafter, Earing et al. (2014) designed an effort sensation paradigm which instructed participants to generate an inspiratory pressure (using the Powerbreathe device) at a fixed effort perception of 14 on the 6-20 Borg Scale. The effort perception was assessed after each completed set of 20 breaths at an intensity of 50% of maximal inspiratory pressure in OSA patients and healthy individuals. The study revealed that OSA patients with a higher AHI

(OSA severity) were found to generate lower inspiratory forces with the Powerbreathe device at a fixed RPE of 14 following bouts of loading. Whilst, the total force production was revealed to be elevated which suggested a possible recruitment of the accessory muscle as a power source. These results formed the premise of conducting this thesis with the overall aim of gaining a better understanding of the perceptual and muscular response to loading in OSA patients. With this information in mind, we must also consider the potential inspiratory and skeletal muscular adaptations that are observed in OSA patients.

2.4.4 Inspiratory and skeletal muscle adaptations in OSA

Respiratory muscles are morphological and functional skeletal muscles responsible for a range of complex functions such as respiration, posture, and bodily movement (Green & Moxham, 1985). The inspiratory muscles are composed of the diaphragm, intercostal muscles (parasternal and external intercostal), and the so called 'accessory muscles' (Luce & Culver, 1982). The primary muscle responsible for inspiration is the diaphragm, which is a thin, dome shaped, sheet of musculotendinous structure that separates the thoracic cavity from the abdominal wall. Patients with obstructive lung disease are known to possess a mechanically inefficient diaphragm that shifts the ventilatory demand onto the intercostal and accessory muscles (Sharp et al., 1980; Sharp et al., 1977). Obese patients with OSA have further showed lower diaphragmatic EMG activity in response to occluded breathing during sleep compared with normal subjects (Lopata & Onal, 1982; Sharp, Druz, & Kondragunta, 1986). These observations have led the authors to speculate the length-tension disadvantage, an overstretching of the diaphragm muscle as a consequence of obesity, is the proposed mechanism that underlies the development of hypoventilation and respiratory failure (Buyse et al., 2003). Therefore, an altered recruitment strategy of the respiratory muscles may be adopted in individuals that possess neuromuscular and/or mechanical constraints in order to maintain efficacy of the muscle pump.

The inspiratory muscles (intercostals and accessory) work simultaneously with the diaphragm to produce changes in thoracic volume and pressure. The intercostal muscles are composed of two thin layers of muscle which occupy the intercostal spaces (De Troyer et al., 1998). The parasternal and external intercostal muscles are responsible for co-ordinating the expansion of the rib cage and abdominal wall during quiet breathing and loading (Campbell & Newsom Davis, 1970; De Troyer et al., 1998; Macklem, Macklem, & De Troyer, 1983). The

inspiratory muscles are also postulated to trigger a protective reflex that causes the upper airways to open when the interlaryngeal pressure threshold is reached during sleep in OSA patients (Vincken et al., 1987). Furthermore, McNicholas et al., (1984) revealed that patients with untreated OSA have an impaired ability to sense narrowing and occlusion of the upper airways which adds to the developing concept that OSA patients demonstrate subtle defects in respiratory regulation during wakefulness and sleep. Therefore, the accessory muscles of inspiration (e.g. scalene, sternocleidomastoid, pectorals, and trapezius) are even more crucial to support and stabilise the ribcage during loaded breathing (Raper et al., 1966; Tokizane, Kawamata, & Tokizane, 1952; Yokoba et al., 2003). However, an overreliance of the accessory muscles during loading in obese populations possibly adds to further inefficiency of the primary inspiratory muscles and increased work of breathing to overcome the elastic resistance (Cherniack & Guenter, 1961; Fritts et al., 1959). These findings have led us to speculate that OSA patients are at a higher risk of developing inspiratory muscle fatigue and increased perception of effort when the respiratory muscles are loaded. However, relatively few studies have assessed the electromyography activity of the inspiratory and peripheral muscles during loading in OSA patients.

Chien et al., (2010) investigated the muscle strength, endurance, and fatigability of the inspiratory muscles and peripheral knee extensors in OSA patients and age-matched controls. The inspiratory and peripheral muscles were assessed to determine whether the effects of OSA were generalised or specific to muscles that were subjected to increased use during sleep. Whilst, the peripheral muscles were studied as they were not considered to be loaded during sleep. The fatiguing inspiratory protocol consisted of maximal voluntary ventilation (MVV) manoeuvres followed by a series of maximal inspiratory pressure (PI_{max}) measures. Whilst the knee extensor loading protocol consisted of repeated cycles of alternative knee extension/flexion isokinetic contractions followed by a series of MVC's. Surface EMG measures of the Diaphragm and Vastus Lateralis muscles were recorded during the re-assessment of PI_{max} and MVC. Patients with OSA demonstrated lower baseline strength (PI_{max} and MVC) and endurance during loading. The EMG amplitude of inspiratory and knee extensor muscles were shown to decline in response to loading in OSA patients. Furthermore, the inspiratory muscles demonstrated greater fatigability during loading and magnetic stimulations in OSA patients. However, the study was limited by differences in the group's baseline strength and fatiguing tasks that were of different intensities. The recurrent deoxygenation-reoxygenation pattern and chronic overloading of the inspiratory muscles that

characterise OSA were speculated to exacerbate and promote the muscle dysfunction within this population.

Recent studies have attempted to establish the link between hypoxia and adverse changes to the structure and function of muscles in animal models and patients living with pathological conditions such as chronic heart failure, peripheral arterial disease, and chronic obstructive pulmonary disease (COPD) (Jakobsson et al., 1995; Lundgren et al., 1989; O'Halloran & Lewis, 2017; Sullivan et al., 1990). Muscle dysfunction is implicated in the pathophysiology of OSA with patients demonstrating altered force production and enhanced fatigability of the pharyngeal dilator muscles and diaphragm during wakefulness (Bradford, McGuire, & O'Halloran, 2005; Eckert et al., 2011; McGuire, MacDermott, & Bradford, 2003). Neurogenic alterations and atrophy of the palatopharyngeal muscle fibres have also been reported in OSA patients (Edstrom, Larsson & Larsson, 1992). A growing body of evidence has found that OSA patients have an abnormal fibre type distribution in the pharyngeal muscles that indicates a transition from slow to fast-twitch muscle fibres (Ferini-Strambi et al., 1988; Sériès et al., 1996). The shift towards type IIA glycolytic muscle fibres promotes fatigability and anaerobic metabolism of the muscles (Sériès et al., 1995). Abnormalities of muscle structure (increased diameter and protein content of type II fibres) and bioenergetic enzymes (upregulated cytochrome oxidase activity - terminal enzyme for electron transport chain and phosphofructokinase activity – rate limiting glycolytic enzyme) were also reported in the quadriceps femoris muscle of OSA patients (Sauleda et al., 2003).

Sleep fragmentation often contributes to the higher levels of subjective fatigue reported by OSA patients (that is independent of excessive daytime sleepiness) at rest and during maximal exercise (Aguillard et al., 1998; Guilleminault et al., 2007; Yue et al., 2009). It has also previously been reported that fatigue produced by high and low-frequency simulation of the diaphragm muscle leads to a reduction in muscle pH and impairs calcium release from the sarcoplasmic reticulum. The altered metabolic state could potentially be the result of an intracellular accumulation of H^+ which influences the contractile properties of the muscles i.e. excitation-contraction coupling (Baylor, Chandler, & Marshall, 1983; Metzger & Fitts, 1987). An altered redox signalling has also emerged as a potential driver of muscle dysfunction in OSA. The excessive production of reactive oxygen species that occurs in response to the heightened activation of inspiratory muscles during obstructive events are thought to be

implicated in the respiratory and skeletal muscle dysfunction observed in OSA (Anzueto et al., 1994; Barreiro et al., 2007; Shortt et al., 2014; Williams et al., 2015). Future research has therefore attempted to identify potential therapeutic modalities for the prevention and treatment of muscle dysfunction. Several pharmacological agents (e.g. vitamin E, C, and N-acetylcysteine) and CPAP treatment are postulated to limit the damage free radicals exert to the muscle fibre type composition. Evidence suggests that in particular CPAP therapy has the ability to normalize upper airway muscle dysfunction (functional and structural alterations of genioglossal muscle) and lower oxidative stress in OSA patients (Alonso-Fernández et al., 2009; Carrera et al., 1999).

2.5 General Aims

With the information above in mind, we firstly examined the ventilatory responses to hypoxia and hypercapnia in healthy individuals with varied cardiovascular fitness levels (Trained and Untrained groups) and patients with newly-diagnosed OSA **(Chapter 4)**. We then investigated the potential contribution of cardiovascular fitness and body characteristics on the ventilatory responses to hypoxia and hypercapnia. Next, we examined the effect of CPAP treatment on the ventilatory responses to hypoxia and hypercapnia in OSA patients. Subsequently, we examined the perception of effort and activation of the Vastus Medialis and Lateralis muscles before and after loading in Trained and Untrained individuals and newly-diagnosed OSA patients **(Chapter 5)**. Thereafter, we examined the perception of effort and activation of the Intercostal and Trapezius muscles before and after loading in Trained and Untrained individuals and newly-diagnosed OSA patients **(Chapter 6)**. Finally, we examined effects of CPAP therapy on the perception of effort before and after repeated bouts of loading.

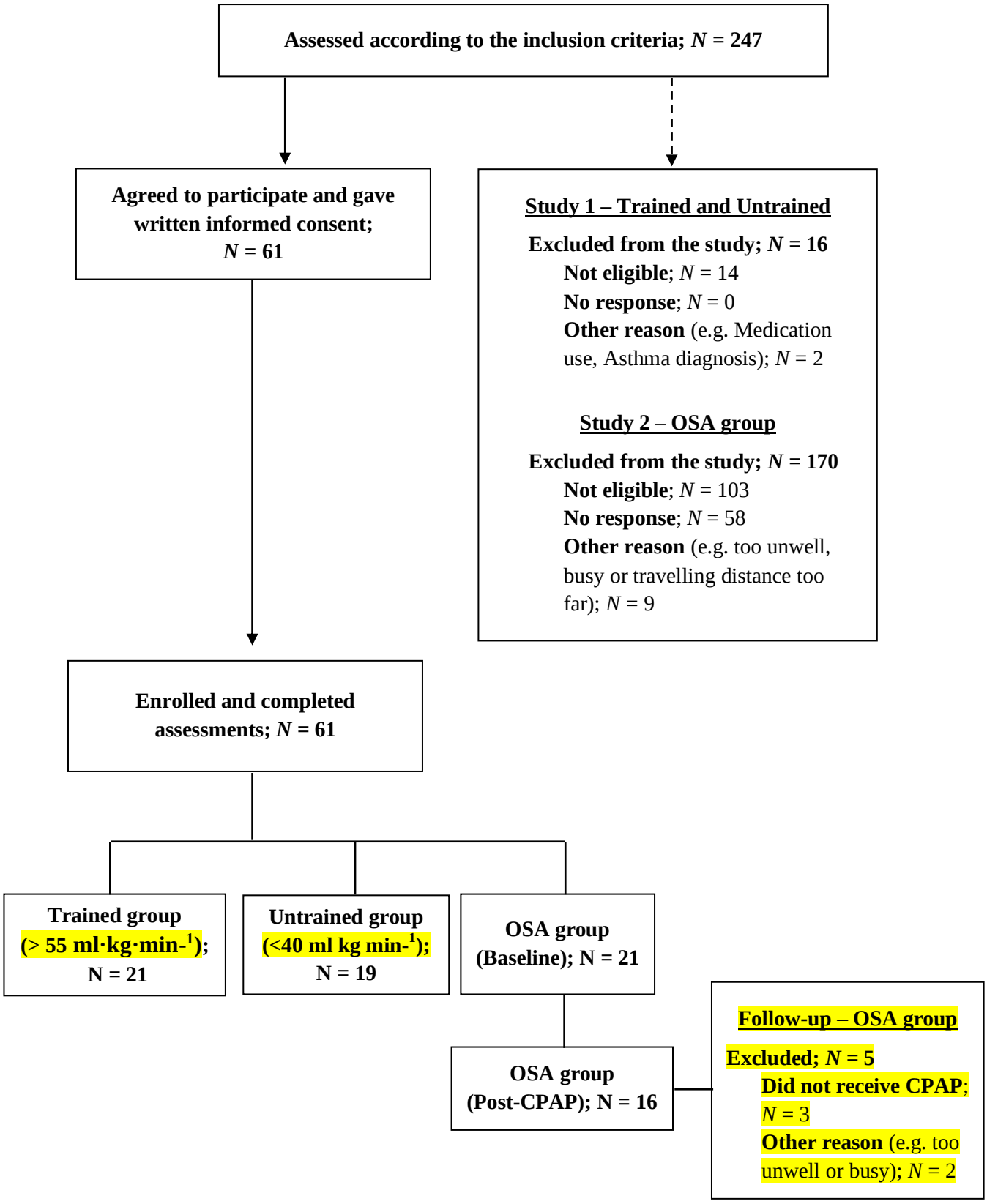
CHAPTER THREE

General Methods

3.1 Ethical Approval

Ethical approval was granted by the Departmental Research Ethics Committee of the School of Sport, Health, and Exercise Sciences (Bangor University; Ref: P01-16/17) for research in healthy participants and the East Midlands - Leicester South Research Ethics Committee (Ref: 17/EM/0162) for research in patients with Obstructive Sleep Apnoea (**Chapter 4, 5, and 6)**. The experimental protocols were performed in accordance with the Declaration of Helsinki for research in humans. Participants received written and verbal explanations of the experimental procedures and were informed they could withdraw at any time, without giving a particular reason, and the decision would not affect their relationship with School of Sport, Health, and Exercise Sciences (Bangor University) or the Pulmonary Function department (Ysbyty Gwynedd, Bangor). After answering any questions they had in relation to the experimental procedures, each participant gave written and verbal informed consent. A flow diagram of the participant recruitment is illustrated in **Figure 3.1.**

Figure 3.1. Flow diagram of participant recruitment (September 2016 - January 2018)



3.2-a Study Design (Healthy participants)

In the cross-sectional study, healthy participants visited the laboratory of the School of Sport, Health, and Exercise Sciences on four separate occasions as outlined in **Figure 3.2-a**. These four visits consisted of a health screen, skeletal muscle loading protocol, inspiratory loading protocol, and ventilatory control test.

Visit 1 - Health screen

Upon arrival to the laboratory, participants received a detailed explanation of the procedures and a checklist to ensure compliance with the eligibility criteria. Written informed consent was obtained from all participants. The general medical questionnaire was then completed to obtain information about each participant's current state of health, medical history, lifestyle, general physical activity levels, and training status (frequency, intensity, duration and type of training). The Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale were also completed to screen participants with undiagnosed sleep disorders. Body characteristics were then measured comprising of; height, weight, body mass index, body surface area, fat percentage, and neck, chest, waist, and hips circumferences. Forced expiratory volume (1 s) and forced vital capacity were measured with a portable hand-held spirometer to determine pulmonary function. Participants then underwent an incremental exercise test on a cycle ergometer to determine maximal oxygen uptake (VO_{2max}). The incremental exercise test began with participants cycling at 70 Watts (W) at a pedalling frequency of 60-90 revolutions per minute. The resistance was increased by 20 W per minute until volitional exhaustion occurred. Participants were then stratified into either Trained (VO_{2max} >55 ml·kg·min^{-1}) and Untrained (VO_{2max} <40 ml·kg·min^{-1}) groups based upon the outcome of the incremental exercise test (Astorino et al., 2012; Hassmén, 1990).

Visit 2 - Skeletal muscle loading protocol

Participants firstly completed a task-specific warm-up before performing a series of maximal voluntary contractions (MVC) using the custom-made isometric chair. Thereafter, participants generated five isometric forces at a clamped effort of 14 on the 6-20 Borg scale using standard procedures (Noble and Robertson, 1996). Participants were instructed to focus all of their efforts to the dominant leg, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as

'*brightest possible light*'. The skeletal muscle loading protocol was adapted from Earing et al., (2014) and consisted of five sets of twenty isometric contractions at 50% of MVC. After each set of isometric contractions, participants generated an isometric force at an effort of 14 for 2-3 seconds followed by a 30 second rest interval. Vastus Medialis and Lateralis muscle activity were recorded with electromyography (EMG) during all the above procedures.

Visit 3 - Inspiratory loading protocol

Participants completed a series of maximal inspiratory pressures (PI_{max}) using the KH2 POWERbreathe device. Thereafter, participants generated five inspiratory pressures at a clamped effort of 14 on the 6-20 Borg scale using standard procedures (Noble and Robertson, 1996). Participants were instructed to focus all of their efforts to the breathing muscles, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as '*brightest possible light*'. The inspiratory loading protocol was later performed which consisted of five sets of twenty breaths at 50% of PI_{max} (Earing et al., 2014). After each set of loaded breaths, participants generated an inspiratory pressure at an effort of 14 for 2-3 seconds followed by a 30 second rest interval. Intercostal and Trapezius muscle activity were recorded with EMG during all the above procedures.

Visit 4 - Ventilatory control test

The protocol was performed according to techniques as reported by Earing et al., (2014). Resting minute ventilation was firstly determined with the participant breathing through the system for 10 minutes whilst focusing on a non-dramatic video or light reading. The series of gas mixtures were then introduced in the following order; hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2) to measure the ventilatory response to each mixture for ~ 3-5 minutes. Ambient air was breathed in the interval between each respective gas mixture and sufficient time (5 minutes) was given to allow ventilation, blood pressure, heart rate, and oxygen saturation to return to baseline values. The process was repeated with the following gas mixture. Upon completion of the testing session, resting minute ventilation during ambient air (average 2 minute period) was subtracted from the maximal minute ventilation achieved for each respective gas (average 30 second period) to calculate the change in minute ventilation.

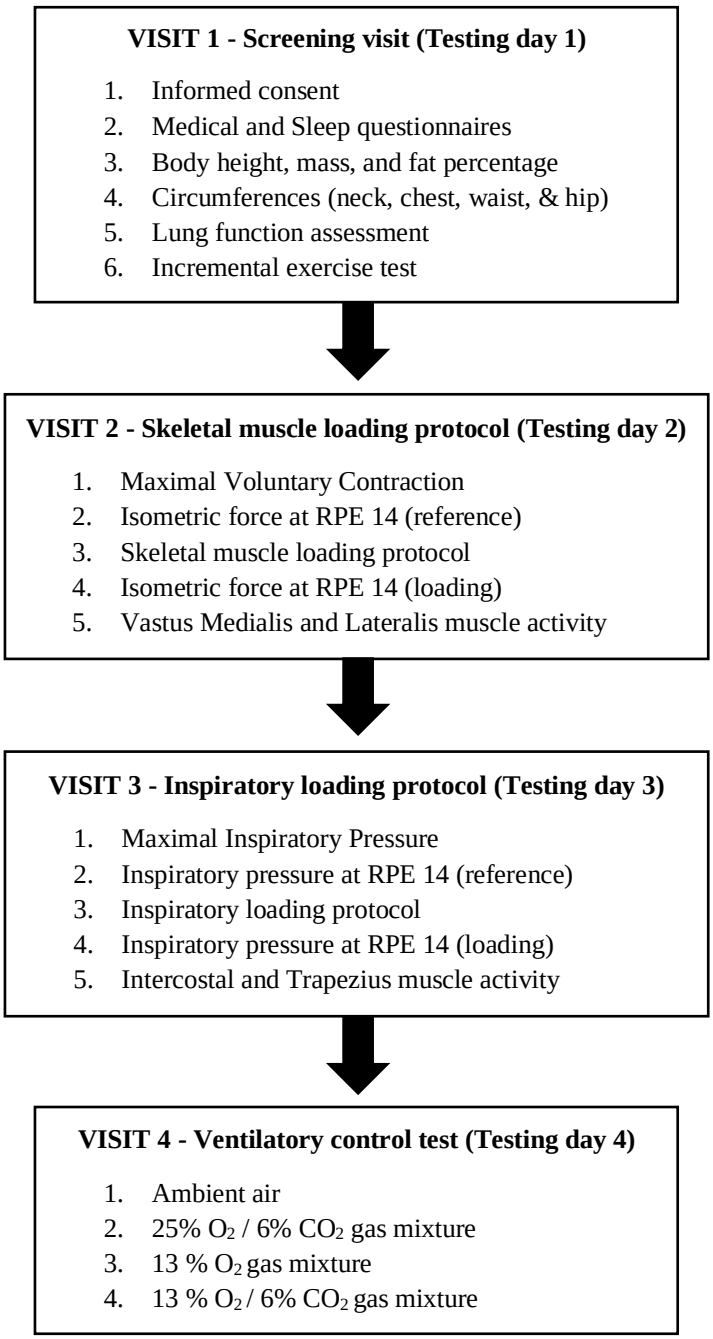


Figure 3.2-a. A schematic overview of the study protocol in healthy participants.

3.2-b Study Design (OSA patients)

In the cross-sectional study, patients with newly diagnosed OSA visited the laboratory at the Pulmonary Function department (Ysbyty Gwynedd) on two separate occasions as outlined below in **Figure 3.2-b**. The first visit consisted of a health screen, ventilatory control test, and skeletal muscle loading protocol. The second visit consisted of a CPAP set-up as part of routine care followed by the inspiratory loading protocol. Upon completion of the second session, OSA patients were given the option to attend the laboratory once more after receiving 12-weeks of Continuous Positive Airway Pressure (CPAP) treatment for the reassessment of health screen, ventilatory control test, and inspiratory loading protocol.

Visit 1 – Health screen, Ventilatory control test, and Skeletal muscle loading protocol

Upon arrival to the laboratory, OSA patients received a detailed explanation of the procedures and a checklist to ensure compliance with the eligibility criteria before written informed consent was obtained. The general medical questionnaire was then completed to obtain information about each participant's current state of health, medical history, and lifestyle. The Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale were also completed to assess each participant's sleep quality and level of sleepiness. Body characteristics were then measured which included; height, weight, body mass index, body surface area, fat percentage, and circumferences of the neck, chest, waist, and hips. Pulmonary function measures were assessed, these included; forced expiratory volume in one second, forced vital capacity, carbon monoxide uptake in the lung, and total lung capacity. Patients received an overnight sleep study as part of routine care to measure the apnoea hypopnea index (**see Chapter 3.10**).

The ventilatory control test was then performed in the second phase of the testing session. The protocol was performed according to techniques as reported by Earing et al., (2014). Resting minute ventilation was firstly determined with the participant breathing through the system for 10 minutes whilst focusing on a non-dramatic video or light reading. The series of gas mixtures were then introduced in the following order; hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2) to measure the maximal ventilatory response to each mixture for ~ 3-5 minutes. Ambient air was breathed in the interval between each respective gas mixture and sufficient time (5 minutes) was given to

allow ventilation, blood pressure, heart rate, and oxygen saturation to return to baseline values. The process was repeated with the following gas mixture. Upon completion of the testing session, resting minute ventilation during ambient air (average 2 minute period) was subtracted from the maximal minute ventilation achieved for each respective gas (average 30 second period) to calculate the change in minute ventilation.

After a 10 minute rest interval, participants completed a task-specific warm-up of the knee extensors of the dominant leg before performing a series of maximal voluntary contractions (MVC) using the custom-made isometric chair. Participants generated five isometric forces at a clamped effort of 14 on the 6-20 Borg scale using standard procedures (Noble and Robertson, 1996). Participants were instructed to focus all of their efforts to the dominant leg, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as '*brightest possible light*'. The skeletal muscle loading protocol was adapted from Earing et al., (2014) and consisted of five sets of twenty isometric contractions at 50% of MVC. Each contraction was performed in a rhythmic manner (3 seconds contraction followed by 3 seconds relaxation). After each set of isometric contractions, participants generated an isometric force at an effort of 14 for 2-3 seconds followed by a 30 second rest interval. Vastus Medialis and Lateralis muscle activity were recorded with EMG during the procedures.

Visit 2 – CPAP set-up and Inspiratory loading protocol

Upon arrival to the laboratory, patients firstly received a demonstration of the CPAP device with a Sleep technician (**see Chapter 3.10**). Thereafter, participants initiated the testing phase of the study by completing a series of maximal inspiratory pressures (PI_{max}) using the hand-held, resistive device. Thereafter, participants generated five inspiratory pressures at a clamped effort of 14 on the 6-20 Borg scale using standard procedures (Noble and Robertson, 1996). Participants were instructed to focus all of their efforts to the breathing muscles, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as '*brightest possible light*'. The inspiratory loading protocol was subsequently performed which consisted of five sets of twenty loaded at 50% of PI_{max} (Earing et al., 2014). After each set of loaded breaths, participants generated an inspiratory pressure at an effort of 14 for 2-3 seconds followed by a 30 second rest interval.

Intercostal and Trapezius muscle activity were recorded with electromyography (EMG) during the procedures.

Visit 3 (optional) - CPAP compliance, Health screen, Ventilatory control test, and Inspiratory loading protocol

During the optional testing session, participants received a progress report from a Sleep physiologist regarding the CPAP compliance and updated AHI. Following this, participants completed the health screen and ventilatory control test in an identical manner as Visit 1. After a rest interval of 10 minute, participants then performed the inspiratory loading protocol as described in Visit 2.

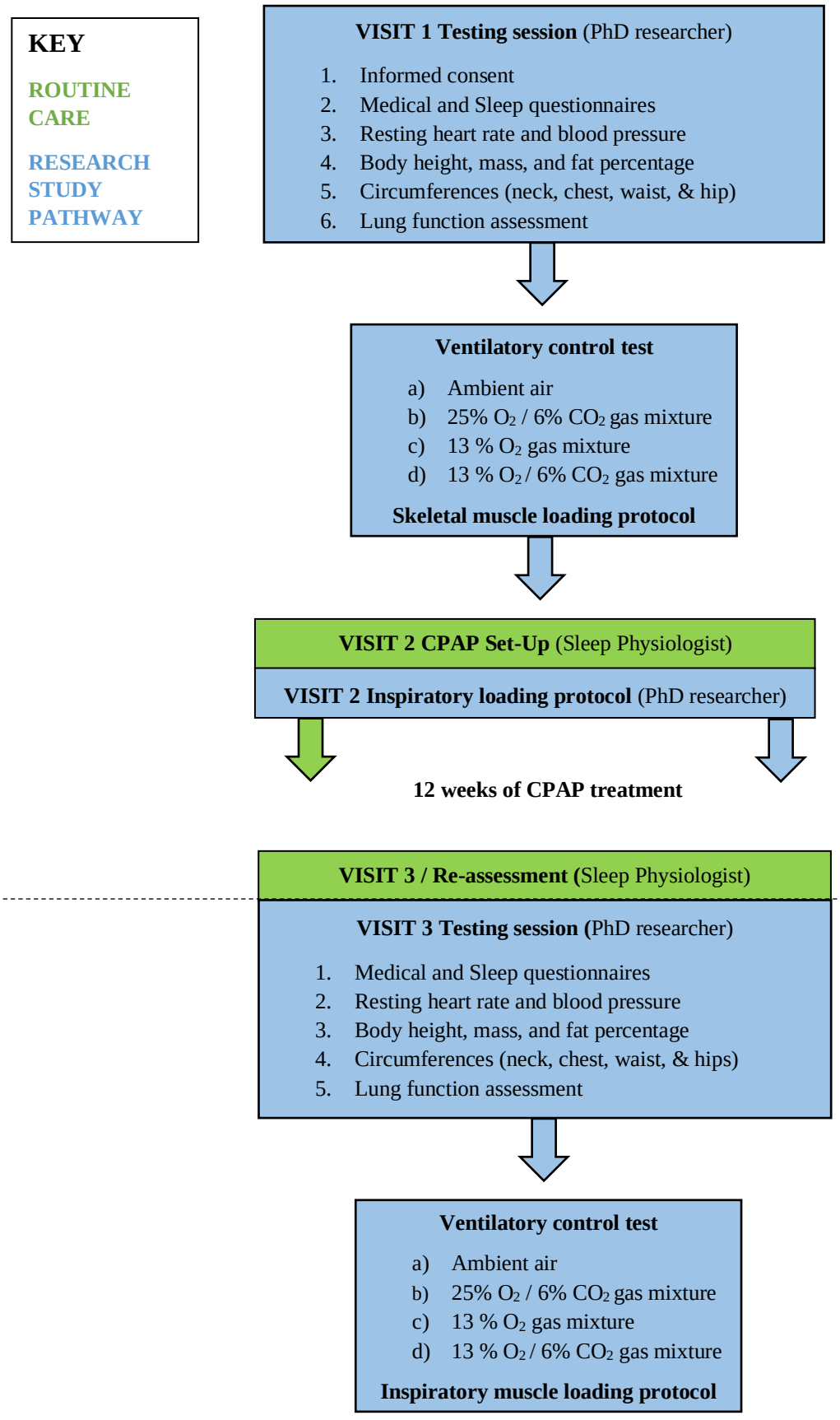


Figure 3.2-b. A schematic overview of the study protocol in OSA patients.

Screening Visit

Overview of testing session

Upon arrival to the laboratory, participants completed the informed consent, checklist, and general medical questionnaire to ensure compliance with the eligibility criteria. The general medical questionnaire was used to obtain information about each participant's current state of health, medical history, lifestyle, general physical activity levels, and training status. Next, participants completed a series of sleep questionnaires. Body characteristics were then recorded followed by a lung function assessment. The incremental exercise test was later performed on a cycle ergometer to determine the maximal rate of oxygen uptake.

3.3 Sleep Questionnaires

The Pittsburgh Sleep Quality Index (PSQI) is a standardised and self-rated measure of sleep quality over a 1 month period (Buysse et al., 1989). The valid and reliable measure was designed to identify 'good' and 'poor' sleepers in healthy and clinical populations (e.g. psychiatric patients). Participants were asked to answer each question with score ranging from 0 *'no difficulty'* to 3 *'severe difficulty'*. The nineteen individual items form the basis of seven component scores, these include; subjective sleep quality, latency, duration, habitual sleep efficiency, disturbances, use of sleep medication, and daytime dysfunction. The seven component scores were summed to produce a global score for each participant that ranges from 0 to 21, with higher PSQI scores (> 15) being indicative of poor sleep quality (**Appendix IV**). The Epworth Sleepiness Scale (ESS) is a valid and widely used measure of sleepiness in healthy and clinical populations (e.g. patients with a range of sleep disorders) (Johns, 1991). The self-administered questionnaire was developed to provide a measure of an individual's likelihood of falling asleep 'dozing' whilst engaged in eight day-to-day situations (e.g. item 3: 'sitting inactive in a public place'; item 2: 'watching TV'; and item 6: 'sitting and talking to someone'). Participants were asked to rate each situation retrospectively on a four point Likert scale (0-3) with 0 being 'unlikely to doze', 1 a 'slight chance of dozing', 2 a 'moderate chance of dozing', and 3 a 'high chance of dozing'. Individual responses were summed to produce a global score that ranges from 0 to 24. ESS scores that exceed 11 are indicative of excessive daytime sleepiness and possibly an underlying sleep disorder (**Appendix IV**). These two measures are routinely used

in the clinical assessment of patients with suspected OSA alongside usual diagnostic methods.

3.4 Anthropometry

Height and body mass were measured using a stadiometer and digital platform scale (SECA 223, SECA gmbh, Hamburg, Deutschland). These measures were performed to the nearest 0.1 cm and 0.1 kg. Participants wore light clothing (t-shirt and shorts) and removed footwear prior to weighing. Body mass index was calculated according to the following equation (BMI: kg / m^2) to produce an estimate of body fat. Each participant stood barefoot on the contact plate of a bioelectrical impedance analysis device in order to assess body fat percentage (BF%) (Model Tanita BC-418 MA, Tanita Corporation of America Inc., Illinois, USA). Due to an underestimation of the DuBois formula at estimating body surface area (BSA) in obese patients (Verbraecken et al., 2006), BSA was calculated with the (Du Bois and Du Bois, 1916) formula for healthy non-obese participants and (Mosteller, 1987) formula for obese OSA participants. Circumferences of the neck, chest, waist, and hips were measured to the nearest 0.1 mm using a retractable measuring tape (Model 201/ST, SECA, Hamburg, Germany).

3.5 Lung Function

Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured using a portable hand-held spirometer (MicroLoop Spirometer, MicroMedical Ltd., UK; Viasys VMAX Encore, SensorMedics Corporation, USA), in accordance with the American Thoracic Society / European Respiratory Society guidelines (Miller et al., 2005). To obtain reproducible results, the two highest values were required to be within 0.1 L (or <5%) and show technically acceptable flow-volume curves. The carbon monoxide uptake in the lung (DLCO) and total lung capacity (TLC) were measured at least twice in accordance with departmental Standard Operating Procedures. These measures were only conducted in OSA patients due to the testing equipment being extensively used by the clinical service during the testing period. Bacterial viral filters with an attached silicon mouthpiece and single-use nose clips were used for all participants. The equipment was calibrated before each day using a 3 litre syringe and the flow sensor disinfected weekly via Perasafe solution as per departmental risk assessment guidelines.

3.6 Maximal Oxygen Consumption in healthy participants

Healthy participants performed an incremental exercise test to volitional exhaustion on an electronically braked cycle ergometer to determine maximal oxygen uptake (VO_{2max}) according to Chien et al., (2013) (Excalibur sport; Lode, Groningen, The Netherlands). Each exercise test was performed at room temperature (18-20 °C) with a relative humidity of (< 70 %). Participants were given a full explanation of the testing procedure and use of the Borg 6-20 ratings of perceive exertion (RPE) scale (Borg, 1998). Following a 5 minute warm-up cycling at 50 Watts [60-90 revolutions per minute (RPM)], participants were asked to maintain cycling at 70 W (60-90 RPM) with increments of 20 W per minute until volitional exhaustion occurred. Heart rate and RPE were recorded during the final 15 seconds of each 1 minute stage and at the point of exhaustion (Polar Electro Oy, Kempele, Finland; Borg, 1998). Expired gases were measured breath-by-breath using a metabolic analyser (Metalyzer 3B, CORTEX Biophysik GmbH, Leipzig, Germany) with the VO_{2max} defined as the highest VO_2 (average 30 seconds). The criteria for attainment of VO_{2max} included the following; plateau in oxygen uptake despite an increase in workload, final heart rate (HR) within 10 beats of maximum HR, respiratory exchange ratio of ≥ 1.15, RPE of ≥ 18, cadence < 60 RPM (> 5 seconds despite strong verbal encouragement), and volitional exhaustion (Edvardsen, Hem, & Anderssen, 2014; Howley, Bassett, & Welch, 1995; Midgley et al., 2007). Healthy participants were stratified into Trained (VO_{2max} > 55 ml·kg·min^{-1}) or Untrained groups (VO_{2max} < 40 ml·kg·min^{-1}) based upon the VO_{2max} (Astorino et al., 2012; Hassmén, 1990). Patients with newly-diagnosed OSA did not complete the incremental exercise test.

3.7 Ventilatory response to moderate hypoxia and hypercapnia

Overview of testing session

To evaluate the ventilatory response to moderate hypoxia and hypercapnia the following protocol was performed. Firstly, resting minute ventilation was determined with the participant breathing through the system for 10 minutes whilst focusing on a non-dramatic video or reading. The following gas mixtures were then introduced in the fixed order; hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2). After each gas mixture, participants breathed ambient air in the interval

(minimum of 5 minutes) to allow ventilation, oxygen saturation, heart rate, and blood pressure to return to baseline.

Experimental set-up of the breathing system

The protocol was performed according to techniques as reported by Earing et al., (2014) in healthy and clinical populations. Participants were given clear instructions about the experimental procedure and potential symptoms they may experience whilst breathing the gas mixtures. Each assessment was conducted with the participant awake and relaxed in a seated upright position. Participants wore a fingertip pulse oximeter (Nonin Onyx Vantage 9590 Finger Pulse Oximeter, USA) and blood pressure cuff to allow measurements of heart rate, oxygen saturation, and blood pressure to be taken manually at 2 minute intervals. Participants were fitted to the breathing system (e.g. face mask and hairnet) and connected to the volume transducer and gas sampling port of the metabolic analyser (MetaMax® 3B, Cortex Biophysik, Germany; **Figure 3.3**). The metabolic analyser was calibrated with ambient air and the gas mixture consisting of 13% O_2 / 6% CO_2 before each testing session. A two-way valve was attached to the volume transducer and gas sampling port allowing gases to be inspired from a 250 L Douglas bag and expired into the atmosphere. The Douglas bag was specifically fed with gas concentrations balanced with Nitrogen (BOC Ltd., England) via a slow flowing regulator of a gas canister. The gas mixtures were inspired in the following order; hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2). Participants were blinded to the composition and order of the gas mixtures to prevent conscious control of breathing. Each participant was instructed to raise their hand to cease the testing session due to hypercapnia related symptoms for example, excessive breathlessness (air hunger), headache, or dizziness.

General procedure to measure ventilatory responsiveness

Resting minute ventilation was determined with the participant breathing through the system for 10 minutes whilst focusing on a non-dramatic video or light reading. The decision was made in order to prevent individuals from consciously controlling their breathing during testing. A potential order effect would be reduced by having participants watch a video or read whilst breathing the particular gas mixture. By preventing participants from concentrating on their breathing this would further attenuate the influence of psychological

factors (CO2-induced stress/anxiety etc). The risk of breathing two gas mixtures containing CO_2 in succession (which could happen with the randomized order) would risk an additive effect and might be more relevant than the order effect we implemented in the protocol. However, any order effect cannot be totally excluded.

After establishing resting breathing parameters, the Douglas bag was emptied using a vacuum and fed with the required gas concentration, before connecting a tube from the Douglas bag to the breathing system. Once a plateau in minute ventilation was achieved after ~ 3-5 minutes of breathing the gas mixture participants reported their degree of dizziness using a visual analogue scale. Ambient air was breathed in the interval between each respective gas mixture and sufficient time (5 minutes) was given to allow ventilation, blood pressure, heart rate, and oxygen saturation to return to baseline values. The process was repeated with the following gas mixture.

Data analysis of ventilatory response measurements

Expired gas concentrations and minute ventilation were averaged over a period of 5 seconds. Baseline measurements of minute ventilation were averaged over a period of 2 minutes. A moving average filter was utilised to highlight the plateau within the ventilatory response to each gas mixture. The maximal ventilatory response observed over a period of 30 seconds (with no obvious outliers) was averaged. Resting minute ventilation during ambient air was then subtracted from the minute ventilation achieved for each respective gas (~ 30 second average of raw data) in order to calculate the change in minute ventilation (ΔV X% CO_2 / Y% O_2). All minute ventilation data was normalised according to the BSA (**as described in Chapter 3.4**) to account for body mass related differences. A **s**chematic diagram of the ventilatory response to 25% O_2 / 6% CO_2 is presented in **Figure 3.4.**

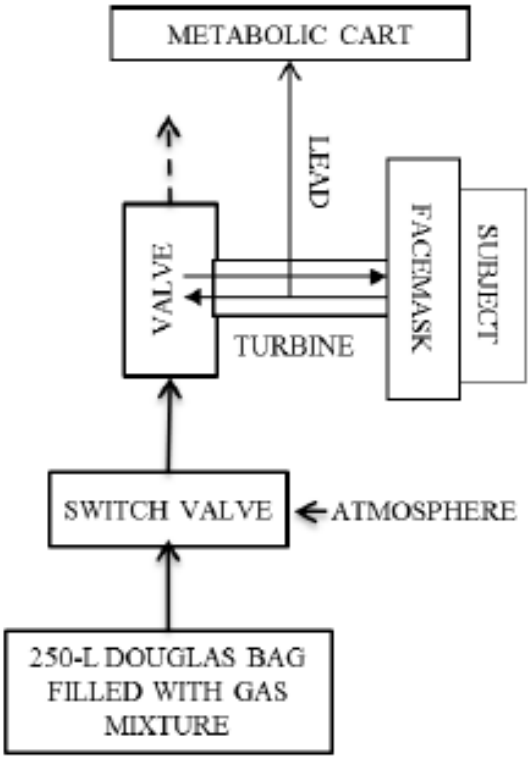


Figure 3.3. Experimental set-up of the breathing system used to assess the ventilatory response to hypoxia and hypercapnia. Photograph of a participant seated in the chair with the breathing system attached (Image used with permission).

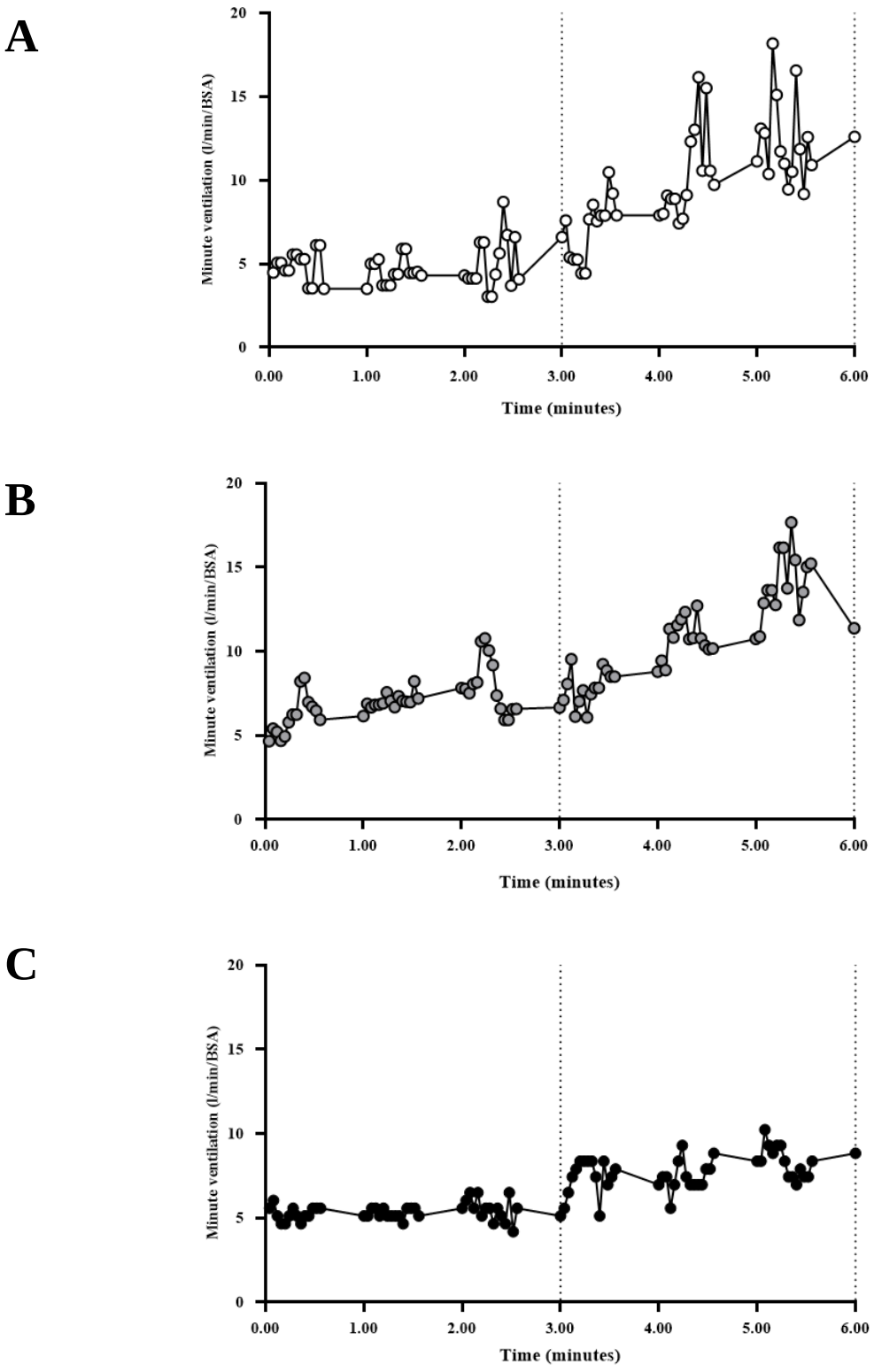


Figure 3.4. Schematic diagram of the ventilatory response to 25% O_2 / 6% CO_2 in a Trained (**A** - VO_{2max}: 66.7 ml·kg·min^{-1}), Untrained (**B** - VO_{2max}: 41.2 ml·kg·min^{-1}), and OSA participant (**C** - AHI: 109.8 events/hr). The dashed lines represent the phase of breathing the gas mixture.

3.8 Skeletal muscle loading protocol

Overview of testing session

To evaluate the impact of skeletal muscle loading on perception of effort and muscle activity of the knee extensor muscles we used the following protocol. Participants completed a task-specific warm-up before performing a series of maximal voluntary contractions using the custom-made isometric chair. Thereafter, participants generated five isometric forces at a clamped effort of 14 on the 6-20 Borg scale. The skeletal muscle loading protocol was later performed which consisted of five sets of twenty isometric contractions at 50% of MVC. After each set of isometric contractions, participants generated an isometric force at an effort of 14. Vastus Medialis and Lateralis muscle activity were recorded during the procedures.

Experimental set-up of the skeletal muscle loading protocol

After completing a 5 minute warm-up on a cycle ergometer at 50-100 W (60-90 RPM), participants sat on the custom-made isometric chair with an immovable pad positioned just proximal to the ankle joint of their dominant leg (Model 615, Vishay Tedea-Huntleigh, CA; **Figure 3.5).** The knee joint angle was fixed at a 90° angle with the level arm length set at 58 cm. The non-dominant leg was left hanging freely next to the chair. A harness was secured in order to prevent upper body movement during the protocol. Vastus Medialis and Lateralis electromyography (EMG) signals were then acquired by applying bipolar silver/silver chloride electrodes (Ambu® Neuroline 720, Ballerup, Denmark) longitudinally on shaved, cleaned, and abraded skin of the dominant leg in accordance with Surface EMG for Non-Invasive Assessment of Muscles recommendations (Hermens et al., 2000). A ground electrode was placed on the superior aspect of the patella and the inter-electrode distance minimised to < 2cm.

Participants then completed a task-specific warm-up consisting of 10 submaximal isometric contractions with the dominant leg for 3 seconds at approximately 50% of their maximal strength. The isometric force generated against the immovable pad during all procedures was measured using a load cell affixed to the base of the isometric chair. The force transducer was connected to a bridge amplifier which fed signals into the sixteen channel data recording unit (ML110 PowerLab; Powerlab-16 SP, AD Instruments, Australia).

General procedure of the skeletal muscle loading protocol

Maximal Voluntary Contraction (MVC) was subsequently measured to determine quadriceps muscle strength and set the intensity of the skeletal muscle loading protocol. Participants were asked to produce a maximal effort for 3 seconds with the dominant leg. A rest interval of ~ 1 minute separated each maximal effort. The procedure was repeated until seven isometric MVC's were performed in total. Participants received strong verbal encouragement during each maximal effort. The greatest isometric force produced with a technically acceptable trace was recorded as the MVC. However, the mean value was taken from the largest two MVC values if found within 10% of each other (McConnell, 2007). The isometric force data was measured in millivolts (mV) and converted into Newtons (N) using known weights (range 0 - 46.3 kg / 454 N).

After 5 minutes of rest, participants were instructed to produce an isometric force that was equivalent to the perceived effort of 14, which corresponded to the verbal anchors of *'somewhat hard'* to *'hard/heavy'* on the 6-20 Borg scale. The guidelines of Noble and Robertson (1996) were used to explain the concept behind using the RPE scale to each participant. Participants were instructed to focus all of their efforts to the dominant leg, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as '*brightest possible light*'. Earing et al., (2014) subjectively perceived that participants related better to an RPE of 14 (6-20 Borg scale) than 6 on the Borg CR-10 scale. The maximal force generated during the series of MVC's were interpreted as an RPE of 20 which familiarised participants to using the scale. Participants were informed there were no right or wrong answers and were encouraged to ask any questions. Five isometric forces at a clamped effort of 14 were then performed for 2-3 seconds and separated by a 30 second rest interval. These isometric forces were later averaged and referred to as the IF_{14} reference **(Appendix V)**. No visual feedback was given during IF_{14} measures in order to force participants to rely upon their perceptions as a gauge.

Following the assessment of MVC and IF_{14} reference, each participant completed the skeletal muscle loading protocol which consisted of five sets of twenty isometric contractions at a resistance equivalent to 50% of pre-recorded MVC. This target force (50% MVC) was set up on a monitor in full view of the participant. Each contraction was held for 3 seconds followed by a rest interval of 3 seconds and repeated in a continuous manner. Visual feedback was

available to participants during the MVC trials and loading protocol. Between each set of isometric contractions, participants were asked to reproduce the IF_{14} measure for 2-3 seconds followed by a 30 second rest interval. The five isometric forces were later averaged and referred to as the IF_{14} loading. All IF_{14} measures were normalised according to the MVC and expressed as percentage of MVC. The percentage difference was later calculated by subtracting each IF_{14} loading measure from the mean of IF_{14} reference. The experimental protocol is illustrated in **Figure 3.6.**

Data Processing of Vastus Medialis and Lateralis muscles

EMG signals were analysed offline using commercially available software (Power Lab Chart V 4.2.3, ADI Instruments, Bella Vista, Australia). The EMG signals of Vastus Medialis and Lateralis muscles were amplified (gain x 1000), band-pass filtered 35-500 Hz, and digitised at a sampling rate of 1000 kHz (Deschenes, Holdren, & Mccoy, 2008). Root Mean Square (RMS) amplitudes with a time constant of 100 ms were analysed over the 0.5 second window (the point at which peak pressure was generated). Data were normalised according to the maximal EMG and expressed as percentage of RMS (% RMS). Neuromuscular efficiency was calculated by dividing the isometric force generated during the IF_{14} with the average EMG activity for that contraction (Deschenes et al., 2008).

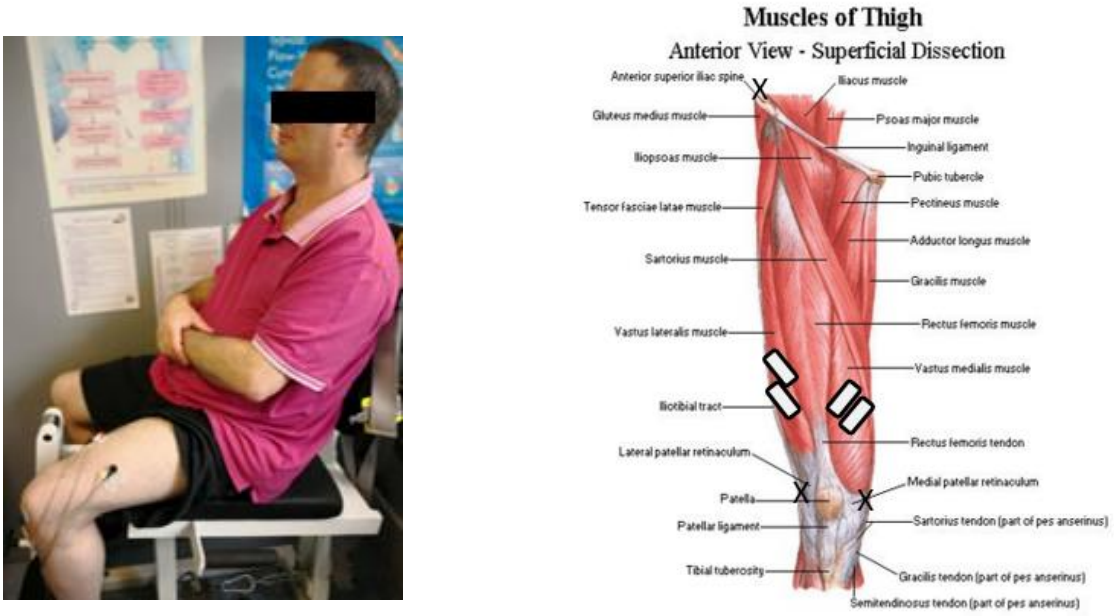


Figure 3.5. EMG electrode placement for Vastus Medialis and Lateralis muscles. Participant seated in the isometric chair during the skeletal muscle loading protocol (Image used with permission).

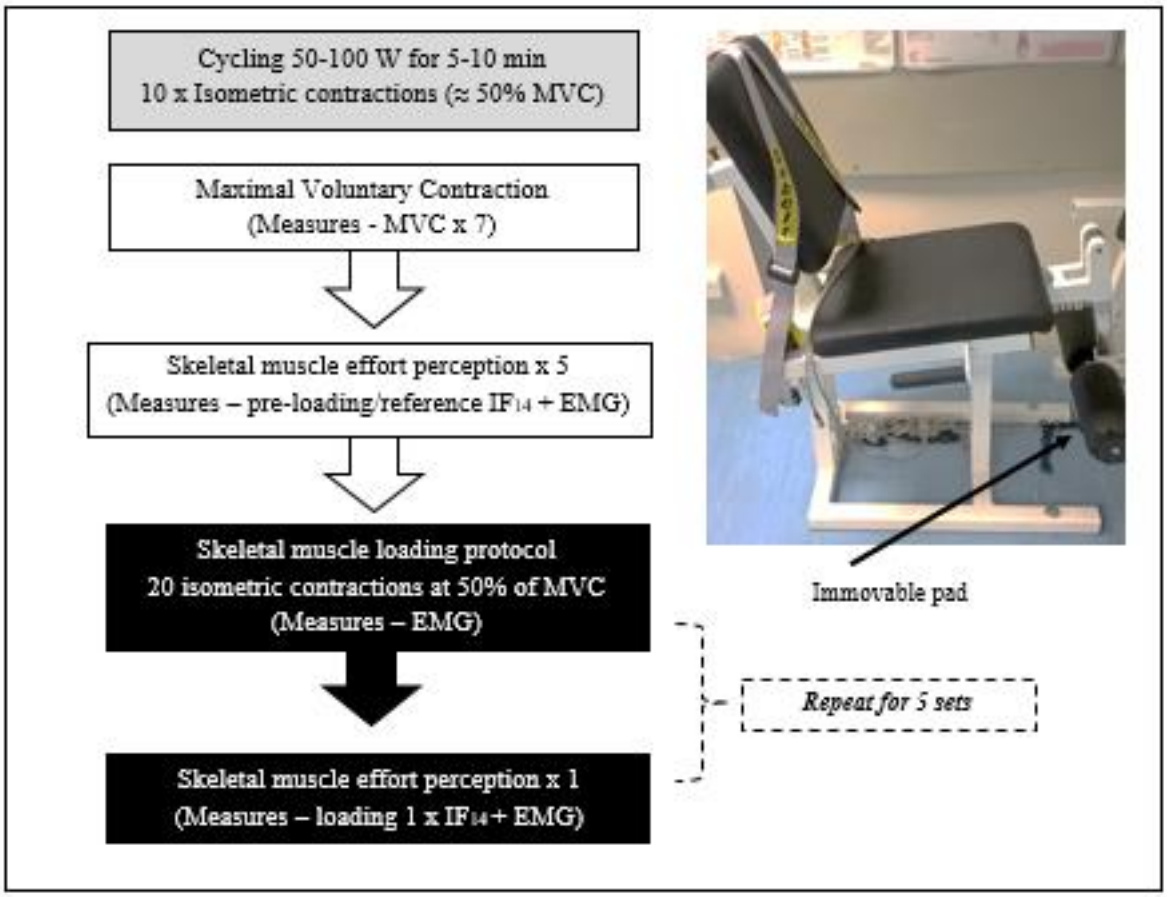


Figure 3.6. Schematic diagram of the skeletal muscle loading procedure.

NOTE: MVC, Maximal Voluntary Contraction; RPE, Rating of Perceived Exertion; IF_{14}, Isometric Force at RPE 14; EMG, Electromyography.

3.9 Inspiratory loading protocol

Overview of testing session

To evaluate the impact of inspiratory muscle loading on perception of effort and muscle activity of the inspiratory muscles we used the following protocol. Participants completed a series of maximal inspiratory pressures (PI_{max}) using the hand-held, resistive device. Thereafter, participants generated five inspiratory pressures at a clamped effort of 14 on the 6-20 Borg scale. The inspiratory loading protocol was subsequently performed which consisted of five sets of twenty breaths at 50% of PI_{max}. After each set of loaded breaths, participants generated an inspiratory pressure at an effort of 14. Intercostal and Trapezius muscle activity were recorded during the procedures.

Experimental set-up of the inspiratory loading protocol

All measures were performed with the participant in a seated position with the knees flexed to 90° angle. Participants were instructed to adopt a forward leaning posture throughout the procedures in order to engage the primary inspiratory muscles and minimise the activation of accessory muscles (**Figure 3.7**). Intercostal and Trapezius EMG signals were acquired by applying bipolar pairs of silver/silver chloride electrodes on cleaned and abraded skin of the ribcage and shoulder region (Ambu® Neuroline 720, Ballerup, Denmark). For the intercostal muscle, electrodes were placed in the fifth intercostal space (midaxillary line), and for the upper trapezius muscle, between the spinous C7 process and acromion process (Cescon, Rebecchi, & Merletti, 2008; Hawkes, Nowicky, & McConnell, 2007) (**Figure 3.7**). The ground electrodes were placed on the sternum and C7 process and the inter-electrode distance was minimised to < 2cm. All measures were completed with the electrodes positioned on the right side of the body to minimise interference from electrocardiogram currents. Further attempts were made to align electrodes with the orientation of muscle fibres. The EMG signals were acquired and processed using the BIOPAC MP150 system (BIOPAC Systems Inc., Aero Camino Goleta, CA, US). Once a clear EMG signal was obtained (via deep inspiration and shoulder shrug) clear surgical tape was used to secure cables to reduce motion artefact. Participants were instructed to refrain from intentionally moving the right side of the body during the procedures. A single-use filter was then attached to the valve head of the hand-held, resistive breathing device (POWERbreathe International Ltd, Warwickshire, UK) and participants wore a nose clip during the procedures. The inspiratory

pressure (cmH_2O) generated against the mouthpiece was measured using the triple-aperture rotary valve system which is contained within the KH2 POWERbreathe device. The electronically controlled, rapid-response valve also operates to produce a resistance against inhalation which was utilised during the loading protocol.

Reliability and validity of inspiratory loading protocol

Despite the clear importance of assessing inspiratory muscle fatigue in clinical populations, little consensus existed regarding an optimal loading protocol capable of fatiguing the inspiratory muscles. A pilot study was previously conducted to systematically explore the test-retest reliability of the novel inspiratory loading protocol in healthy participants (thesis of Earing et al., 2014). Sixty-three males volunteered to participate in the study which included 37 normal BMI participants (age: 29.1 ± 8.1 years; height: 174.9 ± 6.3 cm; mass: 70.6 ± 7.0 kg; BMI: 23.1 ± 1.5 kg/m^2; PI_{max}: 107.2 ± 29.1 cmH_2O) and 26 overweight participants (age: 32.2 ± 7.2 years; height: 174.5 ± 6.0 cm; mass: 86.0 ± 10.0 kg; BMI: 28.2 ± 2.5 kg/m^2; PI_{max}: 113.3 ± 26.4 cmH_2O). Test-retest intraclass correlation coefficients (ICC) were assessed during the inspiratory pressures generated at a clamped effort of 14 on the Borg scale (IP_{14}) after each completed set of 20 breaths with the Powerbreathe Plus device (POWERbreathe International Ltd, Warwickshire, UK). A second assessment was conducted after 1 week under similar testing conditions. The inspiratory loading protocol produced an acceptable test-retest reliability of 0.967 with a 95% confidence interval (CI) of 0.953 - 0.978. The observed test-retest reliability (ICC) indicated that the novel inspiratory loading protocol could produce repeatable measures of IP_{14} in healthy populations.

General procedure of the inspiratory loading protocol

Maximal inspiratory pressure (PI_{max}) was firstly measured from residual volume to determine participants respiratory muscle strength with a hand-held, resistive breathing device (KH2 POWERbreathe International Ltd, Warwickshire, UK). Participants were asked to produce a maximal effort for 3 seconds whilst maintaining the correct form. A rest interval of ~ 1 minute separated each maximal effort. The procedure was repeated until seven PI_{max} manoeuvres were performed in total. Participants received strong verbal encouragement during each maximal effort. The highest pressure (cmH_2O) generated with a technically acceptable trace was defined as the PI_{max}. However, the mean value was taken from the

largest two PI_{max} values if found within 10% of each other (McConnell, 2007). No significant differences were found between the groups CV% for PI_{max} measures recorded (Trained: 19.27%; Untrained: 20.06%; OSA: 19.09%, $p > .05$).

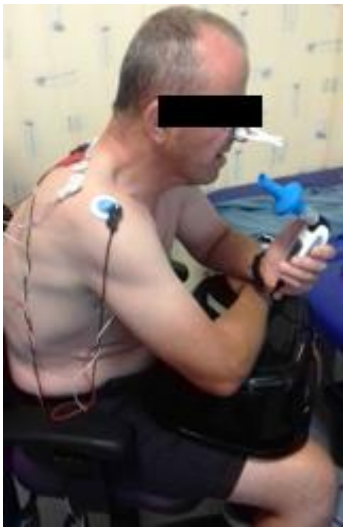
After 5 minutes of rest, participants were instructed to produce an inspiratory pressure that was equivalent to perceived effort of 14, which equated to an effort between the verbal anchors of *'somewhat hard'* to *'hard/heavy'* on the 6-20 Borg scale. The guidelines of Noble and Robertson (1996) were used to explain the concept behind using the RPE scale to each participant. Participants were instructed to focus all of their efforts to the breathing muscles, and anchor their perceptual range according to the following, RPE of 6 defined as a '*light bulb being completely off*' and an RPE of 20 as '*the brightest possible light*'. Earing et al., (2014) subjectively perceived that participants related better to an RPE of 14 (6-20 Borg scale) than 6 on the Borg CR-10 scale. The maximal pressure generated during the series of PI_{max} were interpreted as an RPE of 20 which familiarised participants to using the scale. Participants were informed there were no right or wrong answers and were encouraged to ask any questions regarding the instructions/anchoring. Five inspiratory pressures at a clamped effort of 14 were then performed for 2-3 seconds followed by a 30 second rest interval. These inspiratory pressures were later averaged and referred to as the IP_{14} reference **(Appendix V)**. Participants were given a full explanation of the PI_{max} and IP_{14} measures and use of the 6-20 Borg scale as a gauge of effort before starting the procedures. No visual feedback was given during these measures in order to force participants to become reliant upon their perceptions as a gauge.

Following the assessment of PI_{max} and IP_{14} reference, each participant completed the inspiratory loading protocol which consisted of five sets of twenty breaths at a resistive load equivalent to 50% of the pre-recorded PI_{max} using the KH2 POWERbreathe device. Participants were instructed to adopt a natural breathing style throughout the loading protocol. The real-time target (50% PI_{max}) was viewed using the Breathe-Link Medic PC software set up on the monitor to ensure participants met the required pressure on a consistent basis during the loading protocol. Between each set of loaded breaths, participants were asked to re-produce the IP_{14} measure for 2-3 seconds followed by a 30 second rest interval. The five inspiratory pressures were later averaged and referred to as the IP_{14} loading. All IP_{14} measures were normalised according to the PI_{max} and expressed as percentage of PI_{max}. The

percentage difference was later calculated by subtracting each respective IP_{14} loading measure from the mean of IP_{14} reference. The experimental protocol is illustrated in **Figure 3.8.**

Data Processing

EMG signals were analysed offline using commercially available software (AcqKnowledge 3.9, BIOPAC systems, Inc., US). The EMG signals of the Intercostal and Trapezius muscles were amplified (gain x 1000), band-pass filtered 35-500 Hz, and digitised at a sampling rate of 2000 kHz **(Figure 3.9)**. Root Mean Square (RMS) amplitude with a time constant of 100 ms were analysed over the 0.5 second window (the point at which peak pressure was generated). Data were normalised according to the maximal EMG and expressed as percentage of RMS (% RMS). Neuromuscular efficiency was calculated by dividing the inspiratory pressure generated during the IP_{14} with the average EMG activity for that contraction (Deschenes et al., 2008).



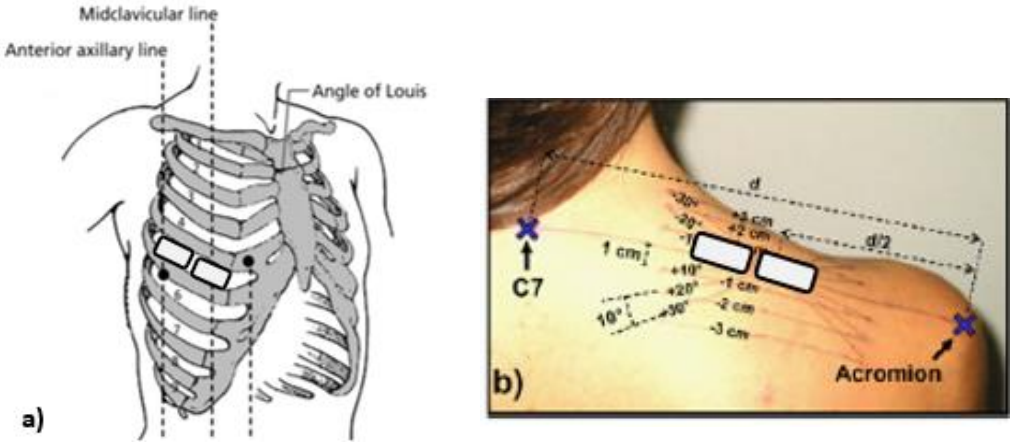


Figure 3.7. Photograph of participant depicting the forward leaning position during the inspiratory loading protocol (Image used with permission). EMG electrode placement for Intercostal (**a**) and Upper Trapezius (**b**) muscles.

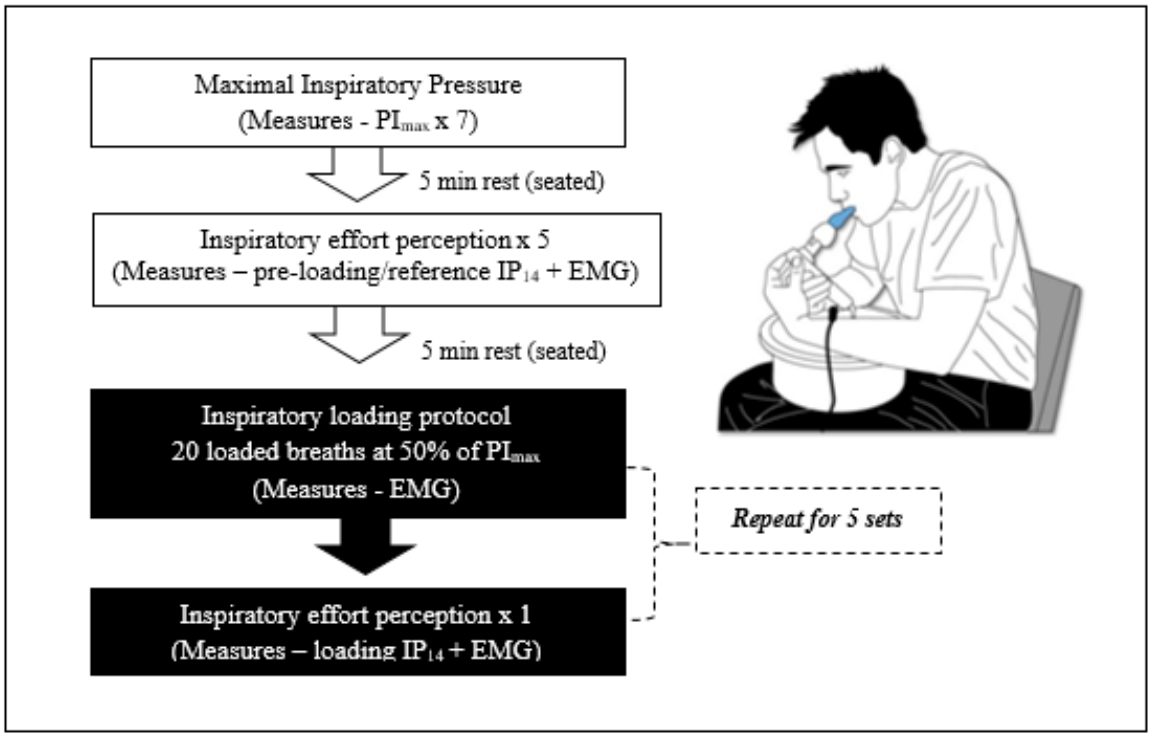


Figure 3.8. Schematic diagram of the inspiratory loading procedure. **NOTE:** PI_{max}, Maximal Inspiratory Pressure; RPE, Rating of Perceived Exertion; IP_{14}, Inspiratory Pressure at RPE 14; EMG, Electromyography.

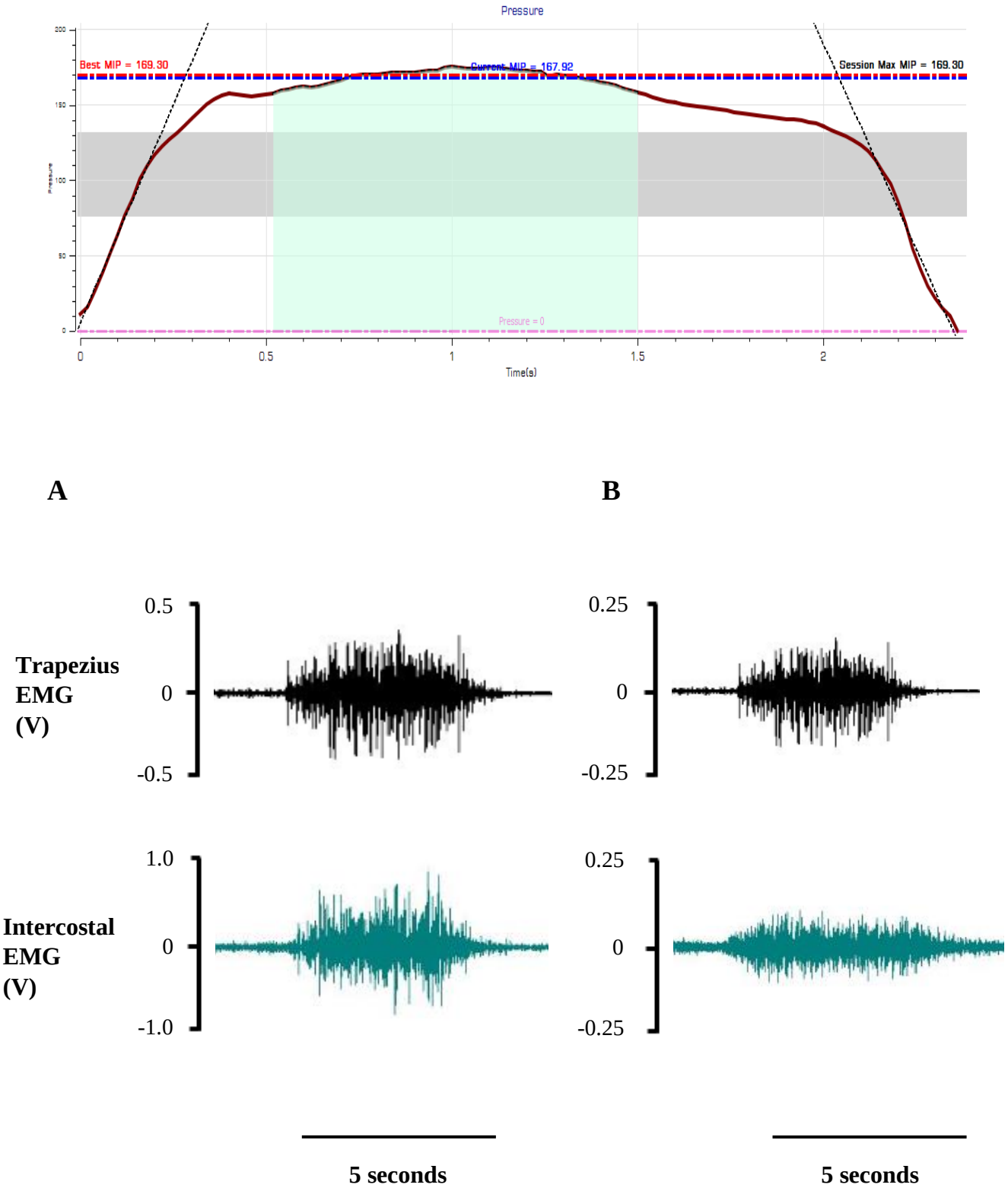


Figure 3.9. Image depicting an individual's mouth pressure during one maximal effort. The blue shaded area illustrates the exact location the PI_{max} was recorded. Raw electromyographic (EMG) activity of the Trapezius (black) and Intercostal muscles (blue) during PI_{max} efforts in Trained (**A**) and OSA (**B**) participants.

3.10 Sleep Assessment and Therapy

Overnight Sleep Study

An unattended overnight sleep study was performed at the patient's home after receiving a demonstration during the hospital appointment with a Sleep Technician. Heart rate and oxygen saturation was continuously measured with a pulse oximeter and attached finger probe (Konica Minolta Pulsox 300i Oximeter, Konica Minolta, Warrington, UK). Nasal airflow and abdominal and chest wall movements were measured using a nasal cannula and plethysmography bands positioned around the chest and abdomen (Nox RIP belts, Nox Nasal Cannula, Nox Medical, Reykjavik, Iceland). The diagnostic process was performed using Noxturnal software (Nox Medical, Reykjavik, Iceland) by an experienced RCCP (Registration Council for Clinical Physiologists) registered Clinical Physiologist or an experienced Sleep Technician.

Continuous Positive Airway Pressure (CPAP) therapy

CPAP therapy is the standard treatment option offered to patients with newly-diagnosed OSA. Each patient is familiarised to the automated CPAP device and set-up of the nasal or full face mask and headgear (AirSense™ 10 AutoSet ™, AirTouch™ F20, AirFit™ N10, ResMed (UK) Limited, Didcot, UK). The mask fit feature is explained and demonstrated in order to determine pressure tolerance (average 10-12 cmH_2O) and ensure minimal mask leak is observed. Airview™ is a cloud-based system that allows the sleep clinic to monitor patients during the first three months of their CPAP treatment.

CHAPTER FOUR

Ventilatory response to hypoxia and hypercapnia in Trained, Untrained, and OSA and the effect of CPAP treatment.

4.1 Abstract

Objectives: Central and peripheral chemosensitivity (ventilatory response to CO_2 and O_2) is thought to be decisive for the ventilatory instability observed in Obstructive Sleep Apnoea (OSA). Cardiovascular fitness is also thought to influence central and peripheral chemosensitivity in healthy individuals. However, no study to date has determined whether cardiovascular fitness and/or body characteristics contribute to the potential alterations observed in OSA. Continuous Positive Airway Pressure (CPAP) is the main treatment recommended for decreasing the frequency of obstructive events. Whether CPAP treatment modifies central and/or peripheral chemosensitivity is currently unknown.

Methods: The ventilatory response to hypoxia and hypercapnia was assessed during wakefulness using the following gas mixtures (25% O_2 / 6% CO_2, 13% O_2, and 13% O_2 / 6% CO_2) balanced with N_2 in Trained (n = 19), Untrained (n = 17), and OSA groups (n = 19). Following 3-months of CPAP therapy the ventilatory response to hypoxia and hypercapnia was re-assessed in OSA patients (n = 15).

Results: Newly diagnosed OSA patients showed a significantly lower ventilatory response to hypoxia and hypercapnia compared to healthy individuals ($p < .05$). The ventilatory response to hypoxia and hypercapnia was also found to be significantly reduced in Untrained individuals compared with Trained individuals ($p < .05$). No differences were observed in the ventilatory response to hypoxia and hypercapnia between Untrained individuals and OSA patients ($p > .05$). Regression analyses showed that body characteristics (BMI, FVC, neck and hip circumference) explained 17.7 % of the variance in central chemosensitivity ($p < .05$). It was found that BMI and VO_{2max} explained 27.6 % of the variance in the peripheral chemosensitivity ($p < .01$). Whilst, body characteristics (BMI, FVC, and hip circumference) explained 25.2 % of the variance in the combined central and peripheral chemosensitivity ($p < .01$). The hypoxic and hypercapnic ventilatory response was found to be unchanged following 3-months of CPAP treatment in OSA patients ($p > .05$).

Conclusion: Central and peripheral chemosensitivity was reduced in patients with newly-diagnosed OSA compared with Trained individuals. Body characteristics and cardiovascular fitness partially explained the alterations in central and peripheral chemosensitivity. The central and peripheral chemosensitivity was unchanged following 3-months of CPAP treatment in OSA patients.

4.2 Introduction

Obstructive sleep apnoea (OSA) is a breathing disorder characterised by repetitive obstruction of the upper respiratory airway during sleep (Carter & Watenpaugh, 2008). The pharyngeal collapse leads to transient episodes of hypoxia, hypercapnia, and strong inspiratory efforts that result in arousals which attempt to normalise the airway patency and blood gas levels (Guilleminault et al., 1976; Somers et al., 1989). The condition is linked with many undesirable health consequences such as cardiovascular disease, hypertension, type II diabetes, stroke, insomnia, and depression (Jean-Louis et al., 2008). Continuous Positive Airway Pressure (CPAP) is the main treatment available for reducing the frequency of obstructive events OSA patients experienced during sleep. It has also been proven to reduce the risk of cardiovascular disease, incidence of fatal and nonfatal events, and road traffic accidents (Barbé et al., 2007; Drager et al., 2017; Siccoli et al., 2008).

The ventilatory control system is regulated by a negative feedback loop that acts to maintain $PaCO_2$ and pH constant. An elevated 'loop gain' has been suggested to contribute to the ventilatory instability of the pharyngeal airways during sleep (Khoo et al., 1982; White, 2005). The ventilatory response to an obstruction is also shown to be critically dependent upon the sensitivity of the central and peripheral chemoreceptors (part of controller gain) (Dempsey, Veasey, Morgan, & O'Donnell, 2010). However, the chronic exposure to intermittent hypoxia during sleep has been shown to influence the chemosensitivity in OSA (Mateika & Narwani, 2009). It has been proposed that intermittent exposure to hypoxia and hypercapnia during sleep leads to a depression or progressive resetting of the carotid body to a lower sensitivity threshold (Forster & Dempsey, 1981; Guilleminault & Cummiskey, 1982). There is further evidence to suggest that cardiovascular fitness is linked with alterations of central and peripheral chemoreceptor sensitivity (Byrne-Quinn et al., 1971).

The ventilatory response to hypoxia and hypercapnia has therefore been investigated during wakefulness in OSA patients and healthy individuals with conflicting results shown. The hypercapnic ventilatory response during wakefulness in OSA patients was shown to be lower (Javaheri et al., 1994; Littner et al., 1984; Osanai et al., 1999), similar (Narkiewicz, et al., 1999; Sin et al., 2000; Verbraecken et al., 2000), or higher than healthy individuals (Verbraecken et al., 1998). Whilst the ventilatory responses to hypoxia in OSA patients was shown to be lower (Garay et al., 1981; Javaheri et al., 1994; Osanai et al., 1999), similar

(Gold et al., 1993; Littner et al., 1984), or higher than healthy individuals (Narkiewicz, et al., 1999). Whereas, the hypercapnic ventilatory response in endurance-trained athletes was shown to be lower (Byrne-Quinn et al., 1971; Miyamura et al., 1976; Scoggin et al., 1978) or similar to non-athletic individuals (Godfrey et al., 1971; Mahler et al., 1982). Whilst, the hypoxic ventilatory response in endurance-trained athletes was reported to be lower (Byrne-Quinn et al., 1971; Scoggin et al., 1978) or similar to non-athletic individuals (Godfrey et al., 1971; Mahler et al., 1982; Sheel et al., 2006). However, most of these studies did not utilise mixed hyperoxic/hypercapnic and hypoxic/hypercapnic gas mixtures for the differentiation of central and peripheral chemosensitivity (Duffin, 2007).

Studies have also investigated the effects of CPAP therapy on the ventilatory response to hypercapnia and/or hypoxia in OSA patients and shown mixed results. The ventilatory response to hypercapnia in OSA patients was shown to be higher (Lin, 1994; Tun et al., 2000) or no different after receiving CPAP treatment (Berthon-Jones & Sullivan, 1987; Foster et al., 2009; Greenberg & Scharf, 1993; Spicuzza et al., 2006; Verbraecken et al., 1995). Whilst the ventilatory response to hypoxia in OSA patients was shown to be higher (Lin, 1994) or reduced following CPAP treatment (Radovanovic et al., 2019; Spicuzza et al., 2006; Tun et al., 2000). The variability in the hypoxic and/or hypercapnic ventilatory response is potentially explained by differences in the methodology, patient population, and duration of CPAP treatment.

Therefore, we examined the ventilatory response to hypoxia and hypercapnia during wakefulness in patients with newly diagnosed OSA and healthy individuals (Trained and Untrained groups) using the novel methodology reported by Earing et al., (2014) which differentiates between central and peripheral chemosensitivity using mixed gases (see Chapter 3.7 for full protocol details). Secondly, we investigated the influence of cardiovascular fitness and body characteristics on potential alterations in central and peripheral chemosensitivity. Thirdly, we examined the impact of 3 months of CPAP therapy on the ventilatory response to hypoxia and hypercapnia in OSA patients. We hypothesized patients with newly diagnosed OSA would show a reduced ventilatory response to hypoxia and hypercapnia during wakefulness compared with healthy individuals. It was also hypothesised the ventilatory response to hypoxia and hypercapnia would be altered following 3-months of CPAP therapy in OSA patients.

4.3 Methods

Participant characteristics

Sixty one males were recruited to participate in the study which included 21 Trained participants (age: 35.1 ± 2.0 years; height: 179.5 ± 1.5 cm; mass: 76.6 ± 2.4 kg; VO_{2max}: 61.1 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$), 19 Untrained participants (age: 28.4 ± 1.8 years; height: 176.8 ± 1.9 cm; mass: 82.0 ± 4.1 kg; VO_{2max}: 39.7 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$), and 21 patients with newly-diagnosed OSA (age: 55.1 ± 2.0 years; height: 173.4 ± 1.3 cm; mass: 98.2 ± 4.3 kg; **Table 4.1-a** and **Table 4.1-b)**. The data of six participants was excluded from the analyses due to talking and technical defects during testing. We therefore present the data of 55 participants which includes 19 Trained, 17 Untrained, and 19 OSA participants. All participants were non-smokers, injury-free, had no history of cardiovascular, pulmonary, neuromuscular, cerebrovascular, neuropsychiatric or neurological disorders, and not receiving pharmacological treatment (e.g. anti-diabetic agents, opiate based painkillers, psychoactive medications). Healthy participants were recruited from the local community and were deemed asymptomatic according to the pre-screening questionnaires (**see Chapter 3.3)**. The Trained group were composed of endurance-trained athletes (cycling, running, or triathlon) who completed on average 8 hours of training per week (weekly distance 41.0 miles). Whilst, the Untrained group were composed of individuals who did not participate in any regular physical exercise.

Fifteen males with diagnosed OSA completed the follow-up assessments after receiving 3-months of CPAP therapy (age: 54.8 ± 2.4 years; height: 173.0 ± 1.3 cm; mass: 100.1 ± 5.9 kg; **Table 4.1-c**). Newly diagnosed OSA patients were recruited from the Virtual Sleep Clinic (Ysbyty Gwynedd, Bangor) between October 2017 and January 2018, and screened for eligibility by assessing medical records (e.g. sleep results and medical history). All patients were assessed with an outpatient overnight sleep study as part of routine care (Nox Medical, Reykjavik, Iceland). Diagnostic results were evaluated by experienced Registration Council for Clinical Physiologists (RCCP) accredited Clinical Physiologists. Apnoea Hypopnea Index (AHI) was calculated from the frequency of apnoeas and hypopneas per hour of sleep and classified according to the criteria set by The American Academy of Sleep Medicine (Berry et al. 2012). Patients were evaluated at baseline and following 3 months of CPAP therapy (AirSense™ 10 AutoSet ™, ResMed Limited, Didcot, UK). The Airview™ feature was utilised to assess AHI and adherence after 3 months of CPAP treatment. After 3 months of

CPAP treatment (mean pressure: 8.67 ± 0.8 cmH_2O, usage: 7.06 ± 0.4 hrs / night (range 4.11-9.23 hrs / night), and days of usage > 4hrs: 93.7 ± 3.5 %). The study was approved by Departmental (SSHES, Bangor University, Ref: P01-16/17) and National Health Ethics Committees (Ref: 17/EM/0162). Participants received a detailed explanation of the procedures and a checklist to ensure compliance with the eligibility criteria **(Appendix I & II)**. Written informed consent was obtained from all participants **(Appendix III)**. The experimental protocols were performed in accordance with the Declaration of Helsinki.

Study design

The ventilatory response to hypoxia and hypercapnia was assessed using a cross-sectional study design. Healthy participants visited the laboratory of the School of Sport, Health, and Exercise Sciences once as outlined in **Figure 3.2-a**. Patients with newly diagnosed OSA were required to visit the laboratory at the Pulmonary Function department (Ysbyty Gwynedd) once as outlined in **Figure 3.2-b**.

Table 4.1-a. Physiological characteristics of all participants

	Trained (*n* = 19)	Untrained (*n* = 17)	OSA (*n* = 19)
Age (years)	36.1 (2.1)*	27.4 (1.9)††	54.5 (2.1)##
Height (cm)	179.6 (1.6)	176.9 (2.2)	172.8 (1.3)#
Weight (kg)	77.3 (2.6)	83.2 (4.5)†	98.1 (4.8)##
Body Mass Index (kg · m^2)	23.9 (.71)	26.4 (.95)††	32.8 (1.5)##
Body Surface Area (cm)	196.2 (3.5)	199.8 (6.0)	215.0 (5.7)#
Body Fat (%)	13.1 (1.1)*	18.6 (1.2)††	31.4 (1.8)##
Neck circumference (cm)	38.1 (.54)	39.3 (.69)††	43.9 (.91)##
Chest circumference (cm)	96.4 (1.9)	100.3 (2.3)††	116.3 (3.1)##
Waist circumference (cm)	84.1 (2.1)	90.1 (2.7)††	112.3 (3.9)##
Hip circumference (cm)	98.9 (1.5)	103.8 (2.2)	108.5 (2.0)##
Apnoea Hypopnea Index (events/h)	N/A	N/A	40.1 (6.5)
Epworth Sleepiness Scale	6.0 (.90)	2.5 (.46)††	12.0 (1.3)##
Pittsburgh Sleep Quality Index	3.6 (.47)	4.0 (.47)††	9.0 (.96)##
Forced Expiratory Volume 1s (l/min)	4.42 (.17)	4.24 (.14)††	3.20 (.12)##
Forced Vital Capacity (l/min)	5.39 (.17)	5.14 (.15)††	4.20 (.18)##
DLCO (ml/min/mmHg)	N/A	N/A	24.0 (1.1)
Total Lung Capacity (l/min)	N/A	N/A	6.07 (.23)
VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	59.9 (1.8)**	39.7 (1.4)	N/A
PI_{max} (cmH_2O)	113.3 (5.0)	113.8 (6.6)	104.0 (4.5)
MVC (N)	424.8 (23.4)	392.4 (32.3)	291.9 (33.8)##

NOTE: DLCO, Diffusing capacity of the lung for carbon monoxide; VO_{2max}, Maximal Oxygen Uptake. PI_{max}, Maximal Inspiratory Pressure (Inspiratory muscles); MVC, Maximal Voluntary Capacity (Quadriceps muscles). Values are Mean ± SEM. * p <.05, ** p <.01 Trained vs Untrained. † p <.05, †† p <.01 Untrained vs OSA. # p <.05, ## p <.01 OSA vs Trained.

Table 4.1-b. Polysomnographic data of OSA patients at baseline (*n* = 21)

Clinical parameters	Mean (SEM)	Median (IQR)
AHI (events / h)	**39.9 (5.9)**	34.1 (16.2-53.1)
Apnoea (events / h)	**21.3 (4.7)**	10.8 (5.7-29.0)
Duration of Apnoea (min)	**37.6 (9.8)**	19.4 (7.2-46.5)
Hypopnoea (events / h)	**20.8 (3.2)**	16.8 (9.2-24.8)
Duration of Hypopnoea (min)	**50.1 (5.8)**	47.3 (26.6-76.5)
TST (min)	**426.9 (25.9)**	442.6 (368.8-509.3)
Snore (%)	**23.4 (3.9)**	25.1 (8.5-33.3)
ODI (events / h)	**34.5 (5.6)**	28.5 (14.7-47.3)
Average SpO_2 (%)	**91.9 (.62)**	92.3 (91.0-93.7)
Minimum SpO_2 (%)	**77.3 (1.8)**	79.0 (73.0-82.5)
Average Desaturation (%)	**5.7 (.66)**	4.7 (3.8-6.2)
Percentage of SpO_2 < 90%	**15.6 (4.5)**	6.9 (1.9-22.0)
Duration of SpO_2 <90% (min)	**59.9 (18.5)**	25.9 (8.6-88.2)
Percentage of SpO_2 < 88%	**11.6 (3.9)**	5.7 (1.3-12.7)
Duration of SpO_2 <88% (min)	**42.8 (15.9)**	15.0 (6.0-46.6)
Percentage of SpO_2 < 85%	**5.2 (2.6)**	.40 (.05-2.6)
Duration of SpO_2 <85% (min)	**17.6 (10.7)**	2.4 (.20-10.0)
Average Heart Rate (bpm)	**63.0 (2.1)**	62.2 (56.6-69.8)

NOTE: AHI, Apnoea Hypopnea Index; TST, Total Sleep Time; ODI, Oxygen Desaturation Index; SpO_2, Peripheral Oxygen Saturation; bpm, Beats per minute; h, hour; min, minute.

Table 4.1-c. Physiological characteristics of OSA participants after CPAP treatment.

	Pre-CPAP OSA (n = 15)	Post-CPAP OSA (n = 15)
Age (years)	54.8 (2.4)	N/A
Height (cm)	173.0 (1.3)	173.0 (1.3)
Weight (kg)	100.1 (5.9)	101.8 (5.9)¶
Body Mass Index ($kg \cdot m^2$)	33.3 (1.7)	33.9 (1.7)¶¶
Body Surface Area (cm)	217.1 (7.1)	219.1 (7.1)¶
Body Fat (%)	32.1 (2.2)	31.8 (1.9)
Neck circumference (cm)	44.0 (1.0)	44.6 (1.0)¶
Chest circumference (cm)	117.4 (3.9)	117.4 (3.9)
Waist circumference (cm)	113.8 (4.7)	113.2 (4.3)
Hip circumference (cm)	109.9 (2.4)	110.6 (2.6)
Apnoea Hypopnea Index (events/h)	38.3 (6.1)	4.5 (.89)¶¶
Epworth Sleepiness Scale	11.6 (1.3)	4.2 (.69)¶¶
Pittsburgh Sleep Quality Index	9.2 (.95)	4.3 (.63)¶¶
Forced Expiratory Volume 1s (l/min)	3.26 (.09)	3.26 (.09)
Forced Vital Capacity (l/min)	4.29 (.15)	4.25 (.14)
DLCO (ml/min/mmHg)	23.2 (1.3)	24.1 (1.4)
Total Lung Capacity (l/min)	6.14 (.24)	6.64 (.25)¶
Maximal Inspiratory Pressure (cmH_2O)	102.4 (4.0)	103.6 (4.2)

NOTE: DLCO, Diffusing capacity of the lung for carbon monoxide. Values are Mean ± SEM. ¶ p <.05, ¶¶ p <.01 Pre-CPAP vs Post-CPAP.

General procedures

The full detailed procedures of the measurements are described in **Chapter 3**; however, the principle techniques are described here in brief. The general medical questionnaire was firstly completed to obtain information regarding participant's current state of health, medical history, lifestyle, physical activity levels, and training status (if applicable). The Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale were then completed to assess participant's sleep quality and sleepiness **(Chapter 3.3)**. The measures were then used to screen healthy participants for undiagnosed sleeping disorders. All patients with suspected OSA underwent an overnight sleep study as part of routine care to measure the OSA severity (AHI) (**Chapter 3.10**). Body characteristics were then measured which included; height, weight, body mass index, body surface area, body fat percentage, and neck, chest, waist, and hip circumferences **(Chapter 3.4)**. Forced expiratory volume (FEV_1) and forced vital capacity (FVC) were measured via spirometry in healthy participants. The following pulmonary function measures were assessed in OSA patients; FEV_1, FVC, carbon monoxide uptake in the lung, and total lung capacity **(Chapter 3.5)**. Healthy participants underwent an incremental exercise test on a cycle ergometer to determine maximal oxygen uptake (VO_{2max}) **(Chapter 3.6)**. The incremental exercise test began with participants cycling at 70 Watts (W) at a pedalling frequency of 60-90 revolutions per minute with the resistance increasing by 20 W per minute until volitional exhaustion. Participants were then stratified into Trained (VO_{2max} > 55 ml·kg·min^{-1}) or Untrained (VO_{2max} < 40 ml·kg·min^{-1}) groups respectively based upon the resulting VO_{2max} (Astorino et al., 2012; Hassmén, 1990). The incremental exercise test was not performed in OSA patients.

The ventilatory response to hypoxia and hypercapnia was measured according to the full detailed procedures as described in **Chapter 3.7**. The protocol was performed according to techniques as reported by Earing et al., (2014) in healthy and clinical populations. Participants breathed the following series of gases (ambient air, 25% O_2 / 6% CO_2, 13% O_2, and 13% O_2 / 6% CO_2) until a plateau in minute ventilation was achieved in 3-5 minutes. Ambient air was utilised to determine the resting minute ventilation. Participants were blinded to the composition and exact time point the gas mixture was inspired. The volume transducer and gas sampling port of the metabolic cart (MetaMax® 3B, Cortex Biophysik, Germany) were attached to a two - way valve that allowed gases to be inspired from the 250 L Douglas bag and expired into the atmosphere. The Douglas bag was fed with the gas

concentrations balanced with N_2 (BOC Ltd., England) via a slow flowing regulator of a gas canister **(see Figure 4.1 for schematic)**.

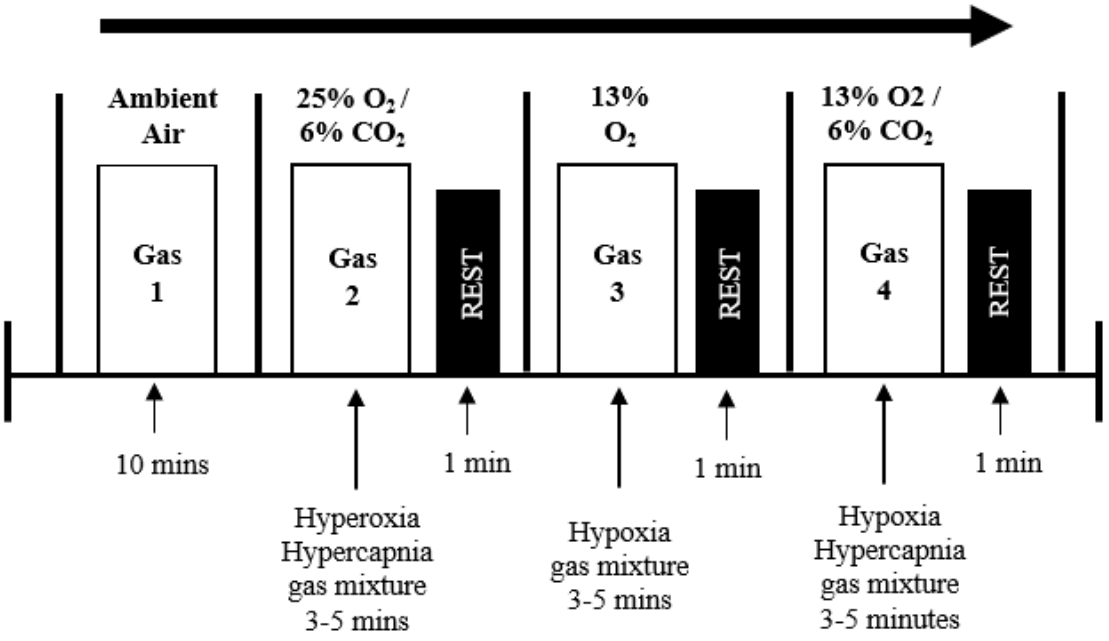


Figure 4.1. Schematic diagram of the ventilatory control assessment.

Data Analysis

Expired gas concentrations and minute ventilation were averaged over 5 s periods. Baseline measurements of minute ventilation was averaged over 2-minute periods. A moving average filter was utilised in order to detect the plateau observed during the maximal ventilatory response to each gas mixture. The maximal ventilatory response observed over a period of 30 seconds (excluding outliers based upon moving average filter) was averaged. Resting minute ventilation was subtracted from the maximal minute ventilation achieved for each gas in order to calculate the change in minute ventilation (ΔV X% CO_2 / Y% O_2). All minute ventilation data was normalised according to BSA equations (**as described in Chapter 3.4**) to account for body-mass related differences (DuBois & DuBois, 1916; Mosteller, 1987).

Statistical Analysis

Mean and standard deviations were taken from a previous study that had examined the ventilatory responses of accomplished marathon runners and control group and was later used to calculate an effect size of 0.56 (Mahler, Moritz, & Loke, 1982). The required sample size for the recruitment of healthy participants was estimated as 32 (G*Power, Version 3.1.2) using alpha (0.05) and beta levels (0.8). However, we aimed to recruit a total of 40 healthy participants (20 per group) to account for potential drop-outs and match the sample size for the recruitment of OSA patients. Future research should aim to recruit a sample size of 50 healthy participants and 25 OSA patients to achieve statistical power and account for drop-outs. Statistical analyses were performed using SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA). Data was expressed as Mean ± SEM with $p < .05$ considered statistically significant. Shapiro-Wilk tests were performed to determine the assumption of normality. One-Way ANOVA and Bonferroni corrections were performed to examine the ventilatory response to hypoxia and hypercapnia. Paired *t*-tests were conducted to investigate the effect of CPAP therapy on the ventilatory response to hypoxia and hypercapnia in OSA patients. Spearman's rho analyses were performed to determine whether correlations exist between body characteristics and the hypoxic and hypercapnic ventilatory responses. Spearman's correlation coefficient was used as many of the body characteristics were not normally distributed. Linear regression analyses were performed to determine the contribution of cardiovascular fitness and body characteristics to central and peripheral chemosensitivity.

4.4 Results

Participant Characteristics

Patients with newly-diagnosed OSA were found to be significantly older and heavier than Trained and Untrained individuals ($p < .05$; Table 4.1-a). Sleep-related measures (ESS and PSQI) were revealed to be significantly higher in OSA patients compared with Trained and Untrained groups ($p < .05$). Lung function parameters (FEV_1 and FVC) were shown to be significantly lower in OSA patients than Trained and Untrained individuals ($p < .05$). Cardiovascular fitness (VO_{2max}) and lower extremity strength (MVC) was significantly greater in Trained individuals compared with Untrained individuals ($p < .05$).

Ventilatory response to hypoxia and hypercapnia

The main aim of our investigation was to examine the ventilatory response to hypoxia and hypercapnia in Trained, Untrained, and OSA groups. Shapiro Wilk tests were initially performed to determine the normality of ventilatory responses to the gas mixtures. The data was revealed to be normally distributed ($p > .05$). We firstly performed a One-way ANOVA analyses to examine the ventilatory response of Trained, Untrained, and OSA groups whilst breathing ambient air. The analyses revealed the minute ventilation to breathing ambient air was similar between the groups (Trained: 5.62 ± .23 l/min/BSA; Untrained: 5.31 ± .24 l/min/BSA; OSA: 5.55 ± .18 l/min/BSA, $p > .05$). Next, we examined the ventilatory response to hypoxia and hypercapnia by calculating the change in minute ventilation for each gas mixture from baseline ventilation. One-Way ANOVA analyses were performed which revealed patients with newly-diagnosed OSA demonstrated a significantly lower ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2; 6.21 ± .36 l/min/BSA vs. 8.20 ± .51 l/min/BSA, $p = .007$), hypoxia (13% O_2; 0.90 ± .36 l/min/BSA vs. 2.05 ± .20 l/min/BSA, $p = .001$), and hypoxia hypercapnia (13% O_2 / 6% CO_2; 7.28 ± .38 l/min/BSA vs. 10.04 ± .52 l/min/BSA, $p = .000$) compared to Trained individuals. Furthermore, Untrained individuals were found to have a significantly lower ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2; 6.46 ± .44 l/min/BSA vs. 8.20 ± .51 l/min/BSA, $p = .026$), hypoxia (13% O_2; 1.12 ± .22 l/min/BSA vs. 2.05 ± .20 l/min/BSA, $p = .007$), and hypoxia hypercapnia (13% O_2 / 6% CO_2; 8.01 ± .39 l/min/BSA vs. 10.04 ± .52 l/min/BSA, $p = .008$) compared to Trained individuals **(Figure 4.2)**. However, no differences were found in the ventilatory response to hypoxia and hypercapnia gas mixtures between Untrained and OSA groups ($p > .05$).

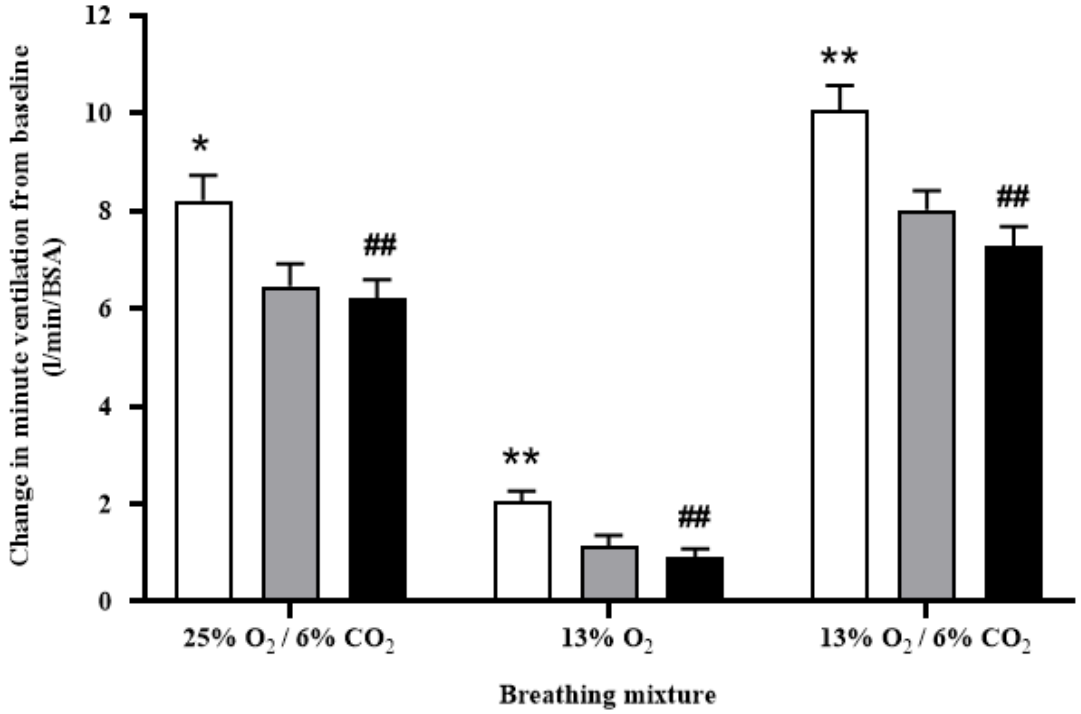


Figure 4.2. Change in minute ventilation from baseline (l/min/BSA) for each breathing mixture. Data are expressed as Mean ± SE. □ = Trained group (*n* = 19); ▒ = Untrained group (*n* = 17); ■ = OSA group (*n* = 19). **NOTE:** Abbreviations: BSA, Body Surface Area; O_2, Oxygen; CO_2, Carbon Dioxide. Hyperoxia Hypercapnia (25% O_2 / 6% CO_2), Hypoxia (13% O_2), and Hypoxia Hypercapnia (13% O_2 / 6% CO_2). * = $p < .05$, ** = $p < .01$ vs. Untrained; # = $p < .05$, ## = $p < .01$ vs. Trained.

We then examined possible correlations between the body characteristics, maximal oxygen uptake, and ventilatory response to each gas mixture **(Table 4.2)**. Spearman's rho analyses revealed the ventilatory response to hyperoxia hypercapnia was found to be significantly correlated with participant's waist circumference (r_s = -.276, p = .041). In addition, the ventilatory response to hypoxia was significantly correlated with participants FEV_1 (r_s = .365, p = .006) and VO_{2max} (litres/min) (r_s = .526, p = .001). Whilst, the ventilatory response to hypoxia hypercapnia was found to be significantly correlated with several body characteristics and lung function measures ($p < .05$). Furthermore, AHI and VO_{2max} was shown to be correlated with body characteristics ($p < .05$). However, the AHI was not correlated with the ventilatory responses to each gas mixture. Linear regression analyses were performed to determine the contribution of body characteristics and cardiovascular fitness for the hypoxic and hypercapnic ventilatory responses **(Table 4.4)**. The analyses revealed that body characteristics (BMI, FVC, Neck and Hip circumference) accounted for 17.7 % of the variance in the hypercapnic ventilatory response ($p < .05$). It was also found that BMI and VO_{2max} explained 27.6 % of the variance in the peripheral response ($p < .01$). It was further revealed that participant's body characteristics (BMI, FVC, and Hip circumference) explained 25.2 % of the variance in the combined central and peripheral response ($p < .01$).

Table 4.2. Spearman's rho correlation matrix of all participants (n = 55)

	AHI	Weight	BMI	BSA	Neck	Chest	Waist	Hip	FEV_1	FVC	VO_2	VO_{2max}	VE Δ 25% O_2 / 6% CO_2	VE Δ 13% O_2	VE Δ 13% O_2 / 6% CO_2
AHI (events/hr)	1.000														
Weight (kg)	.410	1.000													
BMI (kg/m^2)	.572**	.881**	1.000												
BSA (cm)	.348	.956**	.733**	1.000											
Neck (cm)	.465*	.834**	.830**	.779**	1.000										
Chest (cm)	.496*	.887**	.898**	.804**	.875**	1.000									
Waist (cm)	.449*	.876**	.911**	.790**	.872**	.875**	1.000								
Hip (cm)	.451*	.907**	.817**	.875**	.702**	.755**	.824**	1.000							
FEV_1 (l.min)	-.269	-.204	-.395**	-.109	-.302*	-.310*	-.395**	-.154	1.000						
FVC (l.min)	-.456*	-.226	-.476**	-.083	-.297*	-.303*	-.390**	-.186	.860**	1.000					
VO_2 (l.min)	N/A	.302	.019	.425**	.229	.255	.055	.121	.441**	.353*	1.000				
VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	N/A	-.287	-.502**	-.140	-.305	-.236	-.452**	-.411**	.217	.286	.700	1.000			
VE Δ 25% O_2 / 6% CO_2	-.119	-.202	-.171	-.220	-.260	-.230	-.276*	-.191	.245	.141	.185	.205	1.000		
VE Δ 13% O_2	.165	-.096	-.170	-.024	-.170	-.192	-.200	-.059	.365**	.196	.526**	.321	.522**	1.000	
VE Δ 13% O_2 / 6% CO_2	-.061	-.345*	-.350**	-.303*	-.356**	-.414**	-.393**	-.269*	.435**	.356**	.210	.327	.652**	.552**	1.000

NOTE: Abbreviations: AHI, Apnoea Hypopnea Index; BMI, Body Mass Index; FEV_1. Forced Expiratory Volume 1s; FVC, Forced Vital Capacity; VO_2, Maximal Oxygen Uptake (only Trained and Untrained individuals n = 36); VE Δ, Change in ventilatory response to each gas mixture from resting minute ventilation.

Table 4.4. Multiple regression analysis to predict the contribution of body characteristics and cardiovascular fitness in the hypercapnic and hypoxic ventilatory response in Trained and Untrained individuals (n = 36).

	VE Δ 25% O_2 / 6% CO_2					VE Δ 13% O_2					VE Δ 13% O_2 / 6% CO_2				
	B	***SE B***	**β**	***t***	***p***	***B***	***SE B***	**β**	***t***	***p***	***B***	***SE B***	**β**	***t***	***p***
BMI	.504	.233	.862	2.161	.039*	.128	.045	.467	2.809	.008**	.374	.188	.623	1.988	.056
Neck circumference	-.396	.224	-.491	-1.773	.087	-.030	.085	-.080	-.349	.730	-115	.218	-.139	-.528	.602
Hip circumference	-.176	.094	-.668	-1.884	.070	-.025	.034	-.203	-.715	.480	-.260	.090	-.956	-2.888	.007**
FVC	1.297	.715	.400	1.814	.080	-.035	.310	-.025	-.113	.911	1.374	.608	.412	2.260	.031**
VO_{2max}	.032	.044	.172	.721	.477	.045	.014	.532	3.205	.003**	.015	.038	.082	.410	.685

NOTE: Abbreviations: SE, Standard Error; BMI, Body Mass Index; FVC, Forced Vital Capacity; VO_2, Maximal Oxygen Uptake.

CPAP therapy and Ventilatory response to hypoxia and hypercapnia in OSA patients.

For the next phase of our investigation we examined the effect of CPAP therapy on the ventilatory response to the following gas mixtures (25% O_2 / 6% CO_2, 13% O_2, and 13% O_2 / 6% CO_2). Shapiro Wilk tests were initially performed to determine the normality of ventilatory responses to the gas mixtures. The data was revealed to be normally distributed ($p > .05$). Thus, paired *t*-test analyses were performed which revealed the ventilatory response to ambient air was unchanged following 3-months of CPAP treatment in OSA patients (Pre-CPAP: 5.62 ± .19 l/min/BSA vs. Post-CPAP: 5.32 ± .38 l/min/BSA, 95% CI: -1.35 to.77, $t_{(15)} = -.581$, $p = .570$, **Figure 4.3**). There was no observed differences in the ventilatory response to hyperoxia hypercapnia (Pre-CPAP: 6.30 ± .43 l/min/BSA vs. Post-CPAP: 6.86 ± .58 l/min/BSA, 95% CI: -.49 to 1.62, $t_{(14)} = 1.147$, $p = .270$), hypoxia (Pre-CPAP: .97 ± .20 l/min/BSA vs. Post-CPAP: 1.38 ± .30 l/min/BSA, 95% CI: -.31 to 1.13, $t_{(14)} = 1.210$, $p = .246$), and hypoxia hypercapnia in OSA patients (Pre-CPAP: 7.48 ± .44 l/min/BSA vs. Post-CPAP: 7.22 ± .75 l/min/BSA, 95% CI: -2.13 to 1.61, $t_{(14)} = -.296$, $p = .772$).

We then examined potential correlations between participant's body characteristics and the ventilatory response to each gas mixture **(Table 4.3)**. Spearman's rho analyses revealed no correlations were observed between the body characteristics and ventilatory response to each of the gas mixtures.

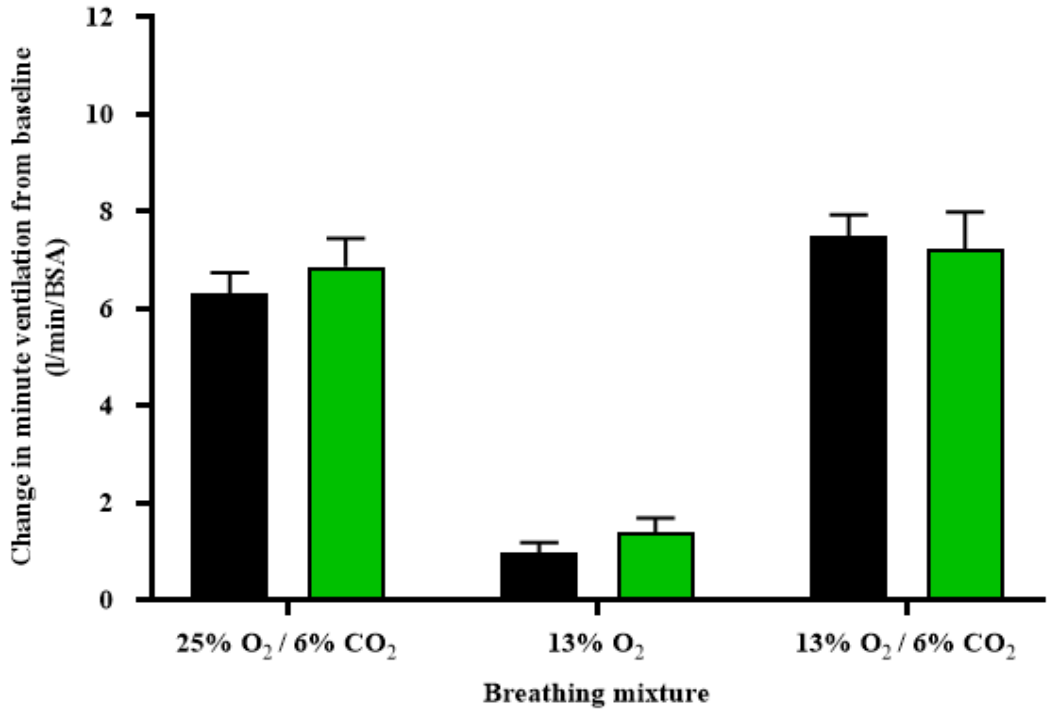


Figure 4.3. Change in minute ventilation from baseline (l/min/BSA) for each breathing mixture at baseline and following CPAP treatment. Data are expressed as Mean ± SE. ■ = Pre-CPAP group (*n* = 15); ■ = Post-CPAP group (*n* = 15). **NOTE:** Abbreviations: BSA, Body Surface Area; O_2, Oxygen; CO_2, Carbon Dioxide. Hyperoxic Hypercapnic gas mixture (25% O_2 / 6% CO_2), Hypoxic ventilatory gas mixture (13% O_2), and Hypoxic Hypercapnic gas mixture (13% O_2 / 6% CO_2).

Table 4.3. Spearman's rho correlation matrix of OSA patients after CPAP treatment (n = 15)

	AHI	Weight	BMI	BSA	Neck	Chest	Waist	Hip	FEV_1	FVC	VE Δ 25% O_2 / 6% CO_2	VE Δ 13% O_2	VE Δ 13% O_2 / 6% CO_2
AHI (events/hr)	1.000												
Weight (kg)	-.007	1.000											
BMI (kg/m^2)	.035	.935**	1.000										
BSA (cm)	.010	.994**	.917**	1.000									
Neck (cm)	.120	.895**	.931**	.880**	1.000								
Chest (cm)	-.006	.918**	.973**	.903**	.942**	1.000							
Waist (cm)	-.009	.920**	.955**	.918**	.938**	.978**	1.000						
Hip (cm)	.128	.898**	.846**	.901**	.802**	.832**	.852**	1.000					
FEV_1 (l.min)	.027	-.050	-.219	-.044	-.271	-.221	-.272	.080	1.000				
FVC (l.min)	.203	-.480	-.633**	-.480	-.525*	-.571*	-.614*	-.330	.708**	1.000			
VE Δ 25% O_2 / 6% CO_2	-.073	-.189	-.075	-.250	.061	-.039	-.070	-.172	-.139	-.071	1.000		
VE Δ 13% O_2	.243	-.400	-.295	-.436	-.190	-.261	-.325	-.301	.293	.382	.582*	1.000	
VE Δ 13% O_2 / 6% CO_2	.134	-.404	-.427	-.450	-.313	-.411	-.418	-.322	.111	.418	.325	.436	1.000

NOTE: Abbreviations: AHI, Apnoea Hypopnea Index; BMI, Body Mass Index; FEV_1. Forced Expiratory Volume 1s; FVC, Forced Vital Capacity; VE Δ, Change in ventilatory response to each gas mixture from resting minute ventilation.

4.5 Discussion

The present study examined the ventilatory response to hypoxia and hypercapnia during wakefulness in healthy individuals with varied cardiovascular fitness levels (Trained and Untrained groups) and patients with newly diagnosed OSA. We further examined the influence of cardiovascular fitness and body characteristics on the potential central and peripheral chemosensitivity alterations observed in OSA. The ventilatory response to hypoxia and hypercapnia was also assessed in OSA patients after 3 months of CPAP treatment. The novel technique reported by Earing et al., (2014) was performed in the present study to determine whether the central and peripheral chemosensitivity was different in OSA patients compared with healthy individuals. The contribution of body characteristics and cardiovascular fitness for the hypoxic and hypercapnic ventilatory responses was also examined. The re-assessment was performed to determine whether central and peripheral chemosensitivity was altered following 3 months of CPAP treatment in OSA patients. There are five major outcomes to be drawn from the experiment. First, patients with newly diagnosed OSA show an attenuated ventilatory response to hypoxia and hypercapnia compared with Trained individuals (Figure 4.2). Second, the ventilatory response to hypoxia and hypercapnia was lower in Untrained individuals compared with Trained individuals. Third, no differences were found in the ventilatory response to hypoxia and hypercapnia between Untrained individuals and OSA patients ($p > .05$). Fourth, body characteristics and cardiovascular fitness partially explained the alterations observed in central and peripheral chemosensitivity (Table 4.4). Fifth, the ventilatory response to hypoxia and hypercapnia was unchanged following 3-months of CPAP treatment in OSA patients (Figure 4.3).

We firstly investigated the ventilatory response to hypoxia and hypercapnia by calculating the change in minute ventilation for each gas mixture from the baseline ventilation. Based upon previous research, we expected patients with newly diagnosed OSA to demonstrate an attenuated ventilatory response to hypoxia and hypercapnia compared with Trained and Untrained individuals. In line with our hypothesis, patients with newly diagnosed OSA were found to possess a significantly lower ventilatory response to hypoxia and hypercapnia during wakefulness compared with Trained individuals (Figure 4.2). Our results are in line with previous studies that have investigated the central and peripheral chemoreflex response in OSA patients. Gold et al., (1993) demonstrated a reduced hypercapnic ventilatory response in eucapnic OSA patients compared with age-matched controls. Similarly, Osanai et al., (1999)

reported a decreased hypercapnic and hypoxic ventilatory response in a small group of OSA patients. Whilst, Javaheri et al., (1994) found a reduced hypoxic and hypercapnic ventilatory response in hypercapnic OSA patients. Finally, Garay et al., (1981) showed the hypercapnic ventilatory response was reduced in a mixed group of eucapnic and hypercapnic OSA patients. The findings of the present study and those above provide support for an alteration of central and peripheral chemoreceptor sensitivity in OSA patients. The alteration of ventilatory control in OSA patients could be attributed to the chronic exposure of intermittent hypoxia patients experience during sleep (Mateika & Narwani, 2009; Rey et al., 2004). It has been hypothesised that frequent episodes of intermittent hypoxia during sleep could potentially alter or reset the sensitivity of the carotid body to a lower sensitivity threshold (Forster & Dempsey, 1981; Guilleminault & Cummiskey, 1982). Furthermore, the repetitive deoxygenation reoxygenation pattern observed during sleep may promote an inflammatory cascade (free radical production and oxidative stress) that alters the performance of the respiratory muscles (Jackson & Farrell, 1993). In addition, leptin deficiency and/or resistance has been suggested to modulate central chemosensitivity in animal models of obesity and respiratory disorders such as OHS and OSA (Fitzpatrick, 2002; O'Donnell et al., 2000; Redolfi et al., 2006; Tankersley et al., 1998). Therefore, several factors may play a role in altering the sensitivity of the central and peripheral chemoreceptors in OSA patients.

It was also revealed the ventilatory response to both hypoxia and hypercapnia were not significantly different between Untrained and OSA groups ($p > .05$). Our findings are supported by previous studies that have found no differences in the ventilatory responses between normotensive OSA patients and healthy controls (Narkiewicz et al., 1999; Radwan et al., 1995; Sin et al., 2000). These findings potentially suggest there is a preservation of normal central and peripheral chemoreceptor sensitivity in OSA patients compared with healthy Untrained individuals. The notion has previously been supported by few studies reporting a subset of OSA patients to have central chemosensitivity within the normal range (Breska, Andreas, and Kreuzer, 1992; Rajagopal et al., 1984). The conflicting results demonstrated in the literature possibly reflects the heterogeneity of the population studied with hypercapnic OSA patients reported to have depressed hypercapnic ventilatory responses compared to the normal responses viewed in normocapnic OSA patients (Garay et al., 1981).

Our study further revealed that Untrained individuals possess a significantly lower ventilatory response to hypoxia and hypercapnia compared with Trained individuals (Figure 4.2). The maximal oxygen uptake was also shown to be significantly correlated with the ventilatory response to hypoxia (Table 4.2). These findings suggest that physical fitness is associated with the sensitivity of peripheral chemoreceptors. Few previous studies have examined the ventilatory response to hypoxia and hypercapnia in endurance-trained athletes and healthy controls with conflicting results. The hypoxic and hypercapnic ventilatory responses was shown to be reduced in endurance-trained athletes compared with non-athletic controls (Byrne-Quinn et al., 1971; Miyamura et al., 1976; Scoggin et al., 1978). Other studies have reported no differences between the hypoxic and/or hypercapnic ventilatory responses of endurance-trained athletes and controls (Godfrey et al., 1971; Mahler et al., 1982; Sheel et al., 2006). However, some important methodological differences exist between our study and those above may account for the disparity observed between the results, particularly the state of oxygenation during hypercapnic breathing (normoxia vs. hyperoxia) and the technique chosen to induce hypercapnia (rebreathing vs. steady state). However, based upon our findings and others it's possible to suggest the sensitivity of the central and peripheral chemoreceptors could be related to the cardiorespiratory fitness of tested individuals.

Next, we investigated the contribution of cardiovascular fitness and body characteristics for the potential alterations observed in OSA (Table 4.4). The analyses revealed that body characteristics (BMI, FVC, neck and hip circumference) accounted for 17.7 % of the variance in the hypercapnic ventilatory response ($p < .05$). It was also found that participants BMI and VO_{2max} accounted for 27.6 % of the variance in the hypoxic ventilatory response ($p < .01$). It was further revealed that participant's body characteristics (BMI, FVC, and hip circumference) explained 25.2 % of the variance in the combined central and peripheral response ($p < .01$). Therefore, body characteristics (particularly BMI, FVC and hip circumference) and cardiovascular fitness were found to partially explain the alterations of central and peripheral chemosensitivity in OSA.

We further hypothesised the ventilatory response to hypoxia and hypercapnia would be altered in OSA patients following 3-months of CPAP therapy. The central and peripheral chemoreceptor sensitivity was found to be unchanged following 3-months of CPAP treatment in OSA patients (Figure 4.3). The observation that CPAP therapy did not alter the sensitivity

of the chemoreceptors in the present study is surprising given that our OSA patients reported good compliance with CPAP and the AHI was normalised (< 5 events/hr; Table 4.1-c). It's also important to highlight that our OSA patients experienced weight gain during the 3-month treatment period which could have influenced the chemical drives via leptin deficiency and/or resistance (Tankersley et al., 1998). The duration of CPAP therapy also needs to discussed. Previous studies investigating the effects of CPAP therapy on hypoxic and hypercapnic ventilatory responses have reported conflicting results. Lin (1994) reported that 4-weeks of CPAP therapy increased the ventilatory response to hypoxia and hypercapnia in hypercapnic OSA patients but not in eucapnic OSA patients. Spicuzza et al., (2006) further demonstrated that 4-weeks of nasal CPAP therapy reduced the hypoxic ventilatory response in patients with moderate to severe OSA. However, no changes were observed in the hypercapnic ventilatory response. Tun et al., (2000) found the hypoxic ventilatory response was reduced and the hypercapnic ventilatory response was increased following 3-6 months of nasal CPAP therapy in severe OSA patients. Our findings are consistent with previous studies that have reported no changes in the hypoxic or hypercapnic ventilatory responses following ~ 3 months of CPAP therapy in eucapnic OSA patients (Berthon-Jones & Sullivan, 1987; Foster et al., 2009; Greenberg & Scharf, 1993; Radovanovic et al., 2019; Verbraecken et al., 1995). We therefore deduce that whilst 3-months of CPAP therapy effectively normalises AHI (i.e. reducing the frequency of obstructions to < 5 events / hour) the reduced central and peripheral chemosensitivity remains unaffected.

The strengths of our study include the recruitment of newly diagnosed, untreated OSA patients who were not affected by co-morbidities such as hypertension, diabetes type II etc. known to affect the chemoreflex response. All participants recruited for the study were non-smokers, free of cardiovascular disease, and underwent pulmonary function testing to screen for any underlying pulmonary impairments. The physical characteristics and pulmonary function were also re-assessed following CPAP treatment in OSA patients who did not undertake any diet, lifestyle, or surgical interventions in the 3 month treatment period. Our study reported a mean CPAP usage of 7.06 ± 0.4 hrs per night exceeding the threshold of > 6 hours per night that is deemed good compliance (Bouloukaki et al., 2014; Martínez-García et al., 2012). Furthermore, the ventilatory response to each gas mixture was normalised by participant's body surface area to account for body mass related differences in our tested populations.

While this study provides further insight into the ventilatory responses to hypoxia and hypercapnia in Trained, Untrained, and OSA groups and the effects of CPAP therapy, some limitations warrant discussion. Firstly, we did not conduct sleep studies in our healthy group to exclude the possibility of OSA due to the limited resources available. Instead we performed a series of sleep-related questionnaires to highlight any signs / symptoms of OSA in our healthy group. The Epworth Sleepiness Score (ESS) and Pittsburgh Sleep Quality Index (PSQI) were revealed to be significantly lower in Trained and Untrained individuals compared with OSA patients (Table 4.1), and classified as normal according to normative data (Buysse et al., 1989; Johns, 1991). Therefore, we are confident the healthy participants recruited for the present study were not demonstrating signs / symptoms of OSA. Secondly, despite our best efforts to recruit healthy participants matched for age and weight we found the recruited OSA patients were considerably older and heavier than our Trained and Untrained individuals. Future studies should attempt to recruit older and/or overweight participants without OSA in order to address whether age and weight differences exist. Thirdly, we did not conduct an incremental exercise test to measure the maximal rate of oxygen uptake in OSA patients. Fourthly, the ventilatory response to hypoxia and hypercapnia was only assessed during wakefulness. The additional measurement during sleep could have provided further insight into the chemical insensitivity observed during sleep and strengthened our overall findings. Fifthly, the CPAP treatment period of 3 months is considered short-term when attempting to observe changes in the chemical drives. Future studies should strive to examine the long-term effect of CPAP therapy for further refinement of this study.

In summary, we have demonstrated that patients with newly diagnosed OSA have a reduced ventilatory response to hypoxia and hypercapnia compared to Trained individuals which is not corrected following 3-months of CPAP therapy. Furthermore, the ventilatory response to hypoxia and hypercapnia was attenuated in Untrained individuals compared with Trained individuals. However, no differences were found in the ventilatory response to hypoxia and hypercapnia between Untrained and OSA groups. Body characteristics and cardiovascular fitness were found to partially contribute to the altered central and peripheral chemosensitivity alterations in OSA. The study provides new insight into the link between physical fitness and peripheral chemosensitivity.

CHAPTER FIVE

Perception of effort and activation of locomotor muscles before and after loading in Trained, Untrained, and OSA groups.

5.1 Abstract

Objectives: Obstructive Sleep Apnoea is characterised by repeated inspiratory efforts that result in a chronic overloading of the inspiratory muscles during sleep. Peripheral (lower extremity) muscles are considered to be underloaded as a consequence of disuse leading to further muscle atrophy, weakness, and dysfunction within this condition. Whether perceptual and muscular differences are observed in the skeletal muscles of OSA patients is unknown. Therefore, the study was performed to evaluate the perception of effort and activation of the quadriceps muscles before and after loading in OSA patients compared with healthy individuals (Trained and Untrained groups).

Methods: Forty-nine males (n = 18 Trained, n = 17 Untrained, and n = 14 OSA) completed the skeletal muscle loading protocol which consisted of five sets of twenty isometric contractions of the quadriceps muscles at a resistance equivalent to 50% of participants maximal voluntary capacity (MVC) using a custom-made isometric chair. Each contraction was performed in a rhythmic manner (3 seconds contraction followed by 3 seconds relaxation) and each set was separated by 30 seconds of rest. Isometric force production at a clamped effort of 14 on the Borg 6-20 rating of perceived exertion scale (RPE) was measured before (reference point) and after each completed set of isometric contractions. Electromyography (EMG) amplitudes of the Vastus Medialis and Lateralis muscles was simultaneously recorded.

Results: Patients with newly diagnosed OSA were shown to generate higher relative forces at the reference point compared with Trained and Untrained individuals ($p < .01$). The relative Vastus Medialis and Lateralis EMG amplitudes were increased during loading in healthy and OSA participants ($p < .05$). Repeated loading of the knee extensor muscles at 50% MVC resulted in a decline in the relative forces produced at RPE14 compared with the reference point in OSA patients e ($p < .05$). The relative EMG amplitudes of the Vastus Medialis and Lateralis muscles were shown to be increased at RPE14 following loading in OSA patients ($p < .05$).

Conclusion: The results of the present study provide further evidence that newly diagnosed OSA patients demonstrate an impaired effort perception and heightened activation of the skeletal muscles in response to loading.

5.2 Introduction

Obstructive sleep apnoea (OSA) is a breathing disorder characterised by repeated episodes of narrowing (hypopnea) or collapse (apnoea) of the upper respiratory airways, that occur intermittently, and at regular intervals during sleep (Carter & Watenpaugh, 2008). Recurrent cessations of airflow result in periodic asphyxia, transient periods of hypoxemia, and hypercapnia (Halliwill & Minson, 2002; Mullington et al., 2009). Futile inspiratory efforts against the obstructed upper airway produce changes in intrathoracic pressure and dysregulates blood flow to the respiratory muscles (Remmers et al., 1978; Wiegand & Zwillich, 1994; Drager, Polotsky, & Lorenzi-Filho, 2011). The ventilatory disturbance and intermittent hypoxia result in sleep fragmentation and respiratory effort-related arousals (RERA) which attempt to re-establish the airway patency and normalise blood gases (Guilleminault, Tilkian, & Dement, 1976; Somers et al., 1995).

Another important feature of the chronic condition is the ventilatory response to the occluded airway and subsequent obstructive event (Wiegand & Zwillich, 1994). Repeated inspiratory efforts against the occluded airway and obstructive event, both of which could potentially result in a chronic overloading of the inspiratory muscles during sleep (Wilcox et al., 1990). In contrast, the peripheral (lower extremity) muscles are considered to be underloaded as a consequence of progressive deconditioning, leading to changes in muscle characteristics (i.e. fibre type shifts and reduced oxidative capacity) and promote further muscle atrophy, weakness, and dysfunction within this condition (Barreiro et al., 2007; Chien et al., 2010; Sauleda et al., 2003; Larsson et al., 2008). However, relatively few studies have addressed the perceptual alterations that accompany fatigue and/or dysfunction of the muscles in OSA patients. There is some evidence to suggest that OSA patients demonstrate an augmented perceptual response to inspiratory loading (Earing, 2014; Tun et al., 2000). However, no study to date has examined the perceptual and muscular response to loading of the skeletal muscles in OSA patients compared with healthy individuals.

Therefore, we adapted the effort sensation paradigm as reported by Earing et al., (2014) to assess the perception of effort and quadriceps muscle activity before and after repeated bouts of loading in patients with newly diagnosed OSA compared with healthy Trained and Untrained individuals. The effort sensation paradigm adopted in the current study was not externally controlled (such as holding a weight or tension for a set duration) rather our

participants were instructed to match the isometric force produced at a set rating of perceived exertion (RPE) of 14 before and after repeated bouts of loading. Earing et al., (2014) subjectively perceived that participants related better to an RPE of 14 (between '*somewhat hard' to 'hard/heavy'* on the 6-20 Borg scale) than 6 on the Borg CR-10 scale. The outcome of the experiment would enable us to determine whether effort is perceived differently in OSA patients compared with healthy Trained and Untrained individuals. We hypothesised patients with newly diagnosed OSA would demonstrate an impaired effort perception of the skeletal muscles that reflects higher isometric forces being produced at an RPE of 14 at baseline (the reference point) and following loading. However, we expected newly-diagnosed OSA patients to show a greater decline in the isometric force production at an RPE of 14 in response to loading. Whilst, the respective EMG amplitudes for the Vastus Medialis and Lateralis muscles (% RMS) measured at RPE 14 were expected to remain constant / unchanged following loading in OSA patients. In contrast, healthy individuals were expected to show a lesser decline in the isometric force measured at RPE 14 following loading. Whilst the respective EMG amplitudes for Vastus Medialis and Lateralis muscles (% RMS) at RPE 14 were expected to remain constant / unchanged following loading.

5.3 Methods

Participant characteristics

Sixty one males were recruited to participate in the study which included 21 Trained participants (age: 35.1 ± 2.0 years; height: 179.5 ± 1.5 cm; mass: 76.6 ± 2.4 kg; VO_{2max}: 60.2 ± 1.9 $ml \cdot kg^{-1} \cdot min^{-1}$), 19 Untrained participants (age: 28.4 ± 1.8 years; height: 176.8 ± 1.9 cm; mass: 82.0 ± 4.1 kg; VO_{2max}: 39.7 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$), and 21 patients with newly-diagnosed OSA (age: 55.1 ± 2.0 years; height: 173.4 ± 1.3 cm; mass: 98.2 ± 4.3 kg). The data of twelve participants was excluded from the analysis due to insufficient data collection and a few participants were unavailable for the testing session. We therefore present the data of 49 participants which includes 18 Trained, 17 Untrained, and 14 OSA participants. The Trained group were composed of endurance-trained athletes (cycling, running, or triathlon) who completed on average 8 hours of training per week (weekly distance 41.0 miles). Whilst, the Untrained group were composed of individuals who did not participate in any regular physical exercise. The study was approved by Departmental (SSHES, Bangor University, Ref: P01-16/17) and National Health Ethics Committees (Ref: 17/EM/0162). Participants received a detailed explanation of the procedures and a checklist to ensure compliance with the eligibility criteria **(Appendix I & II)**. Written informed consent was obtained from all participants **(Appendix III)**. The experimental protocols were performed in accordance with the Declaration of Helsinki.

Study design

The effect of skeletal muscle loading on the isometric forces generated at a fixed RPE and electrical activity of the Vastus Medialis and Lateralis muscles were assessed using a cross-sectional study design. Healthy participants visited the laboratory of the School of Sport, Health, and Exercise Sciences as outlined in **Figure 3.2-a**. Patients with newly diagnosed OSA were required to visit the laboratory at the Pulmonary Function department (Ysbyty Gwynedd) as outlined in **Figure 3.2-b**.

Table 5.1. Physiological characteristics of participants

	Trained (n = 18)	Untrained (n = 17)	OSA (n = 14)
Age (years)	36.0 (2.2)	28.7 (1.9)††	56.3 (2.7)##
Height (cm)	178.8 (1.6)	175.2 (1.8)	174.7 (1.5)
Weight (kg)	75.4 (2.6)	77.0 (2.5)††	98.0 (4.7)##
Body Mass Index ($kg \cdot m^2$)	23.5 (.75)	25.0 (.62)††	32.1 (1.5)##
Body Surface Area (cm)	193.4 (3.4)	192.3 (3.8)††	216.1 (5.5)##
Body Fat (%)	13.3 (1.2)	17.2 (.89)††	30.2 (1.8)##
Neck circumference (cm)	37.8 (.53)	38.8 (.62)††	43.1 (1.0)##
Chest circumference (cm)	95.3 (1.9)	97.1 (1.9)††	115.0 (3.2)##
Waist circumference (cm)	83.8 (2.2)	87.0 (1.7)††	113.3 (3.9)##
Hip circumference (cm)	98.0 (1.5)	101.2 (1.4)††	109.4 (2.1)##
Apnoea Hypopnea Index (events/h)	N/A	N/A	34.7 (5.0)
Epworth Sleepiness Scale	4.72 (.92)	3.00 (.49)††	13.2 (1.2)##
Pittsburgh Sleep Quality Index	3.41 (.54)	4.23 (.46)††	9.7 (1.1)##
Forced Expiratory Volume 1s (l/min)	4.34 (.15)	4.14 (.14)††	3.21 (.14)##
Forced Vital Capacity (l/min)	5.31 (.16)	5.02 (.18)†	4.22 (.22)##
DLCO (ml/min/mmHg)	N/A	N/A	24.5 (1.3)
Total Lung Capacity (l/min)	N/A	N/A	6.16 (.29)
VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	60.2 (1.9)**	39.7 (1.4)	N/A
PI_{max} (cmH_2O)	112.1 (5.4)	112.0 (6.8)	104.9 (6.5)
MVC (N)	411.7 (23.0)	382.9 (29.1)†	278.5 (30.6)##

NOTE: DLCO, Diffusing capacity of the lung for carbon monoxide; VO_{2max}, Maximal Oxygen Uptake. PI_{max}, Maximal Inspiratory Pressure (Inspiratory muscles); MVC, Maximal Voluntary Capacity (Quadriceps muscles). Values are Mean ± SEM. * p <.05, ** p <.01 Trained vs Untrained. † p <.05, †† p <.01 Untrained vs OSA. # p <.05, ## p <.01 OSA vs Trained.

Experimental procedures

The full detailed procedures of the following measurements are described in **Chapter 3.8**; however, the main techniques are described here in brief. The following experimental procedure was designed to progressively load the Vastus Medialis and Lateralis muscles under standardised isometric conditions to determine whether the isometric forces at RPE 14 and corresponding EMG amplitudes were different between the groups **(Figure 5.1)**. The EMG amplitude was also recorded during the first and fourth set of loading to determine whether the muscle activity changes over the course of the loading. Vastus Medialis and Lateralis EMG amplitudes were measured by applying bipolar silver/silver chloride electrodes longitudinally on cleaned, abraded skin of the dominant leg in accordance with SENIAM recommendations (Hermens et al., 2000).

Participants firstly completed a standardised warm-up which consisted of 10 isometric contractions (held for 3 seconds) at a force that was assumed to be approximately 50% of maximal voluntary contraction (MVC) based on former data using a custom-made isometric chair. The warm-up was followed by seven consecutive MVC's that were separated by 1 minute of rest. Participants were then introduced to the 6-20 Borg scale and anchoring of the scale according the effort perceived during the series of MVC's. Participants were instructed to produce five isometric forces at a clamped effort of 14 'reference point' using standard procedures (Noble and Robertson, 1996) corresponding to the verbal anchors of '*somewhat hard*' to '*hard/heavy*' on the 6-20 Borg scale . This allowed participants to gauge how much force was required to represent the target RPE prior to loading. The EMG amplitudes of Vastus Medialis and Lateralis muscles were also recorded during these tasks to determine the muscle activity before loading. The skeletal muscle loading protocol was then performed which consisted of five sets of twenty isometric contractions at 50% of MVC. Each contraction was performed in a rhythmic manner (3 seconds contraction followed by 3 seconds relaxation) with 30 seconds of rest between each set. The EMG amplitudes of the Vastus Medialis and Lateralis muscles were measured during the first and fourth set of loading . After each set of twenty isometric contractions, participants generated an isometric force at RPE 14 that allowed us to reassess the isometric force at RPE14 and corresponding EMG activity.

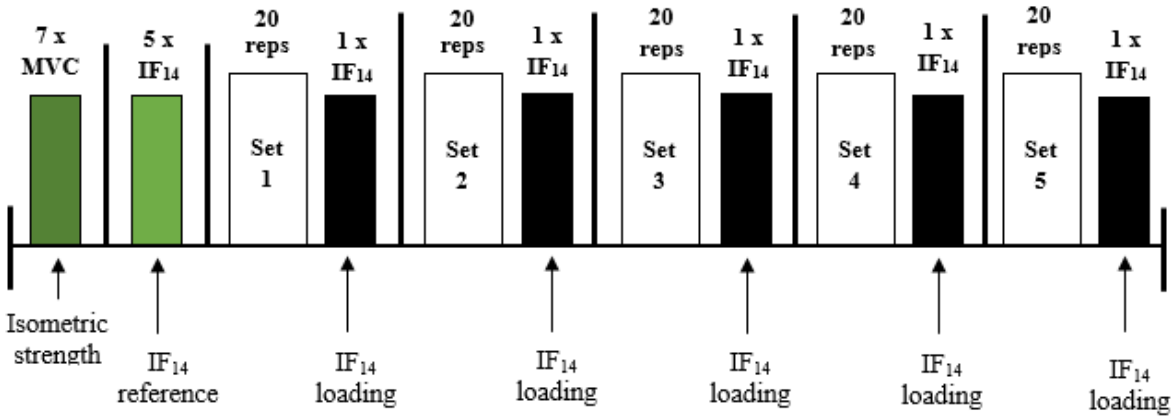


Figure 5.1. Schematic diagram of the skeletal muscle loading protocol. **NOTE:** MVC, Maximal Voluntary Contraction; IF_{14}, Isometric force at RPE 14.

Data Analysis

Isometric forces at a fixed RPE 14 (IF_{14} reference and loading) were studied according to the absolute (Newton) and relative values (normalised according to the MVC and expressed as % MVC). The matching of isometric forces at RPE 14 was calculated by subtracting the average IF_{14} (measured after loading) from the average IF_{14} (reference point measured before loading). Coefficient of variation (CV %) for the IF_{14} reference and loading measures were assessed. Electromyography (EMG) data was recorded and analysed offline using commercially available software (Power Lab Chart V 4.2.3, AD Instruments, Bella Vista, Australia). EMG signals were amplified (gain x 1000), band-pass filtered 35-500 Hz, and digitised at a sampling rate of 1000 kHz. Root mean square (RMS) amplitudes with a time constant of 100 ms were analysed over the 0.5 s window for the MVC, loading protocol (1st and 4th set), IF_{14} reference and loading measures. The EMG data was studied according to absolute (µV) and relative amplitudes (normalised according to the maximal EMG and expressed as % RMS). Neuromuscular efficiency was calculated by dividing the isometric force generated during the IF_{14} measured after loading with the average EMG amplitude for that contraction (Deschenes et al., 2008). Data measured after the fifth set of loading (100 repetitions) was excluded from the analysis due to participants knowing they were facing the last measure. Participants would respond by altering their behaviour either through giving a 'last push', or disengage altogether from the task, which resulted in a higher or lower isometric force at RPE14 being produced at the conclusion of the protocol.

Statistical Analysis

Statistical analyses were performed using SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA). Data was expressed as mean ± SEM with $p < .05$ considered statistically significant. Shapiro-Wilk tests were performed to determine the data's assumption of normality. One-Way ANOVA analyses were performed to determine whether loading had an influence on the relative isometric forces at RPE 14 (IF_{14}) and the corresponding Vastus Medialis and Lateralis EMG amplitudes. Kruskal-Wallis tests and follow-up Mann-Whitney U tests were performed to analyse differences between the groups given the non-parametric nature of the absolute IF_{14} values, EMG amplitudes, and the inability to transform the data. Paired *t*-tests were performed to examine potential differences in the IF_{14}, CV %, and EMG amplitudes in response to loading for each respective group. One-Way ANOVA analyses were performed to assess the CV% of IF_{14} reference and loading measures. Effect sizes were calculated using the Cohen *d* equation and interpreted as 0.2 small, 0.5 moderate, and 0.8 large effect size. Linear regression analysis was conducted to examine the slope of EMG amplitude measured during IF_{14} loading measures for each respective group. One-way ANOVA was also performed to determine the neuromuscular efficiency (IF_{14} / EMG amplitude) of the Vastus Medialis and Lateralis muscles in response to repeated loading. One-Way ANOVA analyses were performed to determine whether the Vastus Medialis and Lateralis EMG amplitudes was influenced during the first and fourth set of the loading protocol. Further linear regression analyses were performed to examine the slope of EMG amplitude during the first and fourth set of loading protocol for each respective group.

5.4 Results

Participant characteristics

Patients with newly-diagnosed OSA were found to be significantly older and heavier than both Trained and Untrained groups ($p < .05$; Table 5.1). Sleep-related measures (ESS and PSQI) were revealed to be significantly higher in OSA patients compared with Trained and Untrained groups ($p < .05$). Lung function parameters (FEV_1 and FVC) were shown to be significantly lower in OSA patients than Trained and Untrained individuals ($p < .05$). Cardiovascular fitness (VO_{2max}) and lower extremity strength (MVC) was significantly greater in Trained individuals compared with Untrained individuals ($p < .05$).

Isometric forces produced at RPE 14 before and after loading

The primary aim of our investigation was to examine the isometric forces produced at an RPE of 14 (IF_{14}) on the Borg scale before and after repeated bouts of loading in Trained, Untrained, and OSA populations. We firstly focused upon the isometric forces generated at an RPE of 14 during unloaded conditions that is referred to as the reference point of effort perception. Shapiro Wilk tests were initially performed to determine the normality of the absolute forces generated at RPE 14 for the reference point. The data was revealed to be normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA and Bonferroni corrections to determine whether the absolute isometric forces produced by the skeletal muscles at a set RPE of 14 were different between the groups. The analyses revealed the absolute isometric forces produced at a fixed effort of 14 were not different at the reference point ($p > .05$; **Table 5.2**). Subsequently, we performed Shapiro Wilk tests which revealed the relative isometric force at RPE 14 (normalised for MVC) was normally distributed ($p > .05$). One-Way ANOVA analyses were performed demonstrating the relative isometric forces produced at RPE 14 was significantly different at the reference point ($F_{(2, 46)} = 8.597$, $p = .001$). Follow-up analyses revealed that OSA patients produce significantly greater relative isometric forces at the reference point compared with Trained ($p = .001$) and Untrained individuals ($p = .003$; **Figure 5.4.1 A**). These results were reinforced by the large effect size observed in OSA patients versus Trained ($d = 1.25$) and Untrained individuals ($d = 1.18$). In addition, the maximal isometric force generated with the dominant leg during unloaded conditions was found to be noticeably lower in OSA patients compared with Trained ($U = 51$, $p = .004$) and Untrained individuals ($U = 63$, $p = .026$).

Next, we examined the isometric forces produced by the skeletal muscles at a clamped effort of 14 (IF_{14}) on the Borg scale following repeated bouts of loading. Shapiro Wilk tests were revealed the absolute forces generated at RPE 14 following loading were not normally distributed ($p < .05$). Therefore, we performed a Kruskal Wallis test which revealed the absolute isometric forces produced at RPE 14 were significantly different following loading ($\chi^2 = 8.781$, $df = 2$, $p = .003$). Mann-Whitney U tests revealed the absolute isometric forces produced at a fixed RPE of 14 were significantly lower in OSA patients compared with Trained individuals after 20 ($U = 49$, $p = .003$), 40 ($U = 68$, $p = .028$), 60 ($U = 42$, $p = .001$), and 80 repetitions ($U = 61$, $p = .014$; **Table 5.2**). The analyses further revealed a significantly lower absolute isometric force generated at RPE 14 following loading 'IF_{14} loading' in OSA patients compared with Trained individuals ($U = 48$, $p = .003$). Our experiment also demonstrated that repeated loading of the quadriceps muscles at 50% MVC resulted in a decline of isometric force production at RPE 14 in OSA patients compared with the reference force measured during unloaded conditions (reference: 161.4 ± 15.4 N vs. loading: 135.4 ± 14.0 N, 95% CI: -9.4 to -42.7, $t_{(13)} = -3.377$, $p = .005$). We then normalised the absolute IF_{14} generated following loading according to participant's MVC. Shapiro Wilk tests showed the relative IF_{14} data was normally distributed ($p > .05$). Therefore, we performed One-Way ANOVA analyses which demonstrated no between-group differences were observed in the relative isometric forces generated by the skeletal muscles at a clamped effort of 14 following loading ($p > .05$; **Figure 5.4.1 B**).

Table 5.2. Isometric forces produced at a fixed RPE of 14 before and after repeated bouts of loading

	Trained (*n* = 18)		Untrained (*n* = 17)		OSA (*n* = 14)		Effect size (Cohen *d*)		
Absolute forces	Force N	Median (IQR) N	Force N	Median (IQR) N	Force N	Median (IQR) N	T v U	T v O	U v O
MVC	411.7 (23.0)	415.5 (351.3-464.2)	382.9 (29.1)†	386.9 (312.2-475.1)	278.5 (30.5)$^{\#\#}$	226.2 (193.4-414.3)	0.26	1.25	0.89
IF_{14} reference (avg)	177.1 (15.6)	158.2 (127.2-215.9)	169.8 (18.6)	149.6 (105.1-220.2)	161.4 (15.4)	163.2 (118.1-190.1)	0.10	0.25	0.12
IF_{14} 20 repetitions	184.7 (10.4)	178.2 (154.5-200.7)	154.9 (14.0)	159.2 (91.2-198.2)	132.2 (14.6)$^{\# \Psi\Psi}$	121.0 (91.4-165.1)	0.57	1.05	0.57
IF_{14} 40 repetitions	180.3 (11.5)	174.7 (148.0-215.1)	164.9 (18.6)	164.8 (96.7-205.2)	141.4 (15.5)$^{\#}$	122.3 (110.7-180.3)	0.23	0.72	0.34
IF_{14} 60 repetitions	180.2 (14.0)	162.8 (138.4-201.1)	162.4 (17.3)	160.1 (104.6-219.3)	131.8 (11.5)$^{\#}$	118.5 (101.3-141.5)	0.27	0.93	0.52
IF_{14} 80 repetitions	179.3 (14.5)	167.8 (130.9-218.4)	152.9 (17.3)	146.6 (91.5-211.4)	136.1 (17.9)$^{\#}$	110.8 (93.8-155.7)	0.39	0.66	0.24
IF_{14} loading (avg)	181.1 (11.5)	169.2 (143.7-222.9)	158.8 (15.9)	152.7 (93.8-213.5)	135.4 (14.0)$^{\# \Psi\Psi}$	114.2 (104.2-161.9)	0.38	0.90	0.38

NOTE: Values are Mean ± SEM unless otherwise stated. MVC, Maximal Voluntary Contraction; N, Newton; IF_{14}, Isometric Force at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea; T, Trained; U, Untrained; O, OSA.

$^{\#\#}$ = p <.01 Significantly lower MVC in OSA vs Trained. † = p <.05 Significantly higher MVC in Untrained vs OSA. $^{\#}$ = p <.05, $^{\#\#}$ = p <.01 Significantly lower IF_{14} produced after 20, 40, 60, 80 repetitions in OSA vs Trained. $^{\#\#}$ = p <.01 Significantly lower IF_{14} loading produced in OSA vs Trained. $^{\Psi\Psi}$ = p <.01 Significantly lower IF_{14} produced after 20 repetitions compared with IF_{14} reference in OSA. $^{\Psi\Psi}$ = p <.01 Significantly lower IF_{14} loading than IF_{14} reference in OSA.

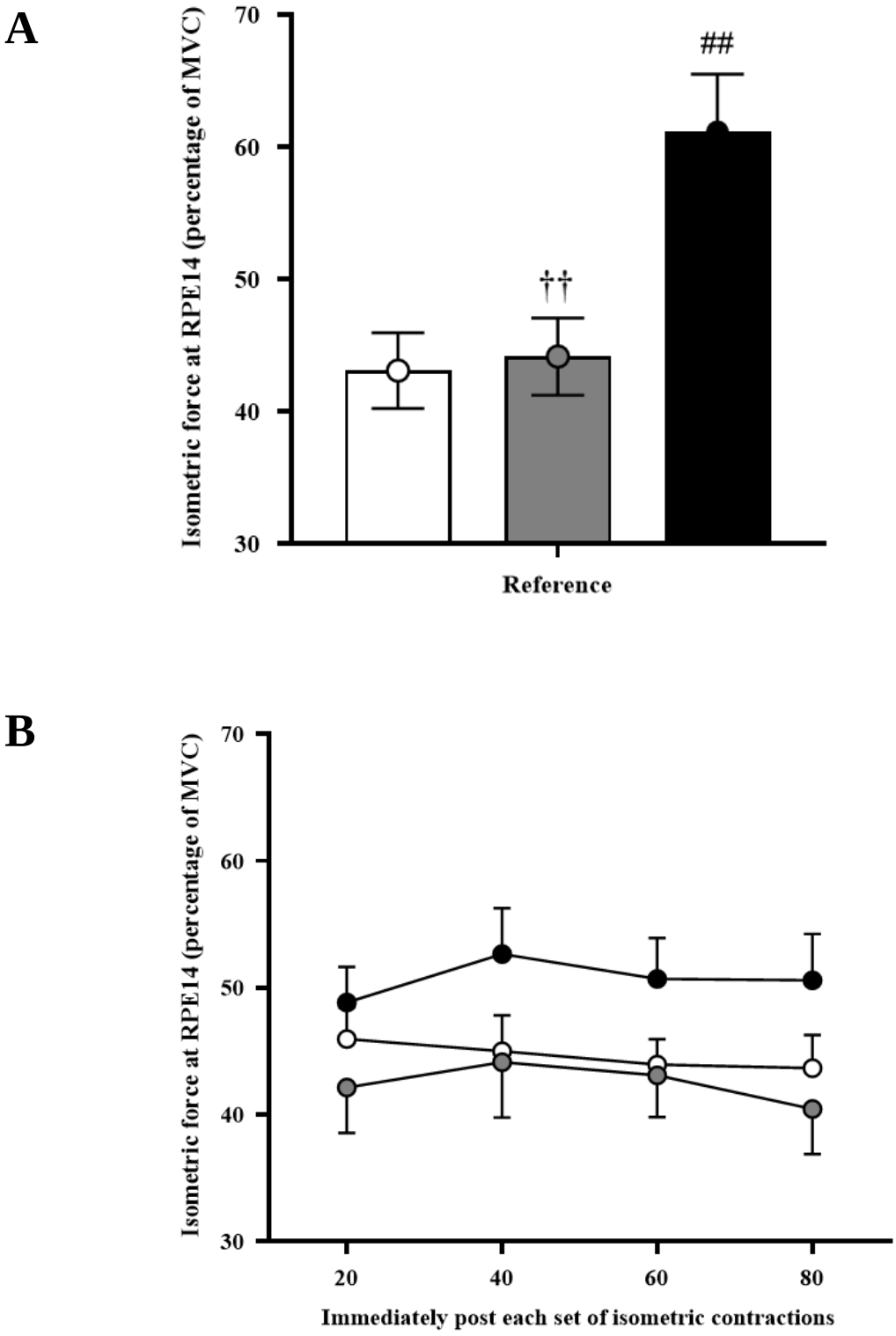


Figure 5.4.1. Isometric force at an RPE of 14 (IF_{14}) measured at the reference point **(A)** and after repeated bouts of loading **(B)**. Force data is expressed as Mean ± SE. ○ = Trained group (*n* = 18); ◍ = Untrained group (*n* = 17); ● = OSA group (*n* = 14). **NOTE:** Abbreviations: MVC, Maximal Voluntary Contraction; $IF_{14,}$ Isometric force at RPE 14. †† = $p < .01$ vs. OSA; $^{\#\#}$ = $p < .01$ vs. Trained.

Subsequently, we examined the coefficient of variation (CV %) to determine whether the variability in IF_{14} measured at the reference point and after loading were different between the groups. Shapiro Wilk tests revealed the CV% for both reference and loading IF_{14} was normally distributed ($p > .05$). One-way ANOVA analyses were therefore performed revealing no between-group differences were observed in the CV% of reference and loading IF_{14} ($p > .05$, **Figure 5.4.2**). Paired *t*-test analyses were subsequently performed to determine whether differences in the CV% of IF_{14} would be observed following loading for each respective group. The results revealed that Untrained and OSA groups respond to loading with a significantly greater CV % when generating isometric forces at RPE 14 (Untrained mean difference: 3.4 %, 95% CI: .78 to 6.0, $t_{(16)} = 2.751$, $p = .014$; OSA mean difference: 6.9%. 95% CI: 3.2 to 10.7, $t_{(13)} = 3.998$, $p = .002$). In contrast, Trained individuals demonstrated no change in the CV% of IF_{14} after loading ($p = .083$).

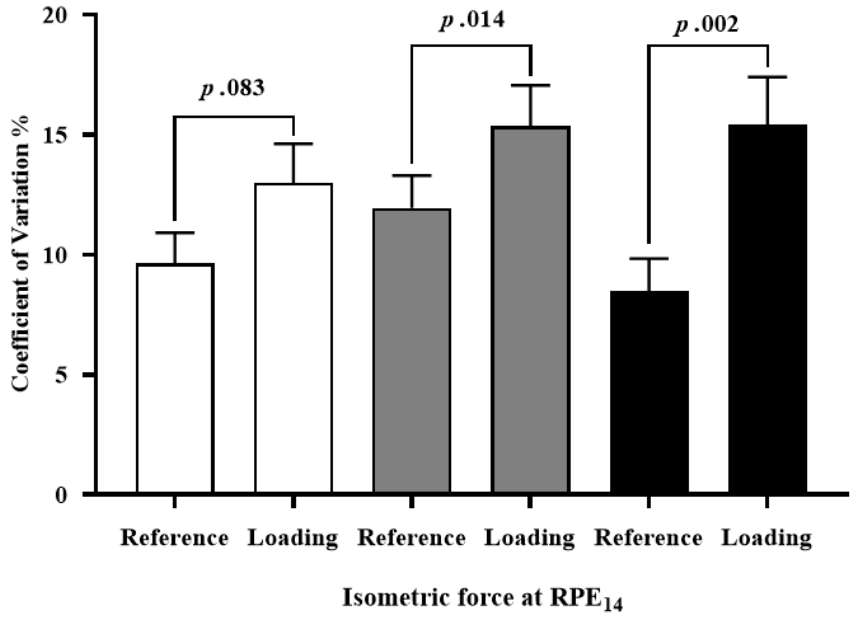


Figure 5.4.2. Coefficient of variation (expressed as percentage) of IF_{14} measured at the reference point and after loading. Data is expressed as Mean ± SE. □ = Trained group (n = 18); ▒ = Untrained group (n = 17); ■ = OSA group (n = 14).

Finally, we examined the matching of isometric forces at a set RPE of 14 by comparing the relative isometric forces measured at the reference point to those performed following loading. Shapiro Wilk tests revealed the percentage difference between reference and loading IF_{14} were normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA which revealed loading influenced the matching of isometric forces at a fixed RPE of 14 ($F_{(2, 46)} = 3.870$, $p = .028$, **Figure 5.4.3**). Follow-up analyses revealed the percentage difference between reference and loading IF_{14} was significantly greater in OSA patients compared with Trained individuals ($p = .027$, $d = 0.95$).

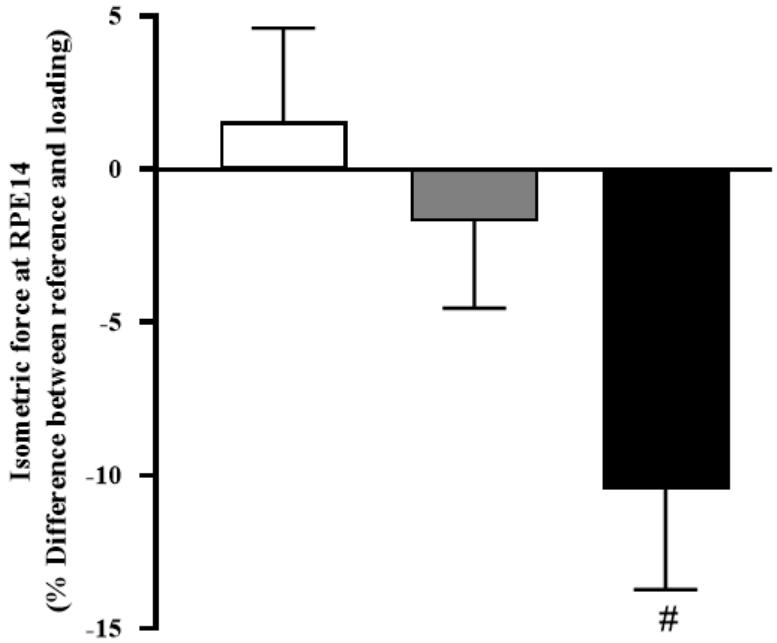


Figure 5.4.3. Isometric force at RPE of 14 (mean percentage difference between IF_{14} reference and loading). Force data is expressed as Mean ± SE. □ = Trained group (n = 18); ▒ = Untrained group (n = 17); ■ = OSA group (n = 14). $^{\#}$ = $p < .05$ vs. Trained group.

Our findings revealed that OSA patients operate at a higher percentage of their MVC for the reference point which reflects the greater relative isometric force generated at the clamped effort of 14 **(Figure 5.4.1 A)**. Whereas, Trained and Untrained individuals produce similar relative isometric force at an RPE of 14 for the reference point. We also found the absolute isometric forces generated at an RPE of 14 were shown to decline in OSA patients after performing repeated bouts of loading **(Table 5.2)**. Furthermore, the matching of isometric forces at RPE 14 were not adjusted to the level of the reference point as reflected by the greater decline in force production following loading in OSA patients **(Figure 5.4.3)**. In contrast, the absolute and relative isometric forces produced at a set RPE 14 were relatively unaffected following repeated bouts of loading in Trained and Untrained individuals **(Figure 5.4.1 B)**. These results were reflected by the closer matching of isometric forces at the fixed effort perception following repeated bouts of loading **(Table 5.2)**.

Electromyography amplitude of the Vastus Medialis and Lateralis muscles during the first and fourth set of the loading protocol

The next phase of our investigation was to examine the EMG activity of the Vastus Medialis and Lateralis muscles during the first and fourth set of the loading protocol in Trained, Untrained, and OSA groups. We firstly focused upon the Vastus Medialis EMG amplitude measured during the first and fourth set of the loading protocol. Shapiro Wilk tests were firstly performed to determine the normality of the Vastus Medialis EMG amplitude during the first and fourth set of the loading protocol. The data was revealed to be normally distributed ($p > .05$). Therefore, One-Way ANOVA analyses were performed revealing no between-group differences were observed in the Vastus Medialis EMG amplitude during the first and fourth set of loading ($p > .05$; **Table 5.3**). Paired *t*-test analyses were subsequently performed to examine potential differences in the EMG amplitude of the Vastus Medialis from the first to fourth set of loading for each respective group. The analyses revealed no differences in the EMG amplitude of Vastus Medialis muscle were observed from the first to fourth set of loading for each respective group ($p > .05$).

Linear regression analyses were then performed to determine whether an increase in EMG amplitude of Vastus Medialis muscles would be observed with loading. The analyses revealed a linear increase in the Vastus Medialis EMG amplitude with loading in OSA

patients (1st set: B = .696 µV per set, p = .000 and 4th set: B = .997 µV per set, p = .000) and Trained individuals (1st set: B = .513 µV per set p = .000 and 4th set: B = .616 µV per set, p = .000; **Figure 5.4.4**). In contrast, Untrained individuals demonstrated a slight reduction in the Vastus Medialis EMG amplitude with loading (1st set: B = .616 µV per set, p = .000 and 4th set: B = .582 µV per set, p = .000). One-Way ANOVA analyses revealed no between-group differences were observed for the slope and intercept of Vastus Medialis EMG amplitude during either the first or fourth set of loading (p > .05). Paired t-test analyses were further performed revealing the intercept of the Vastus Medialis EMG amplitude was significantly increased from the first to the fourth set of loading for each respective group (p = .000).

The following section reports the Vastus Lateralis EMG amplitude during the first and fourth set of the loading protocol. Shapiro Wilk tests were firstly performed to determine the normality of Vastus Lateralis EMG amplitude during the first and fourth set of the loading protocol. The data was revealed to be normally distributed (p > .05). Therefore, One-Way ANOVA analyses were performed revealing no between-group differences were observed in the Vastus Lateralis EMG amplitude during the first and fourth set of loading (p > .05; **Table 5.3**). Paired *t*-test analyses were subsequently performed to examine potential differences in the EMG amplitude of the Vastus Lateralis from the first to fourth set of loading for each respective group. The analyses revealed a significant increase in the Vastus Lateralis EMG amplitude during loading in OSA patients (1st VL: 63.2 ± 3.4 % vs. 4th VL: 72.8 ± 4.2 %, 95% CI: 3.5 to 15.5, $t_{(13)}$ = 3.412, p = .004). No differences were observed in the Vastus Lateralis EMG amplitude during loading in both Trained and Untrained individuals (p > .05).

Linear regression analyses were then performed to determine whether an increase in EMG amplitude of Vastus Lateralis muscles would be observed with loading. The analyses revealed a linear increase in the Vastus Lateralis EMG amplitude with loading in OSA patients (1st set: B = .818 µV per set, p = .000 and 4th set: B = .924 µV per set, p = .000) and Trained individuals (1st set µV per set: B = .241, p = .003 and 4th set: B = .629 µV per set, p = .000; **Figure 5.4.5**). In contrast, there was a slight decrease in Vastus Lateralis EMG amplitude with loading in Untrained individuals, (1st set: B = .628 µV per set, p = .000 and 4th set µV per set: B = .455, p = .000). One-Way ANOVA analyses revealed no between-group differences were observed in the slope and intercept of Vastus Lateralis EMG amplitude during the first set of loading (p >.05). However, further analyses indicated that Untrained

individuals displayed a significant reduction in the slope of Vastus Lateralis EMG amplitude during the fourth set of loading compared with Trained (p = .041, d = 1.02) and OSA groups (p = .011, d = 1.61). Furthermore, the intercept of the Vastus Lateralis EMG amplitude during the fourth set of loading was significantly increased in Untrained individuals compared with Trained individuals (p = .042, d = 1.05) and OSA patients (p = .004, d = 1.85). Paired t-test analyses were further conducted to examine the intercept for the Vastus Lateralis EMG amplitude measured during the first and fourth set of loading. The analyses revealed the intercept of Vastus Lateralis EMG amplitude was significantly increased from the first to fourth set of loading for each respective group (all p = .000). Our findings demonstrated a significant linear increase in the Vastus Medialis and Lateralis EMG amplitude during loading in our tested populations **(Table 5.3; Figure 5.4.4; Figure 5.4.5)**. These findings support the effectiveness of the skeletal muscle loading protocol at inducing fatigue of the quadriceps muscles in our tested populations.

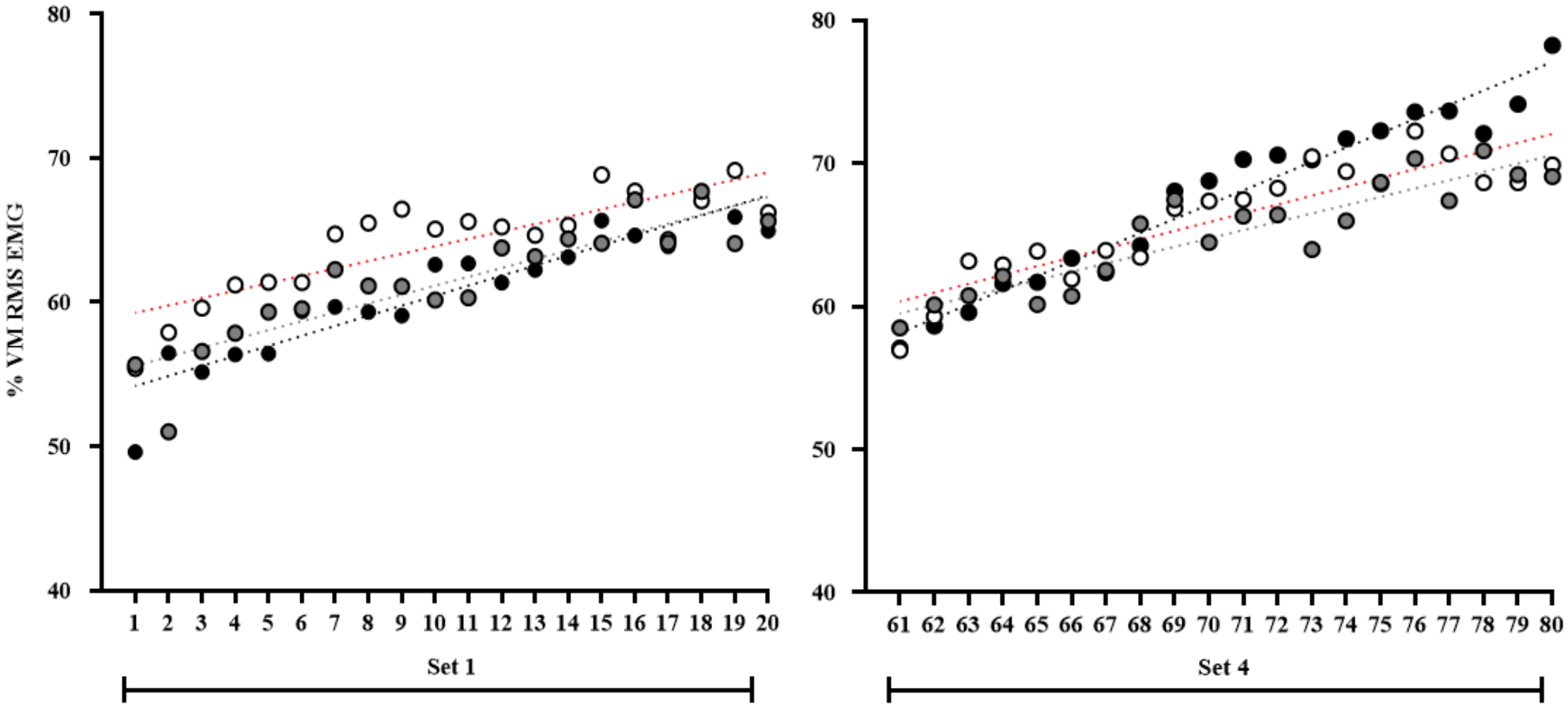


Figure 5.4.4. Vastus Medialis EMG amplitude measured during the first and fourth set of loading. EMG data is expressed as Mean ± SE. ○ = Trained group (*n* = 19); ◍ = Untrained group (*n* = 18); ● = OSA group (*n* = 14). The dashed line represents the linear trend line.

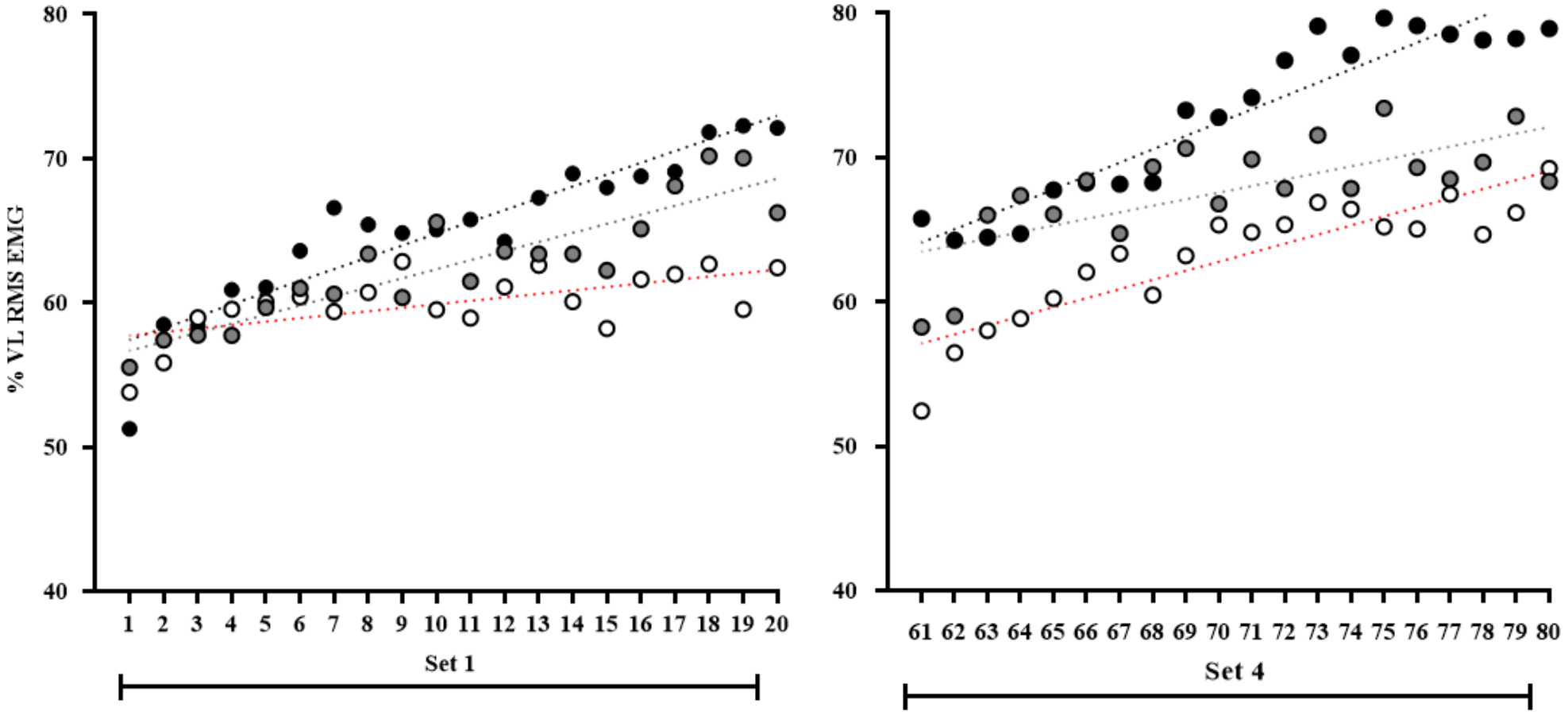


Figure 5.4.5. Vastus Lateralis EMG amplitude measured during the first and fourth set of loading. EMG data is expressed as Mean ± SE. ○ = Trained group (*n* = 15); ◎ = Untrained group (*n* = 10); ● = OSA group (*n* = 14). The dashed line represents the linear trend line.

Table 5.3. Change in slope of Vastus Medialis and Lateralis EMG activity during the first and fourth set of loading

		First set of loading (20 isometric contractions)					Fourth set of loading (80 isometric contractions)				
Outcome	**Group**	***B***	***SE B***	**β**	***t***	***p***	***B***	***SE B***	**β**	***t***	***p***
VM muscle (μV per set)	Trained	.513	.077	.845	6.693	.000	.616	.072	.895	8.523	.000
	Untrained	.616	.074	.891	8.341	.000	.582	.061	.914	9.531	.000
	OSA	.696	.065	.930	10.742	.000	.997	.054	.975	18.595	.000
VL muscle (μV per set)	Trained	.241	.071	.625	3.399	.003	.629	.076	.889	8.240	.000
	Untrained	.628	.069	.906	9.068	.000	.455	.108	.704	4.205	.001
	OSA	.818	.081	.922	10.111	.000	.924	.076	.944	12.163	.000

NOTE: Abbreviations: SE, Standard Error.

Electromyography amplitude of the Vastus Medialis and Lateralis muscles during IF_{14} measured before and after loading

The secondary aim of our investigation was to examine the Vastus Medialis and Lateralis EMG amplitudes measured during reference and loading IF_{14} in Trained, Untrained, and OSA populations. Our investigation firstly focused upon the EMG amplitude of the Vastus Medialis and Lateralis muscles measured during reference IF_{14}. Shapiro Wilk tests were performed to determine the normality of the absolute and relative EMG amplitudes generated at RPE 14 for the reference point. The data was revealed to be normally distributed ($p > .05$). Therefore, One-Way ANOVA analyses were performed which revealed the absolute and relative EMG amplitudes for both Vastus Medialis and Lateralis muscles were similar between the groups at the reference point ($p > .05$; **Table 5.4-a & 5.4-b; Figure 5.4.6 A**).

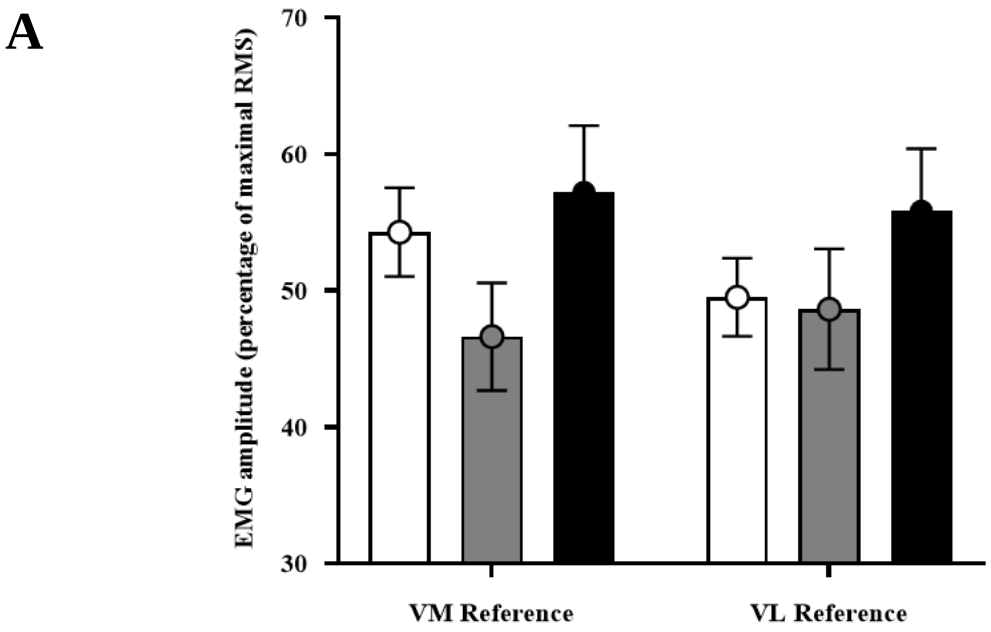


Figure 5.4.6 A. Vastus Medialis and Lateralis EMG amplitude during the IF_{14} measured at the reference point. EMG data are expressed as Mean ± SE. □ = Trained group (n = 18); ▒ = Untrained group (n = 17); ■ = OSA group (n = 14).

Next, we examined the absolute Vastus Medialis and Lateralis EMG amplitudes measured during the IF_{14} following repeated bouts of loading. Shapiro Wilk tests revealed the absolute Vastus Medialis and Lateralis EMG amplitudes was not normally distributed ($p < .05$). Therefore, we performed a Kruskal Wallis test which revealed the absolute Vastus Medialis EMG amplitude measured during loading IF_{14} was shown to be significantly different between the groups ($\chi^2 = 4.745$, $df = 2$, $p = .029$; **Table 5.4-a**). In contrast, the absolute Vastus Lateralis EMG amplitude measured during loading IF_{14} was similar between the groups ($p > .05$; **Table 5.4-b**). We then performed Mann-Whitney U tests to examine the differences in the Vastus Medialis EMG amplitudes during loading IF_{14}. Mann-Whitney U tests revealed the absolute Vastus Medialis EMG amplitudes was shown to be significantly lower in Untrained individuals than Trained individuals after completing 20 ($U = 88$, $p = .032$), 40 ($U = 76$, $p = .011$), 60 ($U = 88$, $p = .032$), and 80 repetitions ($U = 87.5$, $p = .031$). Further analyses revealed a significantly lower absolute Vastus Medialis EMG amplitude was observed at RPE 14 following loading in Untrained individuals compared with Trained individuals ($U = 87$, $p = .029$).

We then normalised the absolute Vastus Medialis and Lateralis EMG amplitudes measured during the IF_{14} following loading according to participant's maximal RMS. Shapiro Wilk tests demonstrated the relative Vastus Medialis and Lateralis EMG amplitudes were normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA which revealed loading did not influence the relative EMG amplitude of the Vastus Medialis and Lateralis muscles measured during the IF_{14} ($p > .05$, **Figure 5.4.6 B** and **5.4.6 C**). Linear regression analyses were then performed to examine changes in slope of Vastus Medialis and Lateralis EMG amplitudes measured during the IF_{14} after loading. The analyses revealed a linear increase in Vastus Medialis and Lateralis EMG amplitude when producing the IF_{14} after loading in OSA patients (VM: $B = .206$ µV per N, $p = .009$; VL: $B = .146$ µV per N, $p = .016$; **Table 5.5**). No changes were observed in the slope of Vastus Medialis and Lateralis EMG amplitude during loading IF_{14} in Trained (VM: $B = .065$ µV per N, $p = .061$; VL: $B = .040$ µV per N, $p = .447$) and Untrained groups (VM: $B = .104$ µV per N, $p = .084$; VL: $B = .035$ µV per N, $p = .370$). No differences were observed for the intercept of both Vastus Medialis and Lateralis EMG during the IF_{14} measured after loading ($p > .05$).

Our findings revealed no between group differences were observed in the EMG amplitude of Vastus Medialis and Lateralis muscles during the isometric forces produced at RPE 14 measured at the reference point **(Figure 5.4.6 A)**. However, OSA patients were shown to respond to repeated bouts of loading with an increased EMG amplitude of both Vastus Medialis and Lateralis muscles during the isometric forces produced at RPE 14 **(Figure 5.4.6 B and C; Table 5.5)**. Whilst, Trained and Untrained individuals demonstrated minimal change in the EMG amplitude of both Vastus Medialis and Lateralis muscles during the IF_{14} measured following loading.

Table 5.4-a. Vastus Medialis electromyography amplitude measured during the IF_{14} before and after loading

	Trained (*n* = 18)		Untrained (*n* = 17)		OSA (*n* = 14)		Effect size (Cohen *d*)		
Absolute RMS	μV	Median (IQR) μV	μV	Median (IQR) μV	μV	Median (IQR) μV	T v U	T v O	U v O
MVC	341.2 (37.5)	323.6 (252.6-362.5)	270.5 (42.1)	184.5 (146.8-372.8)	311.8 (45.5)	224.7 (205.1-467.9)	0.42	0.17	0.24
IF_{14} reference (avg)	196.0 (24.5)	188.7 (121.4-248.4)	123.3 (22.2)	103.7 (66.6-186.0)	190.9 (29.2)	165.8 (118.2-245.1)	0.89	0.05	0.75
IF_{14} 20 repetitions	180.5 (22.5)*	168.7 (106.3-222.2)	132.7 (21.0)	96.6 (75.0-193.1)	152.9 (23.0)	132.8 (86.9-178.6)	0.52	0.30	0.23
IF_{14} 40 repetitions	188.0 (18.8)*	182.6 (116.7-264.8)	128.7 (19.5)	96.4 (70.6-186.2)	162.4 (18.9)	187.3 (86.2-206.1)	0.73	0.33	0.44
IF_{14} 60 repetitions	189.2 (20.3)*	179.2 (121.9-213.0)	142.0 (19.9)	101.4 (86.2-174.9)	168.7 (18.1)	159.3 (138.3-202.8)	0.56	0.26	0.35
IF_{14} 80 repetitions	185.1 (22.2)*	171.9 (108.9-213.7)	135.6 (20.0)	107.6 (75.1-181.2)	182.0 (20.7)	173.8 (141.3-203.0)	0.56	0.03	0.57
IF_{14} loading (avg)	185.7 (20.2)*	174.7 (118.3-223.5)	134.8 (19.2)	106.4 (78.5-193.0)	166.5 (18.6)	172.7 (116.5-196.7)	0.61	0.24	0.42

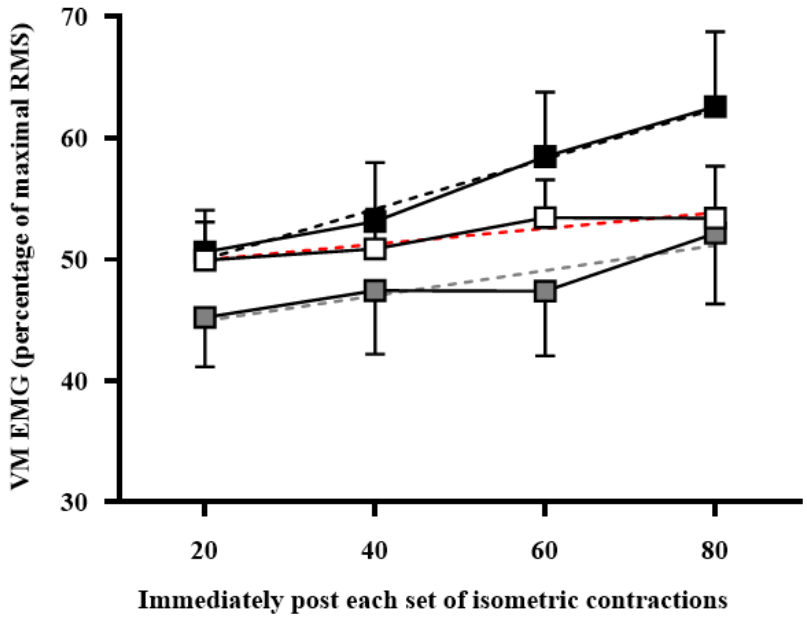
NOTE: Values are Mean ± SEM unless otherwise stated. MVC, Maximal Voluntary Contraction; μV, Microvolt; VM, Vastus Medialis; IF_{14}, Isometric Force at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea; T, Trained; U, Untrained; O, OSA. * = p <.05 Significantly lower VM EMG in Untrained vs Trained.

Table 5.4-b. Vastus Lateralis electromyography amplitude measured during the IF_{14} before and after loading

	Trained (*n* = 18)		**Untrained (*n* = 17)**		**OSA (*n* = 14)**		**Effect size (Cohen *d*)**		
Absolute RMS	μV	Median (IQR) μV	μV	Median (IQR) μV	μV	Median (IQR) μV	T v U	T v O	U v O
MVC	259.7 (21.9)	290.3 (189.2-308.1)	209.6 (32.4)	156.2 (91.5-341.4)	219.3 (28.9)	195.8 (139.2-266.0)	0.43	0.40	0.07
IF_{14} reference (avg)	145.6 (18.7)	120.3 (99.4-192.6)	135.4 (22.4)	109.4 (74.0-203.6)	133.5 (19.9)	105.8 (85.4-151.8)	0.14	0.17	0.03
IF_{14} 20 repetitions	144.4 (11.7)	146.9 (104.4-171.3)	122.7 (17.1)	102.4 (76.7-161.2)	111.0 (16.3)	83.9 (73.1-157.2)	0.35	0.60	0.18
IF_{14} 40 repetitions	154.8 (15.3)	152.1 (100.3-182.8)	116.8 (14.6)	90.4 (76.0-167.5)	118.4 (9.4)	116.2 (85.2-136.2)	0.60	0.69	0.03
IF_{14} 60 repetitions	157.8 (15.9)	135.4 (112.8-183.3)	129.2 (16.9)	113.1 (79.7-167.6)	118.8 (11.9)	101.9 (82.6-168.7)	0.41	0.68	0.18
IF_{14} 80 repetitions	157.1 (18.9)	127.3 (102.2-190.1)	115.4 (13.6)	96.6 (81.6-161.2)	119.4 (13.7)	103.9 (81.7-149.4)	0.60	0.56	0.07
IF_{14} loading (avg)	153.5 (14.5)	138.4 (108.8-185.0)	121.0 (14.5)	109.6 (81.2-165.0)	116.9 (10.7)	103.3 (84.7-145.3)	0.53	0.70	0.08

NOTE: Values are Mean ± SEM unless otherwise stated. MVC, Maximal Voluntary Contraction; μV, Microvolt; VL, Vastus Lateralis; IF_{14}, Isometric Force at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea; T, Trained; U, Untrained; O, OSA.

B



C

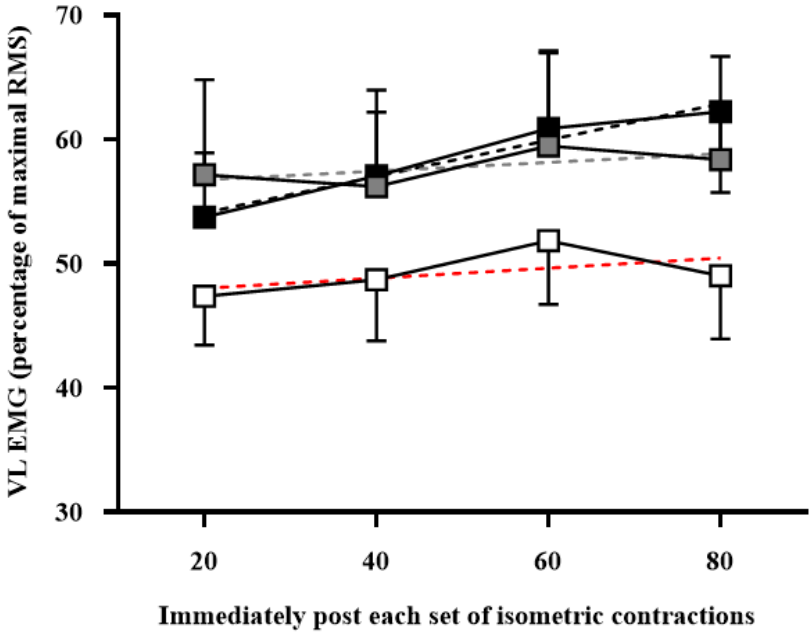


Figure 5.4.6. Vastus Medialis **(B)** and Lateralis **(C)** EMG amplitude measured during the IF_{14} after repeated bouts of loading. EMG data are expressed as Mean ± SE. □ = Trained group (*n* = 18); ▒ = Untrained group (*n* = 17); ■ = OSA group (*n* = 14). The dashed line represents the linear trend line.

Table 5.5. Slope of Vastus Medialis and Lateralis EMG amplitude measured when producing isometric forces at RPE 14 following loading.

Outcome	Group	*B*	*SE B*	β	*t*	*p*
VM muscle (μV per N)	Trained	.065	.017	.939	3.858	.061
	Untrained	.104	.032	.916	3.229	.084
	OSA	.206	.019	.991	10.712	.009
VL muscle (μV per N)	Trained	.040	.043	.553	.940	.447
	Untrained	.035	.030	.630	1.147	.370
	OSA	.146	.019	.984	7.755	.016

NOTE: Abbreviations: IF_{14}, Isometric Force at RPE 14; SE, Standard Error.

Neuromuscular efficiency of the Vastus Medialis and Lateralis muscles during IF_{14} measured before and after loading

A further aim our investigation was to examine the neuromuscular efficiency of the Vastus Medialis and Lateralis muscles during the isometric forces produced at RPE14 before and after repeated bouts of loading in Trained, Untrained, and OSA groups. Neuromuscular efficiency is interpreted as the responsiveness of a working muscle to neural excitation and is quantified by dividing the IF_{14} generated by the knee extensors with the average EMG amplitude measured during the contraction. Our investigation firstly focused upon the neuromuscular efficiency of the Vastus Medialis and Lateralis muscles during the IF_{14} measured as the reference point. Shapiro Wilk tests were performed to determine the normality of the neuromuscular efficiency of both Vastus Medialis and Lateralis EMG amplitudes measured at RPE14 for the reference point. The data was revealed to be normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA which revealed the neuromuscular efficiency of Vastus Medialis EMG amplitude measured at the reference point was significantly lower in Trained individuals compared with OSA patients ($p = .003$, $d = 1.52$) and Untrained individuals ($p = .022$, $d = 1.01$; **Figure 5.4.7 A**). The neuromuscular efficiency of the Vastus Lateralis EMG amplitude was also significantly lower in Trained individuals versus OSA patients ($p = .016$, $d = 1.18$).

A

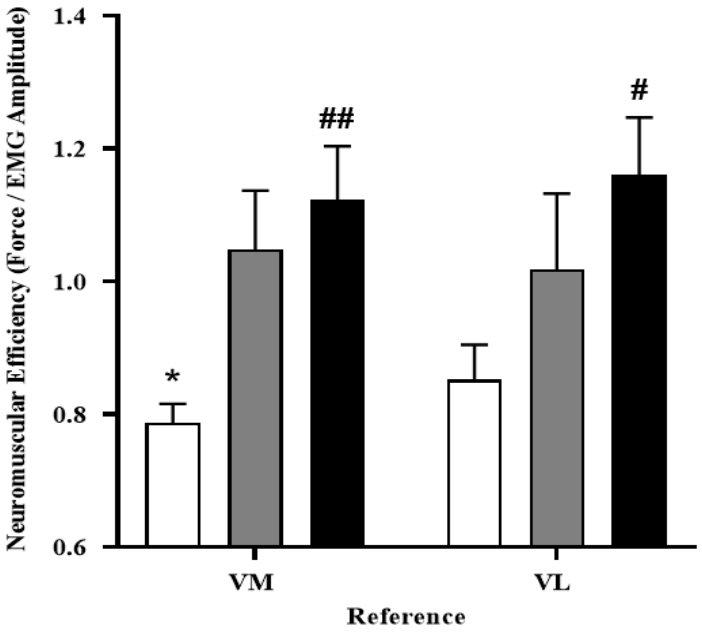


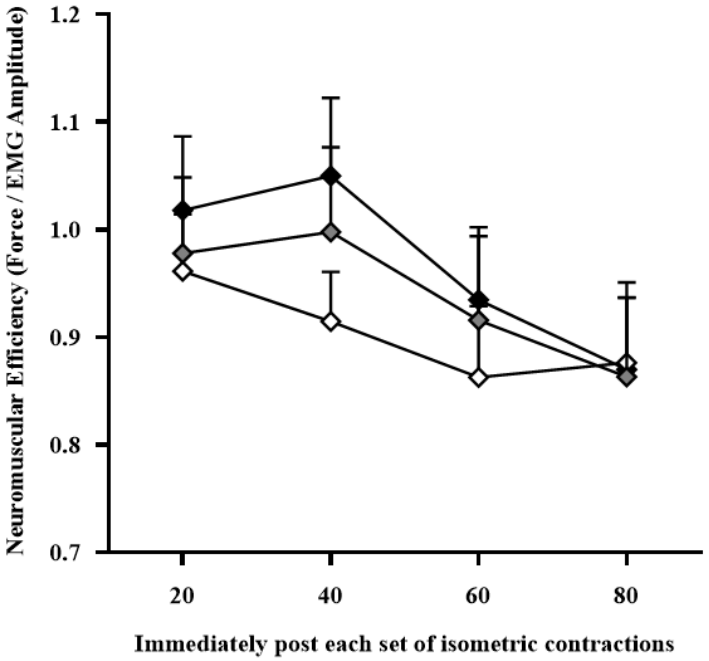
Figure 5.4.7 A. Neuromuscular efficiency of Vastus Medialis and Lateralis EMG amplitudes during the IF_{14} measured at the reference point. All data are expressed as Mean ± SE. □ = Trained group (n = 14); ▒ = Untrained group (n = 10); ■ = OSA group (n = 13). $^{\#}$ = $p < .05$, $^{\#\#}$ = $p < .01$ vs. Trained group; * = $p < .05$ vs. Untrained group.

Next, we examined the impact of loading upon the neuromuscular efficiency of Vastus Medialis and Lateralis EMG amplitudes during IF_{14}. Shapiro Wilk tests revealed the data was normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA analysis which revealed the neuromuscular efficiency of Vastus Medialis and Lateralis EMG amplitude at RPE 14 was similar between the groups ($p > .05$). Paired t-test analyses were subsequently performed to examine differences in the neuromuscular efficiency of Vastus Medialis and Lateralis EMG amplitudes between the reference point and that of the first and fourth set of loading for each respective group. The analyses revealed the neuromuscular efficiency of Vastus Medialis and Lateralis EMG amplitude was significantly increased when producing the IF_{14} after the first set of loading in Trained individuals (reference VM: 0.78 ± .11 vs. 1st set VM: 0.96 ± .21, 95% CI: .06 to .28, $t_{(16)} = 3.352$, $p = .004$; reference VL: 0.87 ± .18 vs. 1st set VL: 1.05 ± .25, 95% CI: .06 to .28, $t_{(13)} = 3.480$, $p = .004$; **Figure 5.4.7 B** and **5.4.7 C)**. In contrast, OSA patients demonstrated a significant decrease in the neuromuscular efficiency of Vastus Medialis EMG amplitude when producing the IF_{14} after the fourth set of loading

(reference VM: 1.12 ± .28 vs. 4th set VM: .87 ± .24, 95% CI: -.41 to -.09, $t_{(12)}$ = -3.521, p = .004; **Figure 5.4.7 B**).

Whereas, the neuromuscular efficiency of Vastus Lateralis EMG amplitude measured during the IF_{14} was significantly lower in OSA patients after the first (reference VL: 1.16 ± .30 vs. 1st set VL: 1.01 ± .32, 95% CI: -.26 to -.03, $t_{(12)}$ = -2.845, p = .015) and fourth set of loading (reference VL: 1.16 ± .30 vs. 4th set VL: .89 ± .22, 95% CI: -.49 to -.04, $t_{(12)}$ = -2.598, p = .023; **Figure 5.4.7 C**). Similarly, Untrained individuals showed a significant decrease in the neuromuscular efficiency of Vastus Medialis EMG amplitude when producing the IF_{14} after the fourth set of loading (reference VM: 1.04 ± .34 vs. 4th set VM: .83 ± .31, 95% CI: -.36 to -.05, $t_{(14)}$ = -2.886, p = .012; **Figure 5.4.7 B**). The neuromuscular efficiency of Vastus Lateralis EMG amplitude measured during the IF_{14} after the first and fourth set of loading was unchanged for Untrained individuals (p >.05). Our findings revealed the neuromuscular efficiency of the Vastus Medialis and Lateralis EMG amplitude was shown to gradually decrease in response to loading in our tested populations.

B



C

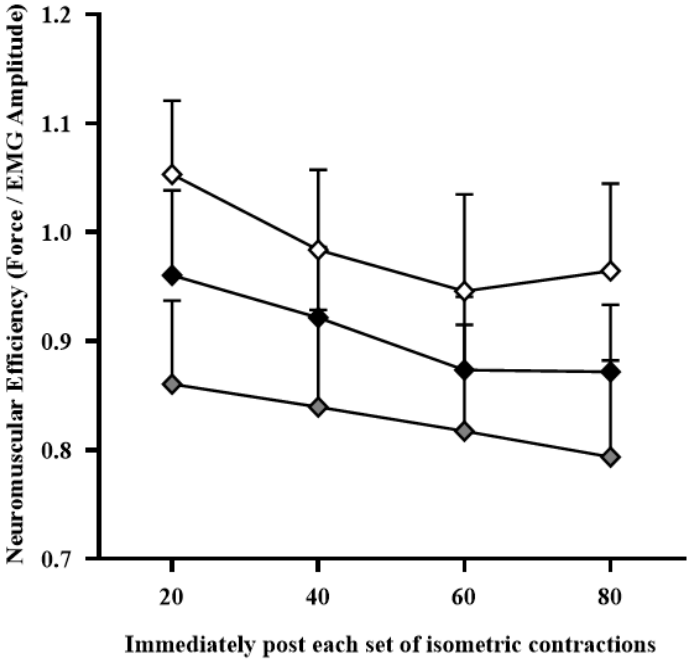


Figure 5.4.7. Neuromuscular efficiency of Vastus Medialis **(B)** and Lateralis **(C)** EMG amplitude during the IF_{14} measured after repeated bouts of loading. All data are expressed as Mean ± SE. □ = Trained group (*n* = 20); ▒ = Untrained group (*n* = 17); ■ = OSA group (*n* = 17).

5.5 Discussion

The present study examined the isometric forces and electromyography activity of the quadriceps muscles recorded at RPE14 before and after repeated bouts of loading in healthy Trained and Untrained individuals and newly diagnosed OSA patients. The experiment was conducted to determine whether effort is perceived differently in OSA patients compared with Trained and Untrained individuals. The effort sensation paradigm adopted in the current study was not externally controlled (such as holding a weight or tension for a set duration) rather our participants were instructed to match the isometric force produced at a set RPE of 14 before and after repeated bouts of loading at 50% MVC. There are four major outcomes to be drawn from the experiment. First, newly diagnosed OSA patients were shown to generate significantly higher relative forces at the reference point compared with healthy individuals (Figure 5.4.1 A). Second, the relative Vastus Medialis and Lateralis EMG amplitudes were significantly increased during loading in healthy and OSA participants (Figures 5.4.4 & 5.4.5; Table 5.3). Third, repeated loading of the knee extensor muscles at 50% MVC resulted in a significant decline in the relative forces produced at RPE14 compared with the reference point in OSA patients (Figure 5.4.3). Fourth, the relative Vastus Medialis and Lateralis EMG amplitudes were significantly increased at RPE14 following loading in OSA patients compared with healthy individuals (Table 5.5).

We firstly examined the isometric forces produced at a clamped effort of 14, which corresponded to the verbal anchors of ‘somewhat hard’ to ‘hard/heavy’ on the Borg scale, to determine the reference point of effort perception during unloaded conditions. We hypothesised patients with newly diagnosed OSA would demonstrate an impaired effort perception of the skeletal muscles that reflects higher relative forces being produced at the reference point. Our study demonstrated that patients with newly diagnosed OSA produced significantly greater relative forces (% MVC) at the reference point compared with healthy individuals ($p < .01$; Figure 5.4.1 A). Whilst, the relative Vastus Medialis and Lateralis EMG amplitudes (% RMS) were revealed to be similar between the groups at the reference point ($p > .05$; Figure 5.4.6 A). These findings suggested that OSA patients utilise a higher percentage of their capacity to generate isometric forces at the reference point despite possessing lower maximal strength of the quadriceps muscles and leading an extremely sedentary lifestyle. The result can be interpreted as OSA patients being less efficient when generating forces at the reference point compared with healthy individuals. Previous studies have reported patients

with neuromuscular conditions (e.g. Muscular Dystrophy and Myasthenia Gravis) exhibit heightened neuromuscular output that is perceived as an increased respiratory effort prior to completing a loading task which consisted of a maximal voluntary ventilation test (Bégin et al., 1982; Spinelli et al., 1992). The outcome of this study was attributed to muscle weakness and fatigability of the respiratory muscles. However, little is known regarding the skeletal muscle response at rest and loading in OSA patients, and as the respiratory system may operate differently, the direct comparison is not possible. Nevertheless, on the basis of our findings, we can presume that OSA patients reduce sensitivity in order to adjust to oncoming external work (i.e. lift objects, moving weights etc) and prevent the workload from becoming too effortful.

The next step of our investigation was to examine the Vastus Medialis and Lateralis EMG amplitudes during the first and fourth set of the skeletal muscle loading protocol to determine whether signs of fatigue would be observed. The relative Vastus Medialis and Lateralis EMG amplitudes were shown to progressively increase during repeated bouts of loading at 50% MVC in healthy individuals and OSA patients ($p < .05$; Figures 5.4.4 & 5.4.5; Table 5.3). Our data was shown to be consistent with previous studies that have demonstrated repeated contractions of the locomotor muscles at 50% MVC leads to an increased EMG amplitude of the skeletal muscles in healthy individuals (Masuda et al., 1999; Smilios, Hakkinen, & Tokmakidis, 2010). Further studies have also reported a reduction in the force-generating capacity i.e. the target force of 50% MVC was no longer maintained following loading in healthy subjects. The loss of isometric force production was shown concurrently with an increased EMG amplitude for both Quadriceps (Vastus Lateralis) and Soleus muscles (Bigland-Ritchie, Furbush, & Woods, 1986). These findings suggest that repeated loading of the skeletal muscles at 50% MVC leads to an altered neural strategy (i.e. muscle activation patterns and/or higher motor command) in order to maximise the force-generating capacity of the muscular system to achieve the target force (Fitting et al., 1988; Roussos et al., 1979). This strategy could reflect an additional recruitment of motor units and/or increased discharge rates of active motor units (Bigland-Ritchie, Cafarelli & Vøllestad, 1986; Maton & Gamet, 1989). Therefore, based upon our outcomes we can infer that repeated loading of the skeletal muscles at 50% MVC possibly leads to an increased recruitment of additional motor units and firing rates, a process that occurs in order to counteract the fatigued muscle fibres in our tested populations (Bigland-Ritchie & Woods, 1984).

The isometric forces and corresponding EMG amplitudes for both Vastus Medialis and Lateralis muscles were then measured at RPE14 after each completed bout of loading. We expected OSA patients to demonstrate a greater decline in the relative force production and the Vastus Medialis and Lateralis EMG amplitudes at RPE14 to remain unchanged following loading. Whereas, healthy individuals were expected to show a lesser decline in the relative force production and the respective Vastus Medialis and Lateralis EMG amplitudes muscles to remain constant at RPE14 following loading. The relative forces produced at RPE14 were found to be significantly reduced following repeated bouts of loading at 50% MVC in patients with newly diagnosed OSA ($p < .05$; Figure 5.4.1 B). Whilst, the normalised isometric forces generated at RPE14 were unchanged following repeated bouts of loading in healthy individuals (Trained and Untrained groups) ($p < .05$). In contrast to the hypotheses stated above, our study revealed the corresponding relative Vastus Medialis and Lateralis EMG amplitudes were shown to progressively increase at RPE14 following repeated bouts of loading in OSA patients compared with Trained and Untrained individuals) ($p < .05$; Figures 5.4.6 B & 5.4.6 C; Table 5.5).

Our findings suggested that OSA patients did not adjust for the loss of isometric forces at RPE14 according to the afferent feedback received from force/length receptors. The lack of force adjustment following loading in OSA patients could therefore be assumed to reflect effort perception being generated based upon corollary discharge which discounts the force/length information received from afferent receptors. However, the loss of isometric force production could theoretically be compensated by altering the neural strategy i.e. increasing the motor unit recruitment and/or discharge rates. This strategy would be seen as the central nervous system attempting to optimise the capacity of the fatigued muscles by allowing force loss to occur despite the increased locomotor muscle activity (Enoka & Stuart, 1992). However, we have shown OSA patients continue to produce lower isometric forces at RPE14 for the remainder of the loading protocol despite the increased recruitment of the quadriceps muscles (Gandevia, 1998; Windhorst & Kokkoroyiannis, 1991). In contrast, healthy individuals demonstrated a lesser decline in the isometric force production and the Vastus Medialis and Lateralis EMG amplitudes were held constant at RPE14 following loading. These results suggest that healthier and physically fit individuals are better than OSA patients at maintaining isometric production at a fixed effort of 14 with the same neural strategy adopted in response to loading.

The neuromuscular efficiency was further examined to determine the isometric force production in relation to the Vastus Medialis and Lateralis EMG amplitudes measured at RPE14 following loading. Our study demonstrated the neuromuscular efficiency of both Vastus Medialis and Lateralis EMG amplitudes was shown to gradually decrease following loading in Trained, Untrained, and OSA groups (Figures 5.4.7 B & 5.4.7 C). The gradual decline in the neuromuscular efficiency of the Vastus Medialis and Lateralis is potentially a consequence of the fatigued muscle fibres being increasingly recruited to generate the same amount of isometric force after repeated bouts of loading. The decline in neuromuscular efficiency in response to fatiguing contractions has also been previously attributed to an impaired excitation-contraction coupling which provides support for the previously held view that loading leads to alteration of the muscle contractile properties (Miller et al., 1987).These findings provide further evidence that fatigue alters the isometric force production at RPE14 relative to the quadriceps muscle activity in OSA patients.

While this study provides new insight into the perceptual response to fatiguing exercise in Trained, Untrained, and OSA groups, some limitations warrant discussion. Firstly, we did not conduct sleep studies in our healthy individuals to exclude the possibility of OSA due to limited resources available. However, sleep-related questionnaires were completed by all participants and classified healthy individuals as normal according to normative data (i.e. no excessive sleepiness and normal sleep quality) (Buysse et al., 1989; Johns, 1991). Secondly, we found the recruited OSA patients were considerably older and heavier than both Trained and Untrained groups. Future studies should attempt to recruit older and/or overweight participants with no diagnosed OSA to address whether age and weight differences exist. Thirdly, the maximal isometric force generated with the dominant leg during unloaded conditions was considerably lower in OSA patients compared with Trained and Untrained individuals. Therefore, we normalised the isometric forces according to participants MVC (% MVC) to determine how participants utilise their force capacity before and after loading. Finally, whilst surface EMG is an advantageous tool for measuring the amplitude of skeletal muscles during loading in a non-invasive manner, the technique is limited by factors such as; noise, cross-talk from nearby muscles, skin-electrode contact (e.g. sweating), and thickness of subcutaneous fat (Reaz, Hussain, & Mohd-Yasin, 2006). In order to reduce the influence of these factors we adopted a thorough cleaning procedure of the skin and followed standardised positions for electrode placement. Participants were further instructed not to excessively

move the dominant leg during the loading protocol. We also followed standardised procedures by normalising the EMG amplitudes according to the maximal RMS (% RMS) (Orozco-Levi et al., 1995; Yokoba et al., 2003).

In summary, we have shown that newly diagnosed OSA patients generate significantly higher relative forces at the reference point compared with Trained and Untrained individuals. The relative Vastus Medialis and Lateralis EMG amplitudes were shown to significantly increase during loading in OSA and healthy groups. Repeated loading of the knee extensor muscles at 50% MVC leads to a significant reduction in the relative force production at RPE14 in OSA patients. Whilst, the normalised EMG amplitudes of the Vastus Medialis and Lateralis muscles were shown to gradually increase at RPE14 following loading in OSA patients compared with healthy individuals. The results of the present study provide further evidence that newly diagnosed OSA patients demonstrate an impaired effort perception and heightened muscle activation of the skeletal muscles in response to loading.

CHAPTER SIX

Perception of effort and activation of respiratory muscles before and after loading in Trained, Untrained, and OSA groups.

6.1 Abstract

Objectives: Obstructive Sleep Apnoea is characterised by a recurrent deoxygenation-reoxygenation pattern and inspiratory efforts during sleep that leads to a chronic overloading of the inspiratory muscles. Whether perceptual and muscular differences are observed before and after loading in OSA patients compared with healthy individuals is unknown. Thus, we examined the effort perception and activation of the inspiratory muscles before and after loading in healthy and OSA groups. The effects of Continuous Positive Airway Pressure (CPAP) therapy on effort perception is also unknown. The effect of CPAP therapy on effort perception was assessed before and after loading in OSA patients.

Methods: Fifty-six males (n = 21 Trained, n = 17 Untrained, and n = 18 OSA) completed the study. Sixteen adults with diagnosed OSA completed the follow-up assessments after receiving 3-months of CPAP therapy. The inspiratory muscle loading protocol consisted of five sets of twenty breaths at a resistance equivalent to 50% of participants maximal inspiratory pressure (PI_{max}) using a KH2 POWERbreathe device. Inspiratory pressure generated at a clamped effort of 14 on the Borg 6-20 rating of perceived exertion scale (RPE) was measured before (reference point) and after each completed set of loading. Electromyography (EMG) amplitudes of the Intercostal and Trapezius muscles was simultaneously recorded.

Results: Newly diagnosed OSA patients were shown to generate similar relative inspiratory pressures to healthy individuals at the reference point ($p > .05$). The normalised Intercostal EMG amplitude was increased during loading in healthy and OSA groups ($p < .05$). Repeated bouts of inspiratory loading at 50% PI_{max} resulted in higher relative pressures generated at RPE14 compared with the reference point in OSA patients ($p < .05$). The relative Trapezius EMG amplitude was increased at RPE14 after loading in OSA patients compared with healthy individuals ($p < .05$). The relative pressures generated at the reference point was increased after 3-months of CPAP therapy in OSA patients ($p < .05$).

Conclusion: The results of the present study provide further evidence that patients with newly diagnosed OSA demonstrate a heightened effort perception and activation of the inspiratory muscles in response to loading. CPAP therapy upregulated the perception of inspiratory effort at rest.

6.2 Introduction

Obstructive sleep apnoea (OSA) is a breathing disorder characterised by repeated episodes of narrowing (hypopnea) or collapse (apnoea) of the upper respiratory airways, that occur intermittently, and at regular intervals during sleep (Carter & Watenpaugh, 2008). Recurrent cessations of airflow result in periodic asphyxia, transient periods of hypoxemia, and hypercapnia (Halliwill & Minson, 2002; Mullington et al., 2009). Futile inspiratory efforts against the obstructed upper airway produce changes in intrathoracic pressure and dysregulates blood flow to the respiratory muscles (Remmers et al., 1978; Wiegand & Zwillich, 1994; Drager, Polotsky, & Lorenzi-Filho, 2011). The ventilatory disturbance and intermittent hypoxia result in sleep fragmentation and respiratory effort-related arousals (RERA) which attempt to re-establish the airway patency and normalise blood gases (Guilleminault, Tilkian, & Dement, 1976; Somers et al., 1995).

Another important feature of the chronic condition is the ventilatory response to the occluded airway and subsequent obstructive event (Wiegand & Zwillich, 1994). Repeated inspiratory efforts against the occluded airway could potentially result in a chronic overloading of the inspiratory muscles during sleep (Wilcox et al., 1990). The chronic overloading of inspiratory muscles during sleep is thought to lead to an increased risk of fatigue during rest and low-intensity exercise (Chien et al., 2013). The inspiratory activity could therefore be alternated between the diaphragm and inspiratory/accessory muscles in order to balance the work of breathing and minimise the development of fatigue (Fitting et al., 1988; Sharp et al., 1964; Yokoba et al., 2003). However, relatively few studies have addressed the perceptual alterations that accompany fatigue and/or dysfunction of the muscles in OSA patients. There is some evidence to suggest that OSA patients demonstrate an augmented perceptual response to inspiratory loading which is modified following nasal CPAP therapy (Earing, 2014; Tun et al., 2000). However, no study to date has examined the perceptual and muscular response before and after inspiratory loading in OSA patients compared with healthy Trained and Untrained individuals. Furthermore, the effect of 3 months of CPAP therapy on the perceptual response to loading has yet to be determined in OSA patients.

Therefore, we utilised the effort sensation paradigm as reported by Earing et al., (2014) to assess the perception of effort and inspiratory muscle activity before and after repeated bouts of loading in patients with newly diagnosed OSA compared with healthy Trained and

Untrained individuals. The effort sensation paradigm adopted in the current study was not externally controlled (such as holding a weight or tension for a set duration) rather our participants were instructed to match the inspiratory pressure generated at a set rating of perceived exertion (RPE) of 14 before and after repeated bouts of loading. The outcome of the experiment would enable us to determine whether effort is perceived differently in OSA patients compared with healthy Trained and Untrained individuals. We hypothesised patients with newly diagnosed OSA would demonstrate a heightened effort perception of the inspiratory muscles that reflects higher inspiratory pressures being produced at an RPE of 14 at baseline (the reference point) and following loading. However, we expected newly-diagnosed OSA patients to show a greater increase in the inspiratory pressure generated at an RPE of 14 in response to loading. The respective EMG amplitudes for the Intercostal and Trapezius muscles (% RMS) at RPE 14 were expected to be increased following loading in OSA patients. In contrast, healthy individuals were expected to show a lesser increase in the inspiratory pressures and lower EMG amplitudes for the Intercostal and Trapezius (% RMS) measured at RPE 14 following loading.

6.3 Methods

Participant characteristics

Sixty one males were recruited to participate in the study which included 21 Trained participants (age: 35.1 ± 2.1 years; height: 179.5 ± 1.5 cm; mass: 76.5 ± 2.4 kg; VO_{2max}: 61.0 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$), 19 Untrained participants (age: 28.4 ± 1.8 years; height: 176.8 ± 1.9 cm; mass: 82.0 ± 4.1 kg; VO_{2max}: 39.7 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$), and 21 patients with newly-diagnosed OSA (age: 55.1 ± 2.0 years; height: 173.4 ± 1.3 cm; mass: 98.2 ± 4.3 kg; **Table 6.1-a**). The data of five participants was excluded from the analysis due to muscle weakness (PI_{max} < 80 cmH_2O), unclear understanding of the protocol, and unavailability during testing. We therefore present the data of 56 participants which includes 21 Trained, 17 Untrained, and 18 OSA participants. The Trained group were composed of endurance-trained athletes (cycling, running, or triathlon) who completed on average 8 hours of training per week (weekly distance 41.0 miles). Whilst, the Untrained group were composed of individuals who did not participate in any regular physical activity on a weekly basis. Sixteen males with diagnosed OSA completed the follow-up assessments after receiving 3-months of CPAP therapy (age: 55.2 ± 2.3 years; height: 173.3 ± 1.3 cm; mass: 99.7 ± 5.6 kg; **Table 6.1-b**). The Airview™ feature of the device was utilised to re-assess the AHI and adherence after 3 months of CPAP treatment (mean pressure: 8.67 ± 0.8 cmH_2O, usage: 7.06 ± 0.4 hrs / night (range 4.11-9.23 hrs / night), and days of usage > 4hrs: 93.7 ± 3.5 %). The study was approved by Departmental (SSHES, Bangor University, Ref: P01-16/17) and National Health Ethics Committees (Ref: 17/EM/0162). Participants received a detailed explanation of the procedures and a checklist to ensure compliance with the eligibility criteria **(Appendix I & II)**. Written informed consent was obtained from all participants **(Appendix III)**. The experimental protocols were performed in accordance with the Declaration of Helsinki.

Study design

The effect of inspiratory loading on the inspiratory pressures generated at a fixed RPE and electrical activity of the Intercostal and Trapezius muscles was assessed using a cross-sectional study design. Healthy participants visited the laboratory of the School of Sport, Health, and Exercise Sciences as outlined in **Figure 3.2-a**. Patients with newly diagnosed OSA were also required to visit the laboratory at the Pulmonary Function department (Ysbyty Gwynedd) as outlined in **Figure 3.2-b**.

Table 6.1-a. Physiological characteristics of all participants

	Trained (n = 21)	Untrained (n = 17)	OSA (n = 18)
Age (years)	35.1 (2.1)*	27.8 (1.9)††	55.9 (2.0)##
Height (cm)	179.5 (1.5)	176.1 (2.0)	173.4 (1.4)#
Weight (kg)	76.5 (2.4)	79.7 (3.9)††	101.5 (4.6)##
Body Mass Index (kg · m^2)	23.7 (.65)	25.6 (.89)††	33.7 (1.4)##
Body Surface Area (cm)	195.2 (3.3)	195.7 (5.2)††	219.2 (5.5)##
Body Fat (%)	13.2 (1.0)	17.7 (1.1)††	32.6 (1.7)##
Neck circumference (cm)	38.1 (.49)	39.0 (.66)††	44.3 (.89)##
Chest circumference (cm)	96.3 (1.8)	98.3 (2.3)††	118.0 (3.0)##
Waist circumference (cm)	83.9 (1.9)	88.5 (2.4)††	115.1 (3.6)##
Hip circumference (cm)	98.5 (1.4)	102.2 (1.9)††	110.2 (2.0)##
Apnoea Hypopnea Index (events/h)	N/A	N/A	42.8 (6.7)
Epworth Sleepiness Scale	5.4 (.89)	2.9 (.51)††	11.5 (1.4)##
Pittsburgh Sleep Quality Index	3.4 (.46)	4.2 (.45)††	8.6 (1.0)##
Forced Expiratory Volume 1s (l/min)	4.48 (.16)	4.17 (.15)††	3.20 (.12)##
Forced Vital Capacity (l/min)	5.44 (.16)	5.04 (.18)††	4.16 (.18)##
DLCO (ml/min/mmHg)	N/A	N/A	24.6 (.92)
Total Lung Capacity (l/min)	N/A	N/A	6.12 (.25)
VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	61.0 (1.8)**	39.7 (1.4)	N/A
PI_{max} (cmH_2O)	111.3 (4.8)	112.7 (6.8)	104.0 (4.5)
MVC (N)	411.7 (23.0)	382.4 (31.0)	280.8 (33.6)##

NOTE: DLCO, Diffusing capacity of the lung for carbon monoxide; VO_{2max}, Maximal Oxygen Uptake. PI_{max}, Maximal Inspiratory Pressure (Inspiratory muscles); MVC, Maximal Voluntary Capacity (Quadriceps muscles). Values are Mean ± SEM. * p <.05, ** p <.01 Trained vs Untrained. † p <.05, †† p <.01 Untrained vs OSA. # p <.05, ## p <.01 OSA vs Trained.

Table 6.1-b. Physiological characteristics of OSA participants after CPAP treatment.

	Pre-CPAP OSA (n = 16)	Post-CPAP OSA (n = 16)
Age (years)	55.2 (2.3)	N/A
Height (cm)	173.3 (1.3)	173.1 (1.3)
Weight (kg)	99.7 (5.6)	101.6 (5.6)¶¶
Body Mass Index ($kg \cdot m^2$)	33.1 (1.7)	33.7 (1.7)¶¶
Body Surface Area (cm)	216.8 (6.7)	219.2 (6.7)¶
Body Fat (%)	32.0 (2.1)	31.8 (1.8)
Neck circumference (cm)	44.0 (.99)	44.6 (.96)¶
Chest circumference (cm)	117.0 (3.7)	117.4 (3.7)
Waist circumference (cm)	113.7 (4.4)	113.2 (4.0)
Hip circumference (cm)	109.8 (2.3)	110.3 (2.4)
Apnoea Hypopnea Index (events/h)	39.2 (5.8)	4.4 (.84)¶¶
Epworth Sleepiness Scale	11.5 (1.2)	4.3 (.65)¶¶
Pittsburgh Sleep Quality Index	9.4 (.90)	4.5 (.62)¶¶
Forced Expiratory Volume 1s (l/min)	3.29 (.10)	3.26 (.08)
Forced Vital Capacity (l/min)	4.34 (.15)	4.25 (.13)
DLCO (ml/min/mmHg)	23.2 (1.3)	24.5 (1.6)
Total Lung Capacity (l/min)	6.14 (.24)	6.75 (.26)¶
Maximal Inspiratory Pressure (cmH2O)	104.6 (4.3)	105.7 (4.4)

NOTE: DLCO, Diffusing capacity of the lung for carbon monoxide. Values are Mean ± SEM. ¶ p <.05, ¶¶ p <.01 Pre-CPAP vs Post-CPAP.

Experimental procedures

The full detailed procedures of the following measurements are described in **Chapter 3.9**; however, the main techniques are described here in brief. The following experimental procedure was designed to progressively load the Intercostal and Trapezius muscles under standardised conditions to determine whether the inspiratory pressures at RPE 14 and corresponding EMG amplitudes were different between the groups **(Figure 6.1)**. The EMG amplitude was also recorded during the first and fourth set to determine the activity changes over the course of loading. Intercostal and Trapezius EMG amplitudes were measured by applying bipolar pairs of silver/silver chloride electrodes on cleaned, abraded skin of the intercostal and trapezius muscles (Cescon et al., 2008; Hawkes et al., 2007).

The experimental protocol was performed according to techniques as reported by Earing et al., (2014) in healthy and clinical populations. A pilot study was conducted to systematically explore the test-retest reliability of the novel inspiratory loading protocol. Sixty three males volunteered to participate in the study which included 37 normal BMI participants (age: 29.1 ± 8.1 years; height: 174.9 ± 6.3 cm; mass: 70.6 ± 7.0 kg; BMI: 23.1 ± 1.5 kg/m^2; PI_{max}: 107.2 ± 29.1 cmH_2O) and 26 overweight participants (age: 32.2 ± 7.2 years; height: 174.5 ± 6.0 cm; mass: 86.0 ± 10.0 kg; BMI: 28.2 ± 2.5 kg/m^2; PI_{max}: 113.3 ± 26.4 cmH_2O). Test-retest intraclass correlation coefficients (ICC) were assessed during the inspiratory pressures generated at a clamped effort of 14 on the Borg scale (IP_{14}), after each completed set of 20 breaths with the Powerbreathe Plus device (POWERbreathe International Ltd, Warwickshire, UK). A second assessment was conducted after 1 week under similar testing conditions. The pilot study produced an acceptable test-retest reliability of 0.967 with a 95% confidence interval (CI) of 0.953 - 0.978.

The maximal inspiratory pressure (PI_{max}) was firstly measured from residual volume to determine participant's respiratory muscle strength with a hand-held, resistive breathing device (KH2 POWERbreathe International Ltd, Warwickshire, UK). Seven consecutive PI_{max} manoeuvres were performed according to ATS/ERS guidelines and separated by 1 minute of rest (Miller et al., 2005). Participants were then introduced to the 6-20 Borg scale and anchoring of the scale according the effort perceived during the series of PI_{max} manoeuvres. Participants were instructed to produce five inspiratory pressures at a fixed effort of 14

'reference point' using standard procedures (Noble and Robertson, 1996) corresponding to the verbal anchors of '*somewhat hard*' to '*hard/heavy*' on the scale. This allowed participants to gauge how much pressure was required to represent the target RPE before loading. The EMG amplitudes of Intercostal and Trapezius muscles were also recorded during these tasks to determine the muscle activity prior to loading. The inspiratory loading protocol was then explained to the participant and thereafter performed which consisted of five sets of twenty loaded breaths at a fixed intensity of 50% of the pre-recorded PI_{max}. The EMG amplitudes of Intercostal and Trapezius muscles was measured during the first and fourth set of loading. Participants were instructed to adopt a natural breathing style throughout the loading protocol. After each set of twenty loaded breaths, participants generated an inspiratory pressure at RPE 14 that allowed us to reassess the IP_{14} and corresponding EMG.

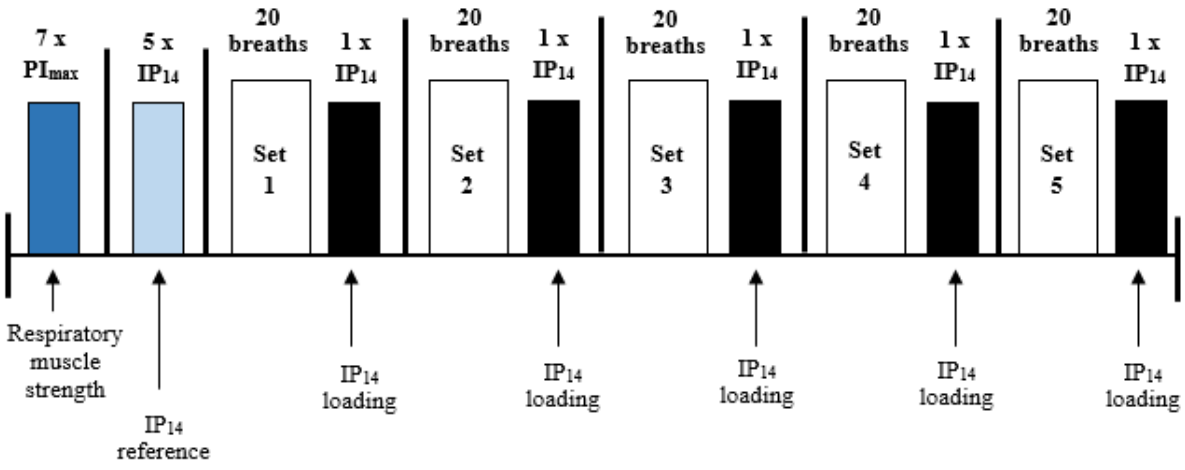


Figure 6.1. Schematic diagram of the inspiratory loading protocol. **NOTE:** PI_{max}, Maximal Inspiratory Pressure; IP_{14}, Inspiratory pressure at RPE 14.

Data Analysis

Inspiratory pressures at a fixed RPE of 14 (IP_{14} reference and loading) were studied according to the absolute (cmH_2O) and relative values (normalised according to maximal inspiratory pressure and expressed as % PI_{max}). The matching of inspiratory pressures at RPE 14 was calculated by subtracting the average IP_{14} (measured after loading) from the average IP_{14} (reference point measured during unloaded conditions). The mean percentage difference was calculated by subtracting the mean of IP_{14} loading from the mean of IP_{14} reference. Coefficient of variation (CV %) for the IP_{14} reference and loading measures were assessed. Electromyography (EMG) data was recorded and analysed offline using commercially available software (AcqKnowledge 3.9, BIOPAC systems, US). EMG signals were amplified (gain x 1000), band-pass filtered 35-500 Hz, and digitised at a sampling rate of 2000 kHz. Root mean square (RMS) amplitudes with a time constant of 100 ms were analysed over the 0.5 second window for the PI_{max}, loading protocol (1^{st} and 4^{th} set), IP_{14} reference and loading measures. The EMG data was studied according to absolute (μV) and relative amplitudes (normalised according to maximal EMG and expressed as % RMS). Neuromuscular efficiency was calculated by dividing the inspiratory pressure generated during the IP_{14} loading measure with the average EMG amplitude for that contraction (Deschenes et al., 2008). Data measured after the fifth set of loading (100 breaths) was excluded from the analysis due to participants knowing they were facing the last measure. Participants would respond by altering their behaviour either through giving a 'last inspiration', or disengage altogether from the task, which resulted in higher or lower inspiratory pressures generated at RPE14 at the conclusion of the protocol.

Statistical Analysis

Statistical analyses were performed using SPSS version 25 for Windows (SPSS Inc., Chicago, IL, USA). Data was expressed as mean ± SEM with $p < .05$ considered statistically significant. Shapiro-Wilk tests were performed to determine the data's assumption of normality. Mixed-model ANOVA and follow-up analyses were performed to determine whether loading had an influence on the relative inspiratory pressures at RPE14 (IP_{14}) and corresponding Intercostal and Trapezius EMG amplitudes. Paired *t*-tests were performed to examine potential differences in response to loading for each group. Kruskal-Wallis tests and follow-up Mann-Whitney U tests were performed to analyse differences between the groups

given the non-parametric nature of the absolute Intercostal and Trapezius EMG amplitudes and the inability to transform the data. Kruskal-Wallis tests were also performed to assess the CV% of IP_{14} reference and loading measures. Follow-up Mann-Whitney U tests with Bonferroni correction were performed to analyse differences between the groups. One-way ANOVA was also performed to determine the neuromuscular efficiency (IP_{14} / EMG amplitude) of the Intercostal and Trapezius muscles in response to the loading protocol. Effect sizes were calculated using the Cohen *d* equation and interpreted as 0.2 small, 0.5 moderate, and 0.8 large effect size. Linear regression analyses were conducted to examine the slope of EMG amplitude measured during IP_{14} loading measures for each respective group. One-Way ANOVA analyses were performed to determine whether the Intercostal and Trapezius EMG amplitude was influenced during the first and fourth set of the loading protocol. Further linear regression analyses were performed to examine the slope of EMG amplitude during the first and fourth set of loading protocol for each respective group. We also conducted a series of Paired-*t* tests to examine the effect of CPAP therapy on the production of inspiratory pressures at RPE 14, CV %, and percentage difference of IP_{14} following loading. Spearman's rho analyses were performed to determine whether correlations exist between participant's physical characteristics, AHI, and the inspiratory pressures at RPE14 following CPAP therapy.

6.4 Results

Patients with newly-diagnosed OSA were found to be significantly older and heavier than Trained and Untrained individuals ($p < .05$; Table 6.1-a). Sleep-related measures (ESS and PSQI) were revealed to be significantly higher in OSA patients compared with Trained and Untrained groups ($p < .05$). Lung function parameters (FEV_1 and FVC) were shown to be significantly lower in OSA patients than Trained and Untrained individuals ($p < .05$). Cardiovascular fitness (VO_{2max}) and lower extremity strength (MVC) was significantly greater in Trained individuals compared with Untrained individuals ($p < .05$). Respiratory muscle strength (PI_{max}) was similar between the three groups ($p > .05$).

Inspiratory pressures generated at RPE 14 before and after loading

The primary aim of our investigation was to examine the inspiratory pressures at an RPE of 14 (IP_{14}) on the Borg scale before and after repeated bouts of loading in Trained, Untrained, and OSA populations. We firstly focused upon the inspiratory pressures at an RPE of 14 generated during unloaded conditions which is referred to as the reference point of effort perception. Shapiro Wilk tests were initially performed to determine the normality of the inspiratory pressures generated at RPE 14 for the reference point. The data was revealed to be normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA and Bonferroni corrections to determine whether the reference inspiratory pressures generated by the respiratory muscles at a set RPE of 14 were different between the groups. The analyses revealed the absolute inspiratory pressures produced at a fixed effort of 14 were similar at the reference point ($p > .05$; **Table 6.2**). However, on closer inspection of the inspiratory data, we observed a trend that showed OSA patients and Untrained individuals produce lower absolute inspiratory pressures at RPE 14 for the reference point compared with Trained individuals. For our next step, we normalised the absolute IP_{14} data according to participant's PI_{max} and performed Shapiro Wilk tests which revealed the relative IP_{14} was normally distributed ($p > .05$). One-Way ANOVA analyses showed the relative inspiratory pressures generated at RPE 14 were similar at the reference point ($p > .05$; **Figure 6.4.1 A**).

Next, we examined the inspiratory pressures produced by the respiratory muscles at a clamped effort of 14 on the Borg scale (IP_{14}) following repeated bouts of loading. Shapiro Wilk tests revealed the absolute pressures generated at RPE 14 following loading were

normally distributed ($p > .05$). One-Way ANOVA analyses were performed which revealed no between-group differences were observed in the absolute inspiratory pressures generated at RPE 14 following loading ($p > .05$). However, our experiment demonstrated that inspiratory loading of the respiratory muscles at 50% PI_{max} leads to an increase of the inspiratory pressure generated at RPE 14 in OSA patients, compared with the reference point measured during unloaded conditions (reference: 57.9 ± 3.1 cmH_2O vs. loading: 71.9 ± 3.8 cmH_2O, 95% CI: 8.4 to 19.6, $t_{(19)} = 5.261$, $p = .000$). Likewise, the inspiratory pressure generated at RPE 14 following loading was noticeably increased compared with the reference point in Trained (reference: 65.2 ± 4.0 cmH_2O vs. loading: 72.5 ± 4.5 cmH_2O, 95% CI: 4.2 to 10.4, $t_{(20)} = 4.902$, $p = .000$) and Untrained individuals (reference: 55.7 ± 5.7 cmH_2O vs loading: 72.0 ± 6.5 cmH_2O, 95% CI: 8.6 to 24.0, $t_{(17)} = 4.487$, $p = .000$; **Table 6.2**). We then normalised the absolute IP_{14} generated following loading according to participant's $PI_{max.}$ Shapiro Wilk tests revealed the relative IP_{14} was normally distributed ($p > .05$). Therefore, we performed One-Way ANOVA analyses which revealed loading did not influence the relative inspiratory pressures generated by the respiratory muscles at a clamped effort of 14 in Trained, Untrained, and OSA groups ($p > .05$; **Figure 6.4.1 B**).

Table 6.2. Inspiratory pressures generated at a fixed RPE 14 measured before and after repeated bouts of loading

	Trained (*n* = 21)		**Untrained (*n* = 17)**		**OSA (*n* = 18)**		**Effect size (Cohen *d*)**		
Absolute pressures	Pressure cmH_2O	Median (IQR) cmH_2O	Pressure cmH_2O	Median (IQR) cmH_2O	Pressure cmH_2O	Median (IQR) cmH_2O	T v U	T v O	U v O
PI_{max}	111.2 (4.7)	110.4 (93.6-122.7)	112.1 (6.5)	110.9 (89.2-138.0)	106.4 (4.3)	106.7 (95.3-120.3)	0.03	0.23	0.23
IP_{14} reference (avg)	65.2 (4.0)	63.7 (49.9-80.8)	55.7 (5.7)	50.6 (39.3-69.8)	57.9 (3.1)	61.0 (45.9-66.2)	0.44	0.44	0.11
IP_{14} 20 breaths	72.9 (5.9)$^{\phi}$	73.6 (58.1-87.1)	72.6 (6.7)§§	78.7 (46.1-95.7)	69.7 (4.1)$^{\psi\psi}$	73.3 (57.4-77.8)	0.01	0.14	0.12
IP_{14} 40 breaths	71.9 (4.8)	69.4 (56.3-88.2)	71.9 (6.6)	70.0 (42.9-100.5)	72.2 (4.0)	72.9 (58.8-82.7)	0.00	0.01	0.01
IP_{14} 60 breaths	73.5 (4.2)	72.0 (58.6-86.5)	71.0 (7.1)	67.3 (45.7-96.8)	71.9 (4.0)	72.6 (59.3-84.8)	0.09	0.08	0.03
IP_{14} 80 breaths	71.8 (4.4)	75.2 (57.3-87.8)	74.3 (6.4)	77.1 (50.7-92.1)	74.0 (4.3)	67.8 (62.1-90.3)	0.10	0.11	0.01
IP_{14} loading (avg)	72.5 (4.5)$^{\phi\phi}$	69.5 (61.0-91.7)	72.0 (6.5)§§	71.9 (43.4-90.9)	71.9 (3.8)$^{\psi\psi}$	70.0 (61.2-86.2)	0.02	0.03	0.00

NOTE: Values are Mean ± SEM unless otherwise stated. PI_{max}, Maximal Inspiratory Pressure; IP_{14}, Inspiratory Pressure at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea; T, Trained; U, Untrained; O, OSA.

$^{\phi}$ = p <.05 Significantly greater IP_{14} after 20 breaths than IP_{14} reference in Trained; $^{\phi\phi}$ = p <.01 Significantly greater IP_{14} loading than IP_{14} reference in Trained; §§ = p <.01 Significantly greater IP_{14} after 20 breaths than IP_{14} reference in Untrained; §§ = p <.01 Significantly greater IP_{14} loading than IP_{14} reference in Untrained; $^{\psi\psi}$ = p <.01 Significantly greater IP_{14} after 20 breaths than IP_{14} reference in OSA; $^{\psi\psi}$ = p <.01 Significantly greater IP_{14} loading than IP_{14} reference in OSA.

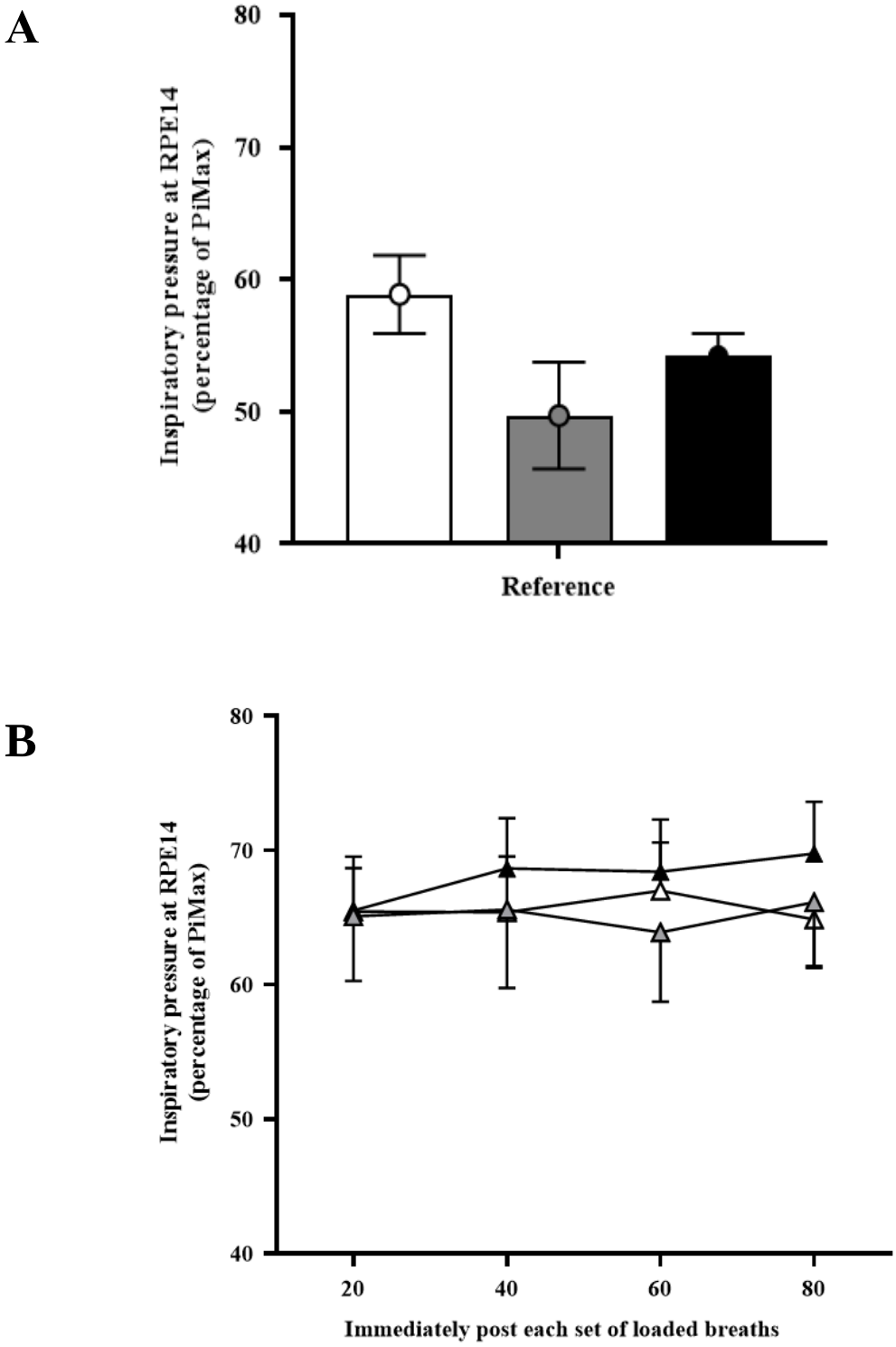


Figure 6.4.1. Inspiratory pressure at an RPE of 14 (IP_{14}) measured at the reference point **(A)** and after repeated bouts of loading **(B)**. Pressure data is expressed as Mean ± SE. △ = Trained group (*n* = 21); ▲ = Untrained group (*n* = 17); ▲ = OSA group (*n* = 17). **NOTE:** Abbreviations: PI_{max}, Maximal Inspiratory Pressure; $IP_{14,}$ Inspiratory pressure at RPE 14.

We then examined the coefficient of variation (CV %) to determine whether the variability in the IP_{14} measured at the reference point and after loading were different between the groups. Shapiro Wilk tests revealed the CV% for both reference and loading IP_{14} were not normally distributed ($p < .05$). Therefore, we performed a Kruskal Wallis test which revealed significant differences were observed in the CV % of reference IP_{14} ($\chi^2 = 6.939$, $df = 2$, $p = .031$). Mann-Whitney U tests revealed that OSA patients produce significantly greater CV % when generating inspiratory pressures at RPE 14 for the reference point compared with Trained individuals ($U = 110$, $p = .009$; **Figure 6.4.2**). No between group differences were observed in the CV% of loading IP_{14} ($p > .05$). Paired *t*-test analyses were subsequently performed to determine whether differences in the CV% of IP_{14} would be observed following loading for each respective group. The results revealed that Untrained and OSA groups respond to loading with a significantly lower CV % when generating inspiratory pressures at RPE 14 (Untrained mean difference: -6.2 %, 95% CI: -10.6 to -1.8, $t_{(17)} = -2.994$, $p = .008$; OSA mean difference: -3.9%. 95% CI: -5.8 to -2.0, $t_{(19)} = -4.395$, $p = .000$). In contrast, Trained individuals showed no change in the CV% of IP_{14} after loading ($p = .450$).

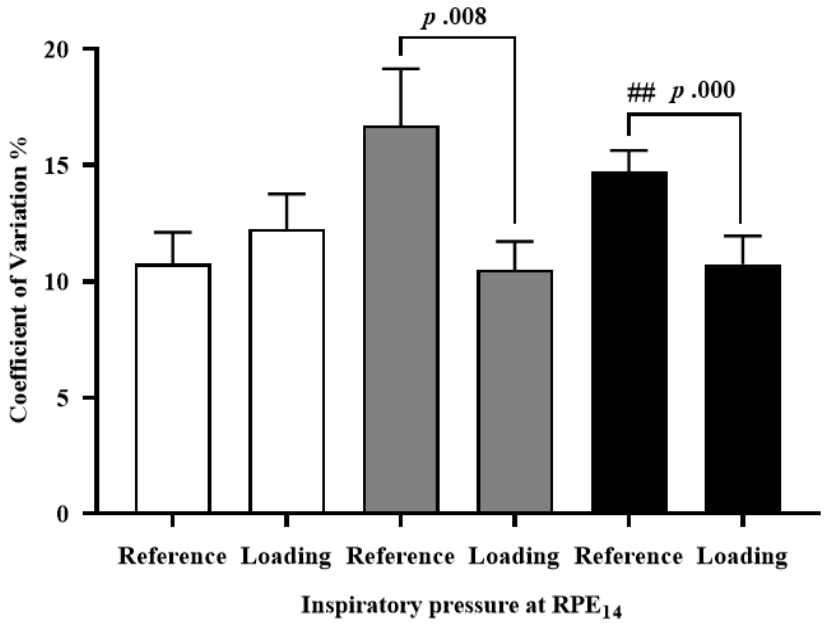


Figure 6.4.2. Coefficient of variation (expressed as percentage) of IP_{14} measured at the reference point and after loading. Data is expressed as Mean ± SE. □ = Trained group ($n = 21$); ▒ = Untrained group ($n = 17$); ■ = OSA group ($n = 18$). ## = $p < .01$ vs. Trained.

Finally, we examined the matching of inspiratory pressures at a set RPE of 14 by comparing the relative inspiratory pressures measured at the reference point to those performed following loading. Shapiro Wilk tests revealed the percentage difference between reference and loading IP_{14} were normally distributed ($p > .05$). Therefore, we performed a mixed-model ANOVA which revealed loading influenced the matching of inspiratory pressures at the clamped effort of 14 (main effect for change in IP_{14}: $F_{(2, 50)} = 4.815$, $p = .012$ and no interaction $p > .05$; **Figure 6.4.3 A**). Follow-up analyses revealed the percentage difference between reference and loading IP_{14} was significantly greater in OSA patients compared with Trained individuals after 80 loaded breaths ($p = .012$). The finding was reinforced by the large effect size observed ($d = 0.96$), and further supports our hypothesis that loading leads to a large increase in IP_{14} in OSA patients. We also performed a One-way ANOVA to examine the mean percentage difference between reference and loading IP_{14}. Newly diagnosed patients with OSA were shown to respond to loading with a significantly greater percentage difference between reference and loading IP_{14} compared with Trained individuals ($p = .037$; $d = 0.89$). No differences were observed between Untrained and OSA groups when examining the mean percentage difference of IP_{14} ($p > .05$; **Figure 6.4.3 B)**.

Our findings demonstrated the relative inspiratory pressures generated at the reference point were similar between the groups **(Figure 6.4.1 A)**. The relative inspiratory pressures generated at RPE14 following loading was also similar between the groups **(Figure 6.4.1 B)**. However, the matching of inspiratory pressures at RPE of 14 was not adjusted to the level of the reference point in OSA patients as reflected by the greater percentage difference between reference and loading IP_{14} **(Figure 6.4.3 A and B)**.

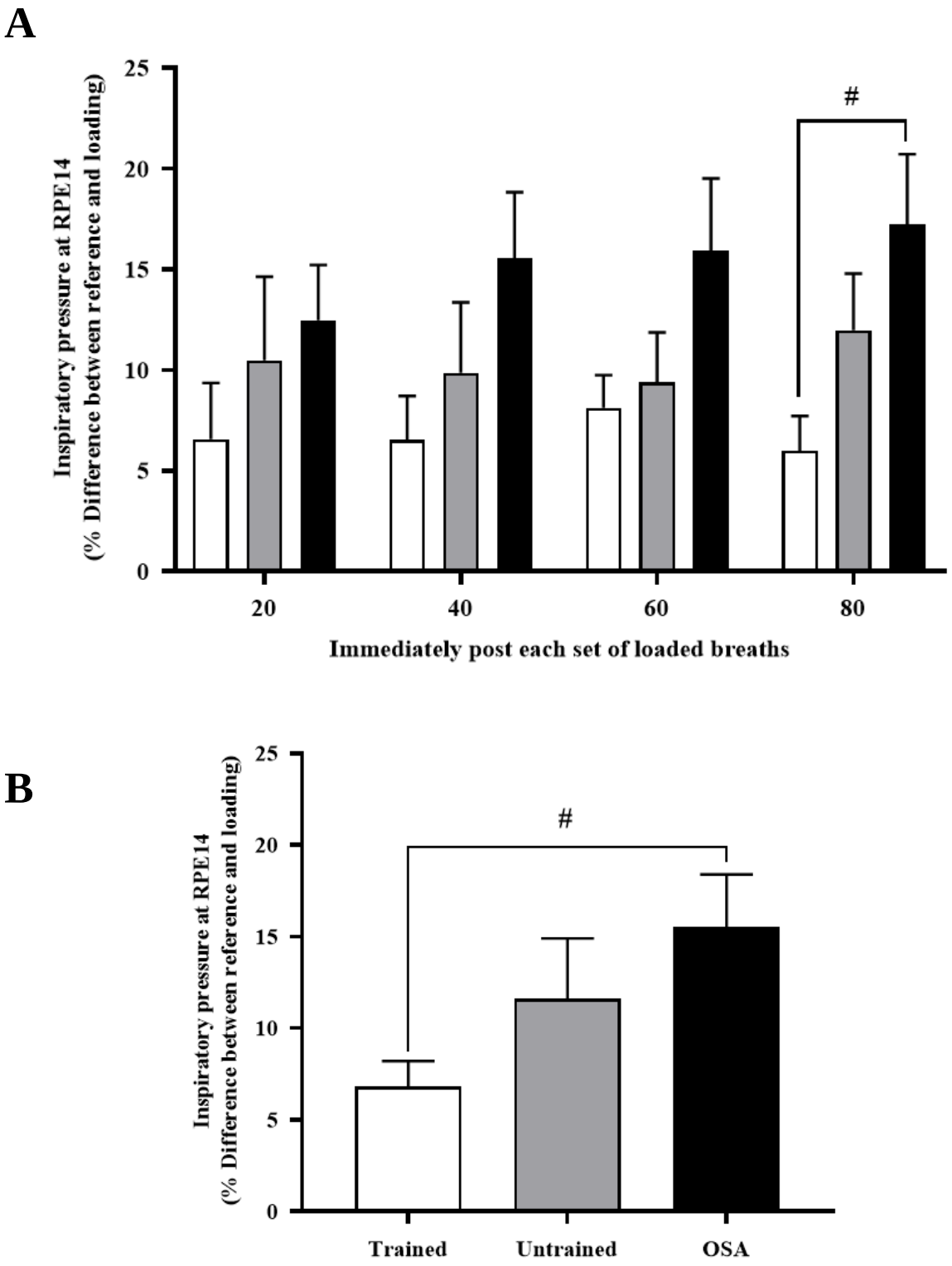


Figure 6.4.3. Inspiratory pressure at RPE 14 (percentage difference between IP_{14} measured at the reference point and after repeated bouts of loading) **(A)**. Inspiratory pressure at RPE 14 (mean percentage difference between IP_{14} reference and loading) **(B)**. Data is expressed as Mean ± SE. □ = Trained group (n = 20); ▒ = Untrained group (n = 17); ■ = OSA group (n = 17). # = p < .05 vs. Trained.

Electromyography amplitude *of the Intercostal and Trapezius muscles during the first and fourth set of the loading protocol*

The next phase of our investigation was to examine the EMG activity of the Intercostal and Trapezius muscles during the first and fourth set of the loading protocol in Trained, Untrained, and OSA groups. We firstly focused upon the Intercostal EMG amplitude measured during the first and fourth set of the loading protocol. Shapiro Wilk tests were firstly performed to determine the normality of the Intercostal EMG amplitude during the first and fourth set of the loading protocol. The data was revealed to be normally distributed ($p > .05$). Therefore, One-Way ANOVA analyses were performed revealing no between-group differences were observed in the Intercostal EMG amplitude during the first and fourth set of loading ($p > .05$; **Table 6.3**). Paired *t*-test analyses were subsequently performed to examine potential differences in the EMG amplitude of the Intercostal muscle from the first to fourth set of loading for each respective group. The analyses revealed no differences in the EMG amplitude of the Intercostal muscle were observed from the first to fourth set of loading for each respective group ($p > .05$).

Linear regression analyses were then performed to determine whether an increase in EMG amplitude of Intercostal muscle would be observed with loading. The analyses revealed an increase in the Intercostal EMG amplitude with loading in Trained individuals (1^{st} set: $B = .152$ µV per set, $p = .199$ and 4^{th} set: $B = .165$ µV per set, $p = .003$; **Figure 6.4.4**) and OSA patients (OSA: 1^{st} set: $B = .380$ µV per set, $p = .000$ and 4^{th} set: $B = .296$ µV per set, $p = .000$). In contrast, and Untrained individuals revealed a decrease in the Intercostal EMG amplitude with loading (Untrained: 1^{st} set: $B = .431$ µV per set, $p = .000$ and 4^{th} set: $B = .229$ µV per set, $p = .016$). One-Way ANOVA analyses further revealed no between-group differences were observed for the slope and intercept of the Intercostal EMG amplitude during either the first or fourth set of loading ($p > .05$).

The following section reports the Trapezius muscle activity during the first and fourth set of the loading protocol. Shapiro Wilk tests were firstly performed to determine the normality of the Trapezius EMG amplitude during the first and fourth set of the loading protocol. The data was revealed to be normally distributed ($p > .05$). Therefore, One-Way ANOVA analyses were performed which revealed significant differences were observed between the groups

Trapezius EMG amplitude following loading (TRA 1st set: $F_{(2, 37)} = 6.462$, $p = .004$; TRA 4th set: $F_{(2, 37)} = 7.803$, $p = .001$). Follow-up analyses demonstrated the Trapezius EMG amplitude was significantly greater in OSA patients compared with Trained individuals during the first and fourth set of loading ($p = .003$ and $p = .001$; **Table 6.3**). Paired *t*-test analyses were subsequently performed to examine potential differences in the EMG amplitude of the Trapezius from the first to fourth set of loading for each respective group. The analyses revealed no differences in the EMG amplitude of the Trapezius muscle were observed from the first to fourth set of loading for each respective group ($p > .05$).

Linear regression analyses were then performed to determine whether an increase in EMG amplitude of Trapezius muscle would be observed with loading. The analyses reported a linear increase in the EMG amplitude of the Trapezius with loading in OSA patients (1st set: $B = .056$ µV per set, $p = .267$ and 4th set: $B = .109$ µV per set, $p = .046$; **Figure 6.4.5**). In contrast, the Trapezius EMG amplitude was unchanged following loading in both Trained and Untrained groups ($p > .05$). One-Way ANOVA analyses further revealed no between-group differences were observed for the slope and intercept of the Trapezius EMG amplitude during either the first or fourth set of loading ($p > .05$).

Our findings demonstrated the Intercostal EMG amplitude was shown to increase with loading in our tested populations **(Table 6.3; Figure 6.4.4)**. Whilst, the Trapezius EMG amplitude was revealed to increase with loading in OSA patients **(Figure 6.4.5)**. These findings support the effectiveness of the inspiratory muscle loading protocol at fatiguing the respiratory muscles given the increased recruitment of the accessory muscles in OSA patients and Untrained individuals. These populations could require further assistance of the accessory muscles during loading to prevent excessive levels of fatigue and eventually task failure. The loading protocol also reveals the different strategies adopted in the activation of the respiratory muscles in our tested populations.

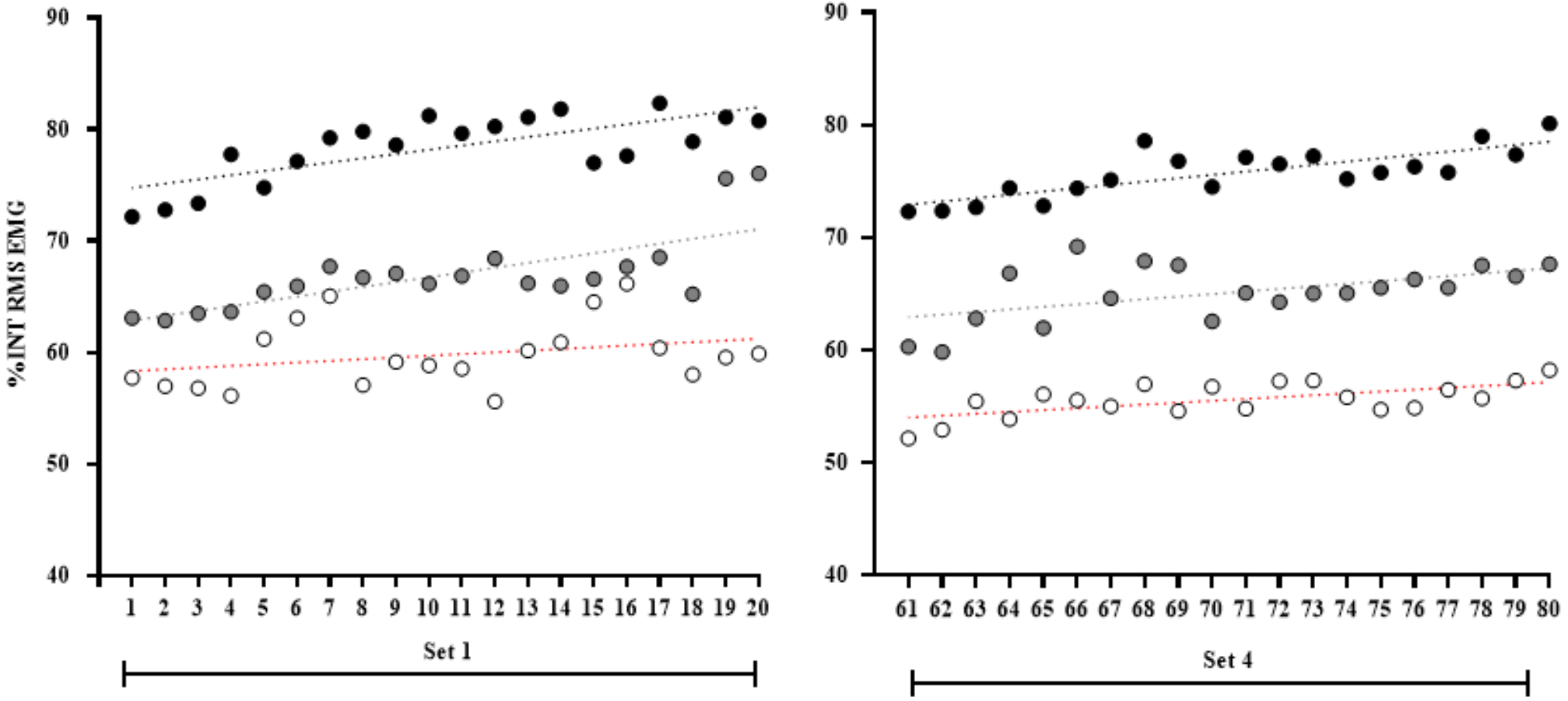


Figure 6.4.4. Intercostal EMG amplitude measured during the first and fourth set of loading. EMG data is expressed as Mean ± SE. ○ = Trained group (n = 15); ◍ = Untrained group (n = 12); ● = OSA group (n = 14). The dashed line represents the linear trend line.

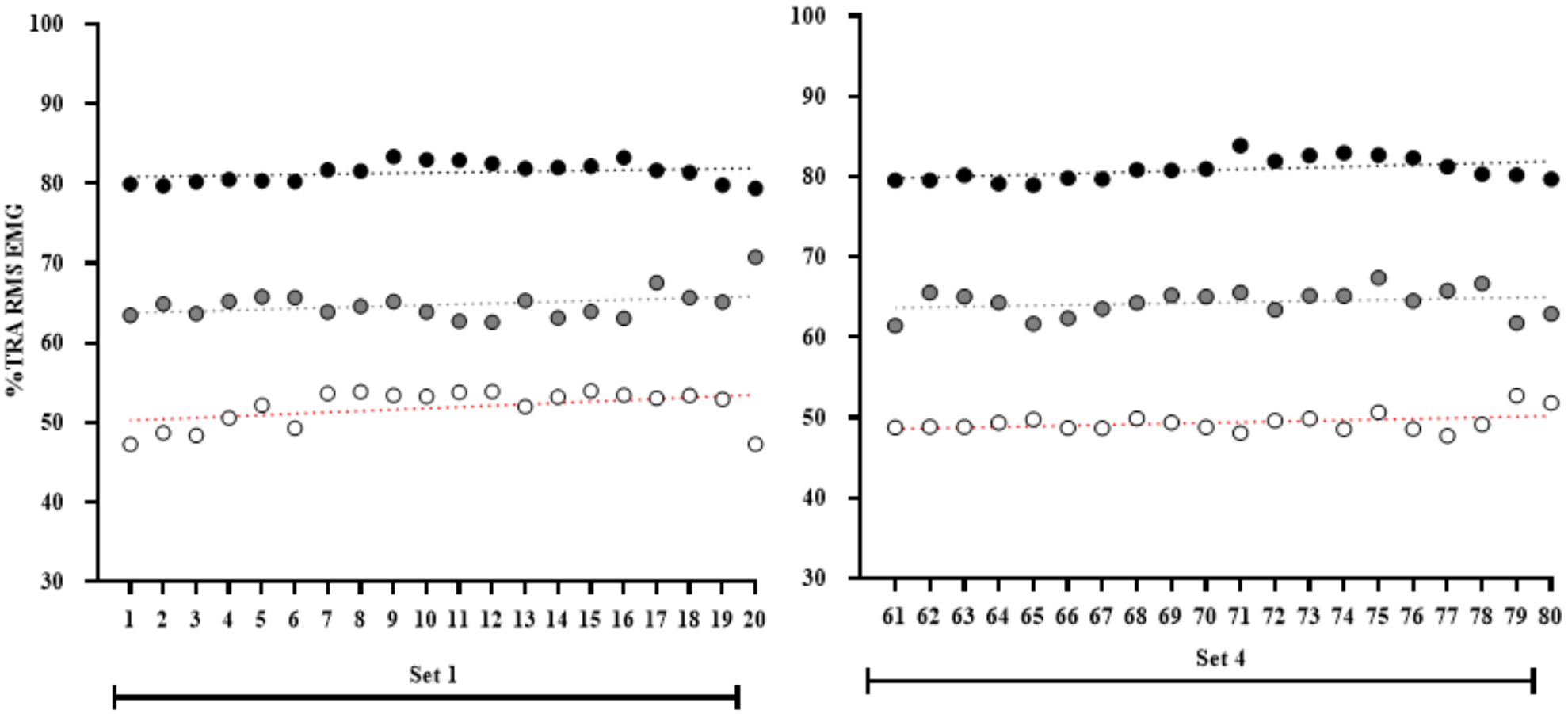


Figure 6.4.5. Trapezius EMG amplitude measured during the first and fourth set of loading. EMG data is expressed as Mean ± SE.

○ = Trained group (n = 14); ◍ = Untrained group (n = 13); ● = OSA group (n = 14). The dashed line represents the linear trend line.

Table 6.3. Change in slope of Intercostal and Trapezius EMG activity during the first and fourth set of loading.

		First set of loading (20 loaded breaths)					Fourth set of loading (80 loaded breaths)				
Outcome	**Group**	***B***	***SE B***	**β**	***t***	***p***	***B***	***SE B***	**β**	***t***	***p***
Intercostal (μV per set)	Trained	.152	.114	.300	1.333	.199	.165	.047	.634	3.477	.003
	Untrained	.431	.093	.738	4.639	.000	.229	.086	.531	2.656	.016
	OSA	.380	.082	.737	4.627	.000	.296	.055	.787	5.413	.000
Trapezius (μV per set)	Trained	.169	.085	.424	1.986	.062	.084	.044	.412	1.916	.071
	Untrained	.110	.070	.349	1.581	.131	.071	.064	.253	1.111	.281
	OSA	.056	.049	.261	1.145	.267	.109	.051	.452	2.147	.046

NOTE: Abbreviations: SE, Standard Error.

Electromyography amplitude of the Intercostal and Trapezius muscles during IP_{14} measured before and after loading

The secondary aim of our investigation was to measure the Intercostal and Trapezius EMG amplitude measured during reference and loading IP_{14} in Trained, Untrained, and OSA groups. Our investigation firstly focused upon the EMG amplitude of Intercostal and Trapezius muscles generated during reference IP_{14}. Shapiro Wilk tests were performed to determine the normality of the absolute EMG amplitudes generated at RPE 14 for the reference point and revealed the data was not normally distributed ($p < .05$). Therefore, we performed a Kruskal Wallis test which revealed the absolute Intercostal and Trapezius EMG amplitudes measured during reference IP_{14} were significantly different between the groups (INT: $\chi^2 = 9.261$, $df = 2$, $p = .010$; TRA: $\chi^2 = 9.031$, $df = 2$, $p = .011$; **Table 6.4-a**).

A

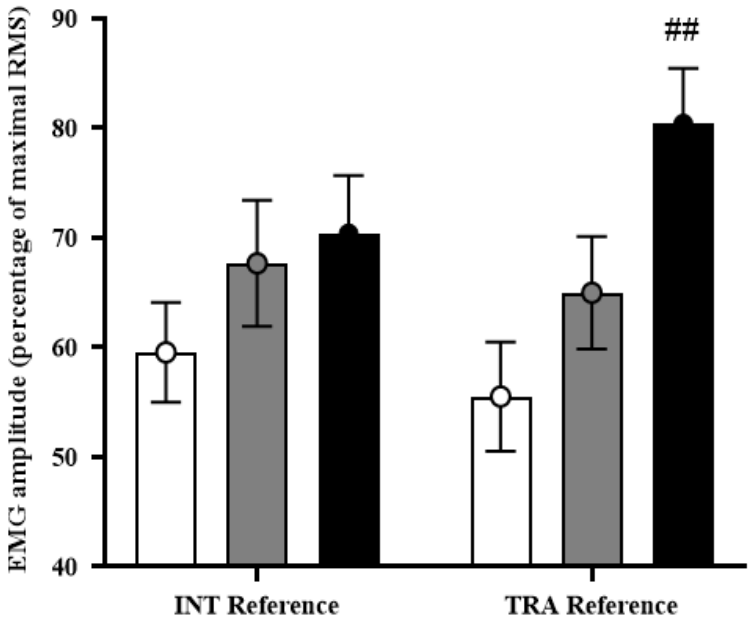


Figure 6.4.6 A. Intercostal and Trapezius EMG amplitude during the IP_{14} measured at the reference point. EMG data are expressed as Mean ± SE. □ = Trained group ($n = 21$); ▒ = Untrained group ($n = 16$); ■ = OSA group ($n = 14$). ## = $p < .01$ vs. Trained.

Mann-Whitney U tests were then performed and revealed the absolute Intercostal and Trapezius EMG amplitudes measured during reference IP_{14} were significantly lower in OSA patients compared with Trained individuals (U = 60, p = .003; U = 65, p = .006). The analyses further revealed a significantly lower absolute Intercostal EMG amplitude produced during reference IP_{14} in OSA patients compared with Untrained individuals (U = 58, p = .025). Whilst, the Trapezius amplitude produced during reference IP_{14} was significantly lower in Untrained versus Trained individuals (U = 87, p = .024). We then normalised the absolute Intercostal and Trapezius EMG amplitudes measured during reference IP_{14} according to participant's maximal RMS. Shapiro Wilk tests revealed the relative EMG amplitudes were normally distributed (p > .05). One-Way ANOVA analyses were performed demonstrating the relative Intercostal EMG amplitudes during reference IP_{14} were similar between the groups (p > .05). However, the relative Trapezius EMG amplitude was shown to be significantly greater during reference IP_{14} in OSA patients compared with Trained individuals (p = .003; **Figure 6.4.6 A and B**).

Next, we examined the absolute EMG amplitudes of both Intercostal and Trapezius muscles measured during the IP_{14} following repeated bouts of loading. Shapiro Wilk tests revealed the EMG amplitudes was not normally distributed (p < .05). Therefore, we performed a Kruskal Wallis test which revealed the absolute Intercostal and Trapezius EMG amplitudes measured during loading IP_{14} was shown to be significantly different between the groups (INT: χ^2 = 14.763, df = 2, p = .001; TRA: χ^2 = 14.624, df = 2, p = .001). Mann-Whitney U tests revealed the absolute Intercostal EMG amplitudes were significantly lower in OSA patients compared with Untrained individuals after 20 (U = 36, p = .001), 40 (U = 37.5, p = .001), 60 (U = 30, p = .000), and 80 loaded breaths (U = 49, p = .010). Further Mann-Whitney U tests revealed the absolute Intercostal EMG amplitude was significantly lower in OSA patients compared with Trained individuals during the IP_{14} measured following 20 (U = 51, p = .002), 40 (U = 51.5, p = .003), 60 (U = 52, p = .004), and 80 loaded breaths (U = 64, p = .010; **Table 6.4-a**). Mann-Whitney U tests also revealed the absolute Trapezius EMG amplitudes were significantly lower in OSA patients compared with Untrained individuals after 20 (U = 45, p = .004), 40 (U = 61, p = .025), 60 (U = 58.5, p = .019), and 80 loaded breaths (U = 57, p = .025). Furthermore, the absolute Trapezius EMG amplitudes were significantly lower in OSA patients compared with Trained individuals following 20 (U = 41, p = .001), 40 (U = 43.5, p = .001), 60 (U = 33, p = .000), and 80 loaded breaths (U = 36, p = .000; **Table 6.4-b)**.

We then normalised the absolute Intercostal and Trapezius EMG amplitudes measured during the IP_{14} following loading according to participant's maximal RMS. Shapiro Wilk tests demonstrated the relative Intercostal and Trapezius EMG amplitudes were normally distributed ($p > .05$). Therefore, we performed a One-Way ANOVA which revealed loading did not influence the relative EMG amplitude of the Intercostal muscle during IP_{14} ($p > .05$; **Figure 6.4.6 B**). Further One-Way ANOVA analyses demonstrated that loading altered the relative EMG amplitude of the Trapezius muscle during the IP_{14} measured after loading ($F_{(2, 41)} = 6.920$, $p = .003$, no interaction $p > .05$). Follow-up analyses revealed that OSA patients had a significantly greater EMG amplitude of the Trapezius muscle during the IP_{14} measured after 20 ($p = .017$, $d = 1.15$), 40 ($p = .007$, $d = 1.22$), 60 ($p = .022$, $d = 1.05$), and 80 loaded breaths ($p = .003$, $d = 1.33$) compared with Trained individuals (**Figure 6.4.6 C**). Similarly, Untrained individuals demonstrated a significantly greater EMG amplitude of the Trapezius muscle during the IP_{14} measured after 20 ($p = .017$. $d = 0.96$) and 40 loaded breaths ($p = .041$, $d = 0.84$) compared with Trained individuals. Linear regression analyses were then performed to examine changes in slope of Intercostal and Trapezius EMG amplitudes measured during the IP_{14} after loading. The analyses revealed no changes in the slope of both Intercostal and Trapezius EMG amplitudes during loading IP_{14} ($p > .05$; **Table 6.5**).

Our findings demonstrated OSA patients activate the Trapezius muscle to a greater extent during the inspiratory pressures generated at RPE 14 for the reference point compared with Trained and Untrained individuals **(Figure 6.4.6-A)**. Newly diagnosed OSA patients were also shown to respond to repeated bouts of loading with an increased EMG amplitude of the Trapezius muscle during the inspiratory pressure generated at RPE 14 **(Figure 6.4.6-C)**. However, the Intercostal EMG amplitude was shown to be similar at the reference point and relatively unaffected following loaded breathing **(Figure 6.4.6-B)**.

Table 6.4-a. Intercostal electromyography amplitude measured during IP_{14} before and after repeated bouts of loading.

	Trained (*n* = 21)		Untrained (*n* = 18)		OSA (*n* = 13)		**Effect size (Cohen *d*)**		
Absolute RMS	μV	Median (IQR) μV	μV	Median (IQR) μV	μV	Median (IQR) μV	T v U	T v O	U v O
PI_{max}	96.1 (12.9)	83.4 (46.4-124.9)	72.4 (8.9)	56.7 (42.1-100.6)	57.8 (5.4)$^{\#}$	54.4 (37.1-75.5)	0.47	0.86	0.48
IP_{14} reference (avg)	53.7 (5.4)	44.0 (37.2-61.1)	45.3 (2.8)†	43.1 (35.3-53.1)	36.9 (1.5)$^{\#\#}$	35.3 (33.4-37.9)	0.44	0.93	0.94
IP_{14} 20 breaths	60.2 (7.1)	47.5 (36.1-84.6)	47.4 (4.0)††	43.4 (38.2-50.4)	35.5 (.95)$^{\#\#}$	34.5 (33.1-36.4)	0.49	1.06	0.98
IP_{14} 40 breaths	49.6 (3.7)$^{\phi}$	44.5 (36.0-57.6)	46.3 (3.4)††	41.2 (37.7-49.3)	35.5 (.84)$^{\#\#}$	34.6 (33.2-36.7)	0.20	1.15	1.04
IP_{14} 60 breaths	48.0 (3.2)	46.5 (36.2-54.0)	46.4 (2.9)††	43.5 (37.2-52.4)	35.4 (.85)$^{\#\#}$	34.0 (33.2-37.0)	0.12	1.21	1.22
IP_{14} 80 breaths	46.9 (4.3)	38.3 (34.5-51.6)	44.4 (2.8)††	40.8 (34.6-50.4)	35.3 (.63)$^{\#\#}$	34.9 (33.4-37.8)	0.15	0.82	1.07
IP_{14} loading (avg)	51.7 (4.1)	46.8 (37.9-59.0)	46.0 (2.6)††	41.7 (38.4-55.4)	35.4 (.77)$^{\#\#}$	34.6 (33.3-37.0)	0.36	1.20	1.32

NOTE: Values are Mean ± SEM unless otherwise stated. PI_{max}, Maximal Inspiratory Pressure; μV, Microvolt; INT, Intercostal; IP_{14}, Inspiratory Pressure at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea.

$^{\#}$ = p <.05 Significantly greater INT RMS during PI_{max} in Trained vs. OSA. † = p <.05 Significantly lower INT RMS during IP_{14} reference in OSA vs. Untrained. $^{\#\#}$ = p <.01 Significantly lower INT RMS during IP_{14} reference in OSA vs. Trained. $^{\phi}$ = p <.05 Significantly lower INT RMS during IP_{14} after 40 breaths than 20 breaths in Trained. †† = p <.01 Significantly lower INT RMS during the IP_{14} after 20, 40, 60, and 80 breaths in OSA vs. Untrained. $^{\#\#}$ = p <.01 Significantly lower INT RMS during the IP_{14} after 20, 40, 60, and 80 breaths in OSA vs Trained; †† = p <.01 Significantly lower INT RMS during IP_{14} loading in OSA vs. Untrained. $^{\#\#}$ = p <.01 Significantly lower INT RMS during IP_{14} loading in OSA vs. Trained.

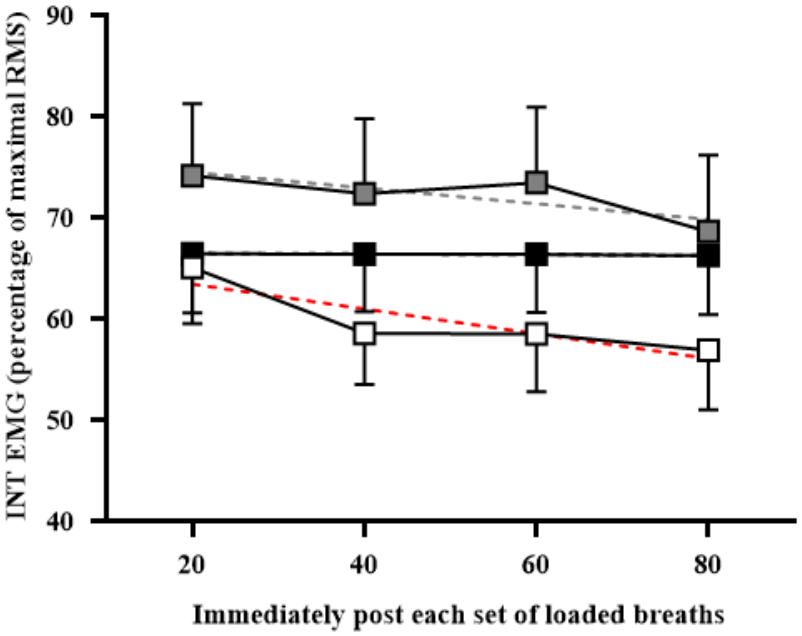
Table 6.4-b. Trapezius electromyography amplitude measured during IP_{14} before and after repeated bouts of loading

	Trained (*n* = 21)		Untrained (*n* = 18)		OSA (*n* = 13)		Effect size (Cohen *d*)		
Absolute RMS	μV	Median (IQR) μV	μV	Median (IQR) μV	μV	Median (IQR) μV	T v U	T v O	U v O
PI_{max}	91.6 (11.0)**	75.9 (50.9-135.5)	56.4 (7.9)	43.1 (34.2-69.2)	40.8 (4.0)$^{\#\#}$	36.8 (30.4-48.9)	0.84	1.42	0.60
IP_{14} reference (avg)	49.9 (7.2)*	37.6 (29.0-50.8)	33.3 (2.7)	28.0 (27.5-31.7)	29.8 (.82)$^{\#\#}$	29.3 (27.5-31.6)	0.67	0.85	0.45
IP_{14} 20 breaths	54.1 (9.8)	36.4 (30.0-53.8)	39.4 (3.7)††	36.8 (28.4-42.1)	28.0 (1.0)$^{\#\#}$	28.0 (26.9-29.3)	0.43	0.82	1.01
IP_{14} 40 breaths	44.8 (5.7)	34.2 (30.9-49.3)	39.9 (6.2)†	30.1 (28.7-36.7)	27.9 (1.2)$^{\#\#}$	28.0 (26.8-30.8)	0.18	0.90	0.63
IP_{14} 60 breaths	57.1 (10.2)	41.9 (29.7-70.0)	42.5 (6.1)†	30.4 (28.2-45.7)	28.8 (.74)$^{\#\#}$	27.9 (26.9-30.2)	0.39	0.87	0.74
IP_{14} 80 breaths	48.2 (4.5)	41.0 (29.5-68.5)	42.1 (5.9)†	30.6 (28.0-49.2)	28.5 (.58)$^{\#\#}$	27.9 (27.1-29.2)	0.26	1.33	0.79
IP_{14} loading (avg)	50.8 (6.7)	41.3 (30.4-60.3)	40.8 (4.8)††	32.5 (29.5-40.2)	28.3 (.80)$^{\#\# \Psi}$	27.8 (27.0-29.6)	0.38	1.03	0.85

NOTE: Values are Mean ± SEM unless otherwise stated. PI_{max}, Maximal Inspiratory Pressure; μV, Microvolt; TRA, Trapezius; IP_{14}, Inspiratory Pressure at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea.

** = p <.01 Significantly greater TRA RMS during PI_{max} in Trained vs. Untrained; $^{\#\#}$ = p <.01 Significantly lower TRA RMS during PI_{max} in OSA vs. Trained; * = p <.05 Significantly greater TRA RMS during IP_{14} reference in Trained vs. Untrained; $^{\#\#}$ = p <.01 Significantly lower TRA RMS during IP_{14} reference in OSA vs. Trained; † = p <.05, †† = p <.01 Significantly lower TRA RMS during the IP_{14} after 20, 40, 60, and 80 breaths in OSA vs. Untrained. $^{\#\#}$ = p <.01 Significantly lower TRA RMS during the IP_{14} after 20, 40, 60, and 80 breaths in OSA vs Trained; †† = p <.01 Significantly lower TRA RMS during IP_{14} loading in OSA vs. Untrained. $^{\#\#}$ = p <.01 Significantly lower TRA RMS during IP_{14} loading in OSA vs. Trained. $^{\Psi}$ = p <.05 Significant lower RMS during IP_{14} loading than IP_{14} reference in OSA.

B



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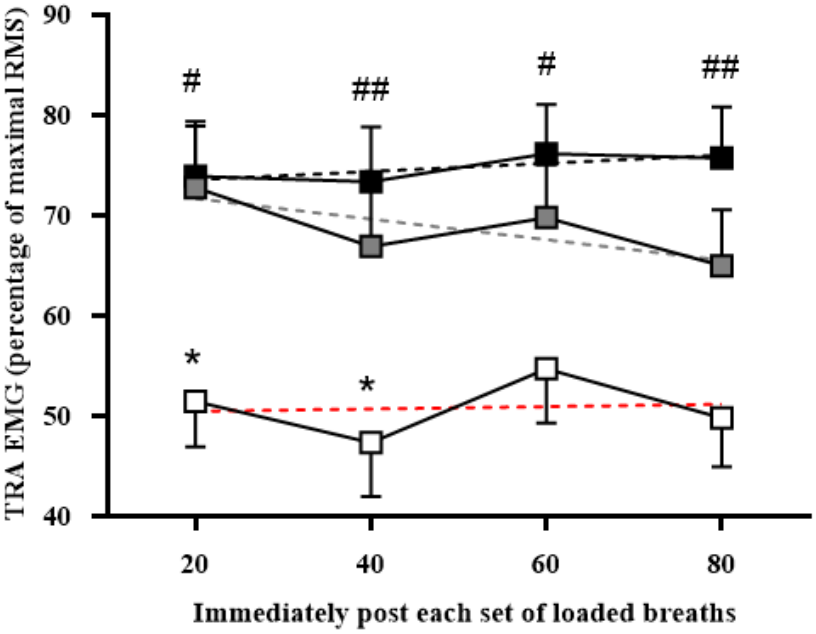


Figure 6.4.6. Intercostal **(B)** and Trapezius **(C)** EMG amplitude measured during the IP_{14} after repeated bouts of loading. EMG data are expressed as Mean ± SE. □ = Trained group (n = 18); ▒ = Untrained group (n = 16); ■ = OSA group (n = 14). The dashed line represents the linear trend line. # = $p < .05$, ## = $p < .01$ vs. Trained. * = $p < .05$ vs. Untrained.

Table 6.5. Slope of Intercostal and Trapezius EMG activity measured when producing inspiratory pressures at RPE 14 following loading.

Outcome	Group	*B*	*SE B*	β	*t*	*p*
Intercostal (μV per cmH_2O)	Trained	-.123	.048	-.875	-2.557	.125
	Untrained	-.078	.039	-.813	-1.973	.187
	OSA	-.003	.001	-.913	-3.163	.087
Trapezius (μV per cmH_2O)	Trained	.012	.084	.098	.139	.902
	Untrained	-.102	.059	-.776	-1.739	.224
	OSA	.041	.023	.779	1.754	.221

NOTE: Abbreviations: IP_{14}, Inspiratory pressure at RPE14; SE, Standard Error.

Neuromuscular efficiency of the Intercostal and Trapezius muscles during IP_{14} measured before and after loading

A further aim of our investigation was to examine the neuromuscular efficiency of the Intercostal and Trapezius muscles during the inspiratory pressures generated at RPE 14 before and after repeated bouts of loading in Trained, Untrained, and OSA groups. Neuromuscular efficiency is interpreted as the responsiveness of a working muscle to neural excitation and is quantified by dividing the IP_{14} generated by the inspiratory muscles with the average EMG amplitude measured during the contraction (pressure / EMG amplitude). Our investigation firstly focused upon the neuromuscular efficiency of the Intercostal and Trapezius muscles during the IP_{14} measured at the reference point. Shapiro Wilk tests were performed to determine the normality of the neuromuscular efficiency of both Intercostal and Trapezius EMG amplitudes measured at the reference point. The data was revealed to be normally distributed ($p > .05$), therefore, we performed One-Way ANOVA analyses which revealed the neuromuscular efficiency of Intercostal EMG amplitude to be similar between the groups at the reference point ($p > .05$; **Figure 6.4.7 A**). However, the neuromuscular efficiency of the Trapezius EMG amplitude was significantly lower at the reference point in OSA patients compared with Trained individuals ($p = .034$, $d = 1.36$). No differences were observed in the neuromuscular efficiency of the Trapezius EMG amplitude between OSA and Untrained groups ($p > .05$).

A

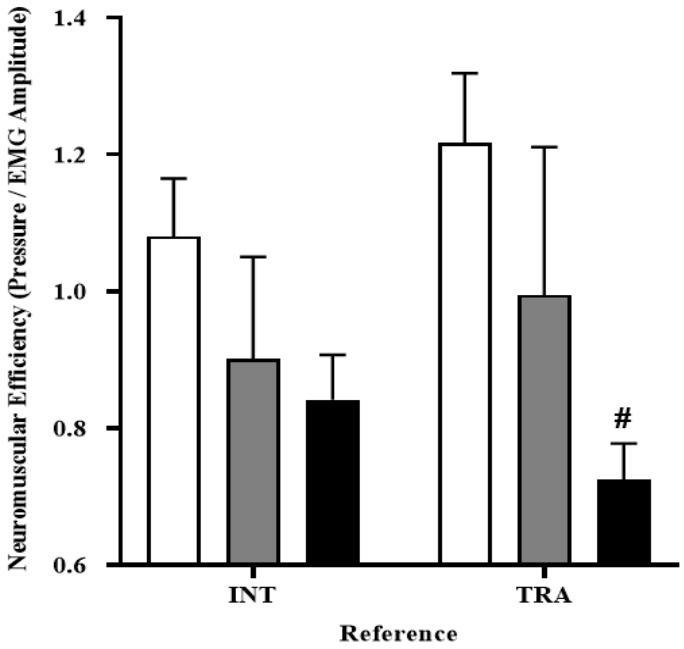


Figure 6.4.7 A. Neuromuscular efficiency of Intercostal and Trapezius EMG amplitudes during the IP_{14} measured at the reference point. All data are expressed as Mean ± SE. □ = Trained group (*n* = 21); ▒ = Untrained group (*n* = 17); ■ = OSA group (*n* = 14).

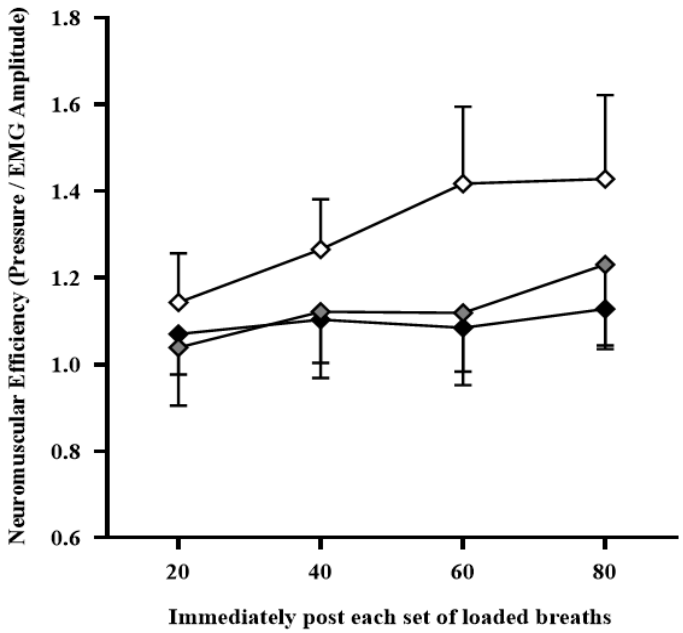
Next, we examined the impact of loading upon the neuromuscular efficiency of Intercostal and Trapezius EMG amplitudes during IP_{14}. Shapiro Wilk tests revealed the data was normally distributed ($p > .05$). We performed a One-Way ANOVA analyses which revealed the neuromuscular efficiency of the Intercostal EMG amplitude at RPE 14 was similar between the groups ($p > .05$; **Figure 6.4.7 B**). Further analyses showed the neuromuscular efficiency of the Trapezius EMG amplitude was significantly greater in Trained individuals compared with OSA patients following the IP_{14} after 20 ($p = .019$, $d = 1.02$), 40 ($p = .008$, $d = 1.13$), 60 ($p = .019$, $d = 1.04$), and 80 loaded breaths ($p = .024$, $d = 0.94$) **(Figure 6.4.7 C**). We also found the neuromuscular efficiency of the Trapezius EMG amplitude during IP_{14} was significantly greater in Trained individuals compared with Untrained individuals after 20 loaded breaths ($p = .012$, $d = 0.99$), 40 ($p = .014$, $d = 0.98$), and 60 loaded breaths ($p = .048$, $d = 0.82$).

Paired *t*-test analyses were subsequently performed to examine potential differences in the neuromuscular efficiency of the Intercostal and Trapezius EMG amplitudes between the

reference point and that of the first and fourth set of loading for each respective group. The analyses revealed the neuromuscular efficiency of Intercostal EMG amplitude was significantly increased when producing the IP_{14} after the first (reference INT: .90 ± .57 vs. 1st set INT: 1.10 ± .54, 95% CI: .02 to .39, $t_{(14)} = 2.409$, $p = .030$), and fourth set of loading in Untrained individuals (reference INT: .90 ± .57 vs. 4th set INT: 1.34 ± .73, 95% CI: .10 to .72, $t_{(14)} = 2.929$, $p = .012$). The neuromuscular efficiency of the Intercostal EMG amplitude measured was also noticeably increased in OSA patients when producing the IP_{14} after the first (reference INT: .85 ± .24 vs. 1st set INT: 1.07 ± .33, 95% CI: .10 to .31, $t_{(12)} = 4.319$, $p = .001$), and fourth set of loading (reference INT: .85 ± .24 vs. 4th set INT: 1.12 ± .33, 95% CI: .13 to .40, $t_{(12)} = 4.359$, $p = .001$). In contrast, the neuromuscular efficiency of the Intercostal EMG amplitude was only shown to increase in Trained individuals when producing the IP_{14} after the fourth set of loading (reference INT: 1.08 ± .38 vs. 4th set INT: 1.42 ± .88, 95% CI: .003 to .69, $t_{(19)} = 2.104$, $p = .048$).

Further paired *t*-test analyses revealed the neuromuscular efficiency of the Trapezius EMG amplitude was significantly increased in OSA patients when generating the IP_{14} after the first (reference TRA: .73 ± .20 vs. 1st set TRA: .94 ± .27, 95% CI: .09 to .32, $t_{(12)} = 4.028$, $p = .002$), and fourth set of loading (reference TRA: .73 ± .20 vs. 4th set TRA: .96 ± .31, 95% CI: .08 to .39, $t_{(12)} = 3.344$, $p = .006$). We also found the neuromuscular efficiency of the Trapezius EMG amplitude was significantly increased in Untrained individuals when generating the IP_{14} after the fourth set of loading (reference TRA: .82 ± .32 vs. 4th set TRA: 1.06 ± .24, 95% CI: .05 to .43, $t_{(11)} = 2.919$, $p = .015$). No differences were observed in neuromuscular efficiency of the Trapezius EMG amplitude measured during IP_{14} after loading in Trained individuals ($p > .05$). Our findings revealed the neuromuscular efficiency of both Intercostal and Trapezius EMG amplitudes were significantly increased in response to loading in our tested populations **(Figure 6.4.7 B and C).**

B



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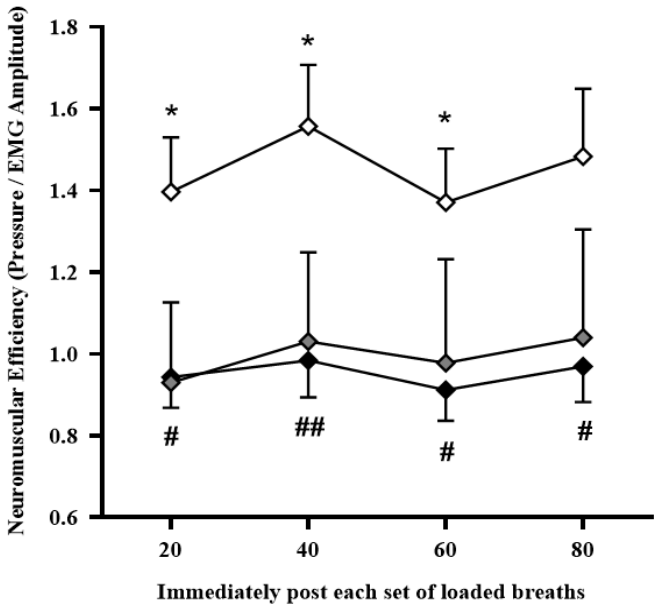

Figure 6.4.7. Neuromuscular efficiency of Intercostal **(B)** and Trapezius **(C)** EMG amplitude during the IP_{14} measured after repeated bouts of loading. All EMG data are expressed as Mean ± SE. □ = Trained group (n = 21); ▒ = Untrained group (n = 16); ■ = OSA group (n = 13). # = p < .05, ## = p < .01 vs. Trained. * = p < .05 vs. Untrained.

Effect of CPAP therapy on the inspiratory pressures generated at RPE 14 before and after loading

For the next phase of our investigation we examined the inspiratory pressures generated by the respiratory muscles at a clamped effort of 14 (IP_{14}) on the Borg scale before and after repeated bouts of loading in OSA patients treated with CPAP therapy. We firstly focused upon the inspiratory pressures at an RPE of 14 generated during unloaded conditions which is referred to as the reference point of effort perception. Shapiro Wilk tests were initially performed to determine the normality of the absolute pressures generated at RPE 14 for the reference point. The data was revealed to be normally distributed ($p > .05$). Therefore, we performed Paired *t*-test analyses which revealed OSA patients treated with 3 months of CPAP therapy were shown to produce significantly higher absolute inspiratory pressures at RPE 14 for the reference point (pre-CPAP: 56.7 ± 2.6 cmH_2O vs. post-CPAP: 77.0 ± 3.5 cmH_2O, 95% CI: 12.3 to 28.3, $t_{(14)} = 5.445$, $p = .000$; **Table 6.6)**. Subsequently, we normalised the absolute IP_{14} according to participant's PI_{max} and performed Shapiro Wilk tests which revealed the relative inspiratory pressures at RPE14 was normally distributed ($p > .05$). After receiving CPAP therapy, OSA patients were reported to produce significantly higher relative inspiratory pressures at RPE 14 for the reference point (pre-CPAP: 54.5 ± 1.8 % vs. post-CPAP: 68.2 ± 3.2 %, 95% CI: 6.6 to 20.8, $t_{(14)} = 4.135$, $p = .001$, **Figure 6.4.8 A**).

Next, we examined the inspiratory pressures generated by the respiratory muscles at a clamped effort of 14 (IP_{14}) on the Borg scale following repeated bouts of loading. Shapiro Wilk tests revealed the absolute pressures generated at RPE 14 following loading were normally distributed ($p > .05$). Paired *t*-test analyses reported that loading did not influence the absolute inspiratory pressures generated by the respiratory muscles at a clamped effort of 14 in OSA patients treated with CPAP therapy ($p > .05$). We then normalised the absolute IP_{14} generated following loading according to participant's PI_{max}. Shapiro Wilk tests demonstrated the relative IP_{14} was normally distributed ($p > .05$). Therefore, we performed Paired *t*-test analyses which revealed loading did not influence the relative inspiratory pressures generated at RPE 14 ($p > .05$; **Figure 6.4.8 B**). However, our experiment demonstrated that repeated loading of the respiratory muscles at 50% PI_{max} leads to an increase of inspiratory pressure generated at RPE 14 in OSA patients, compared with the reference point which is measured during unloaded conditions (pre-CPAP reference: 56.7 ±

2.6 cmH_2O vs. pre-CPAP loading: 72.8 ± 2.8 cmH_2O, 95% CI: 9.7 to 22.4, $t_{(14)} = 5.458$, $p = .000$). Following CPAP therapy, no differences were observed in the absolute and relative inspiratory pressures generated at RPE 14 between the reference point and loading ($p > .05$).

Table 6.6. Effects of CPAP therapy on the inspiratory pressure generated at a fixed RPE of 14 before and after repeated bouts of loading.

	OSA Pre-CPAP (*n* = 15)		**OSA Post-CPAP (*n* = 15)**		**Effect size (Cohen *d*)**
Absolute pressures	Pressure cmH_2O	Median (IQR) cmH_2O	Pressure cmH_2O	Median (IQR) cmH_2O	Pre v Post
PI_{max}	104.6 (4.3)	101.4 (95.0-120.3)	105.7 (4.4)	107.4 (91.7-119.8)	0.06
IP_{14} reference (avg)	56.7 (2.6)	60.4 (45.8-65.9)	77.0 (3.6)¶¶	73.5 (64.7-93.1)	1.67
IP_{14} 20 breaths	68.7 (3.0)ΨΨ	75.6 (55.4-77.4)	74.6 (5.6)	71.2 (56.1-100.1)	0.33
IP_{14} 40 breaths	72.8 (3.0)	73.4 (64.4-81.3)	75.5 (5.7)	77.8 (56.0-86.2)	0.15
IP_{14} 60 breaths	74.3 (3.3)	73.3 (61.9-84.6)	73.6 (5.0)	76.8 (52.9-90.2)	0.04
IP_{14} 80 breaths	74.4 (4.0)	69.5 (63.8-91.2)	73.9 (5.7)	74.7 (53.3-85.1)	0.04
IP_{14} loading (avg)	72.5 (2.8)ΨΨ	71.7 (61.7-83.4)	74.4 (5.2)	75.0 (53.6-97.4)	0.11

NOTE: Values are Mean ± SEM unless otherwise stated. PI_{max}, Maximal Inspiratory Pressure; IP_{14}, Inspiratory Pressure at RPE 14; IQR, Inter Quartile Range; OSA, Obstructive Sleep Apnoea.

ΨΨ = $p < .01$ Significantly greater IP_{14} after 20 breaths than IP_{14} reference (Pre-CPAP). ΨΨ = $p < .01$ Significantly greater IP_{14} loading than IP_{14} reference (Pre-CPAP). ¶¶ = $p < .01$ Significantly greater IP_{14} reference Post-CPAP vs. Pre-CPAP.

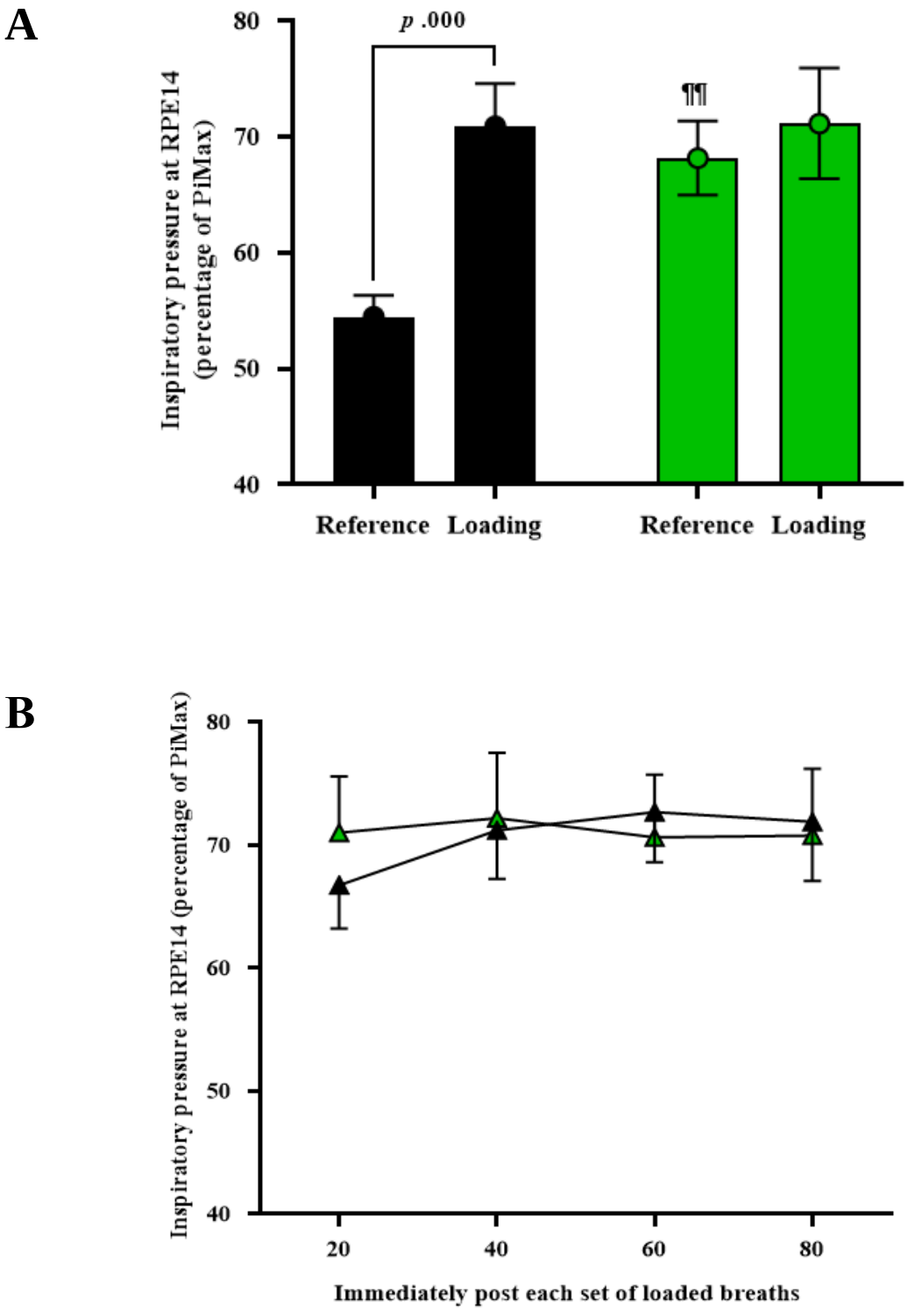

Figure 6.4.8. Inspiratory pressure at an RPE of 14 (IP_{14}) measured at the reference point and after loading **(A)**. Inspiratory pressure at an RPE of 14 (IP_{14}) measured after repeated bouts of loading **(B)**. Pressure data is expressed as Mean ± SE. ▲ = Pre-CPAP group (*n* = 15); △ = Post-CPAP group (*n* = 15). **NOTE:** Abbreviations: PI_{max}, Maximal Inspiratory Pressure; $IP_{14,}$ Inspiratory pressure at RPE 14. ¶¶ = $p < .01$ vs. Pre-CPAP.

Subsequently, we examined the coefficient of variation (CV %) to determine whether the variability in the IP_{14} measured at the reference point and after loading were different following CPAP therapy. Shapiro Wilk tests revealed the CV% for both reference and loading IP_{14} was normally distributed ($p > .05$). Paired *t*-test analyses revealed that OSA patients respond to loading with a significantly lower CV % when generating inspiratory pressures at RPE 14 before receiving CPAP therapy (mean difference: -4.3 %, 95% CI: -6.8 to -1.8, $t_{(14)} = -3.741$, $p = .002$; **Figure 6.4.9**). Following CPAP therapy, no differences were observed in the CV % when producing the reference IP_{14} ($p = .724$). The results further revealed a significant reduction in the CV% of reference IP_{14} in OSA patients treated with CPAP therapy (mean difference: -4.3 %, 95% CI: -7.0 to -1.7, $t_{(14)} = -3.533$, $p = .003$). No differences were observed in the CV % when generating IP_{14} after loading in OSA patients treated with CPAP therapy ($p = .782$).

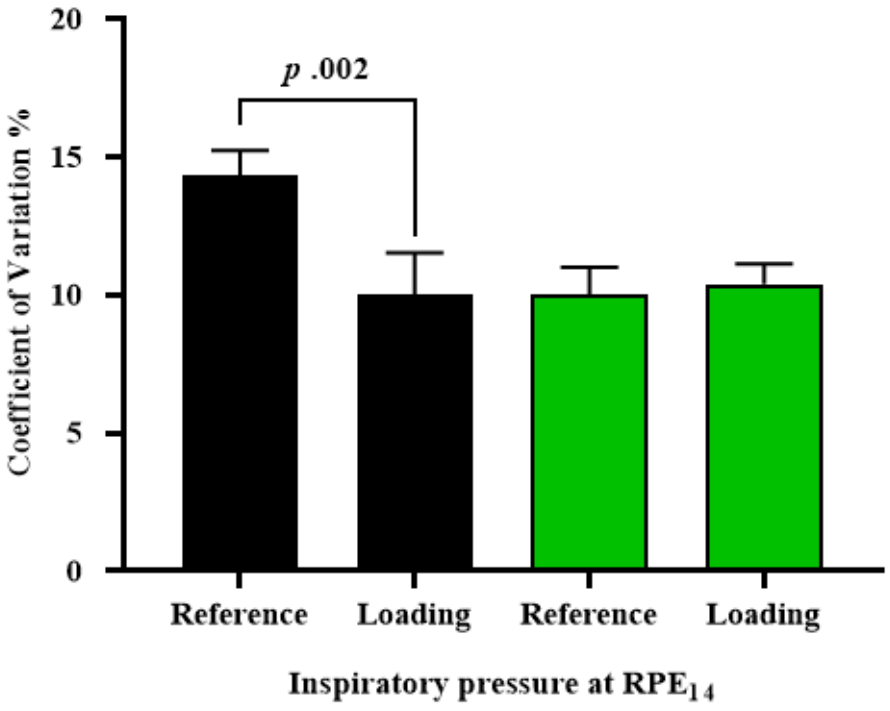

Figure 6.4.9. Coefficient of variation (expressed as percentage) of IP_{14} measured at the reference point and after loading. Data is expressed as Mean ± SE. ■ = Pre-CPAP group ($n = 15$); ■ = Post-CPAP group ($n = 15$).

Finally, we examined the matching of inspiratory pressures at a set RPE of 14 by comparing the relative inspiratory pressures generated for the reference point to those performed following loading. Shapiro Wilk tests revealed the percentage difference between reference and loading IP_{14} were normally distributed ($p > .05$). Paired *t*-test analyses demonstrated the percentage difference between reference and loading IP_{14} was significantly lower in OSA patients treated with CPAP therapy after completing 20 ($p = .030$), 40 ($p = .003$), 60 ($p = .005$), and 80 loaded breaths ($p = .018$; **Figure 6.4.10 A**). Further analyses revealed the percentage difference between reference and loading IP_{14} was significantly lower following CPAP therapy in OSA patients ($p = .000$, **Figure 6.4.10 B**).

Spearman's rho analyses demonstrated a significant correlation between AHI (OSA severity) and the inspiratory pressure generated at the reference point ($r_s = -.567$, $p = .022$) in OSA patients before received CPAP therapy. No correlations were observed after OSA patients received CPAP therapy (all $p > .05$). Our findings revealed that after receiving 3 months of CPAP therapy OSA patients operate at a higher percentage of their PI_{max} at the reference point as shown by the greater relative inspiratory pressure generated at RPE 14 **(Figure 6.4.8 A)**. We also found the absolute and relative inspiratory pressures generated at RPE 14 were not influenced by loading in OSA patients treated with CPAP therapy **(Table 6.6; Figure 6.4.8 B)**. Furthermore, the matching of inspiratory pressures at RPE 14 were improved following CPAP therapy in OSA patients **(Figure 6.4.10 A and B)**.

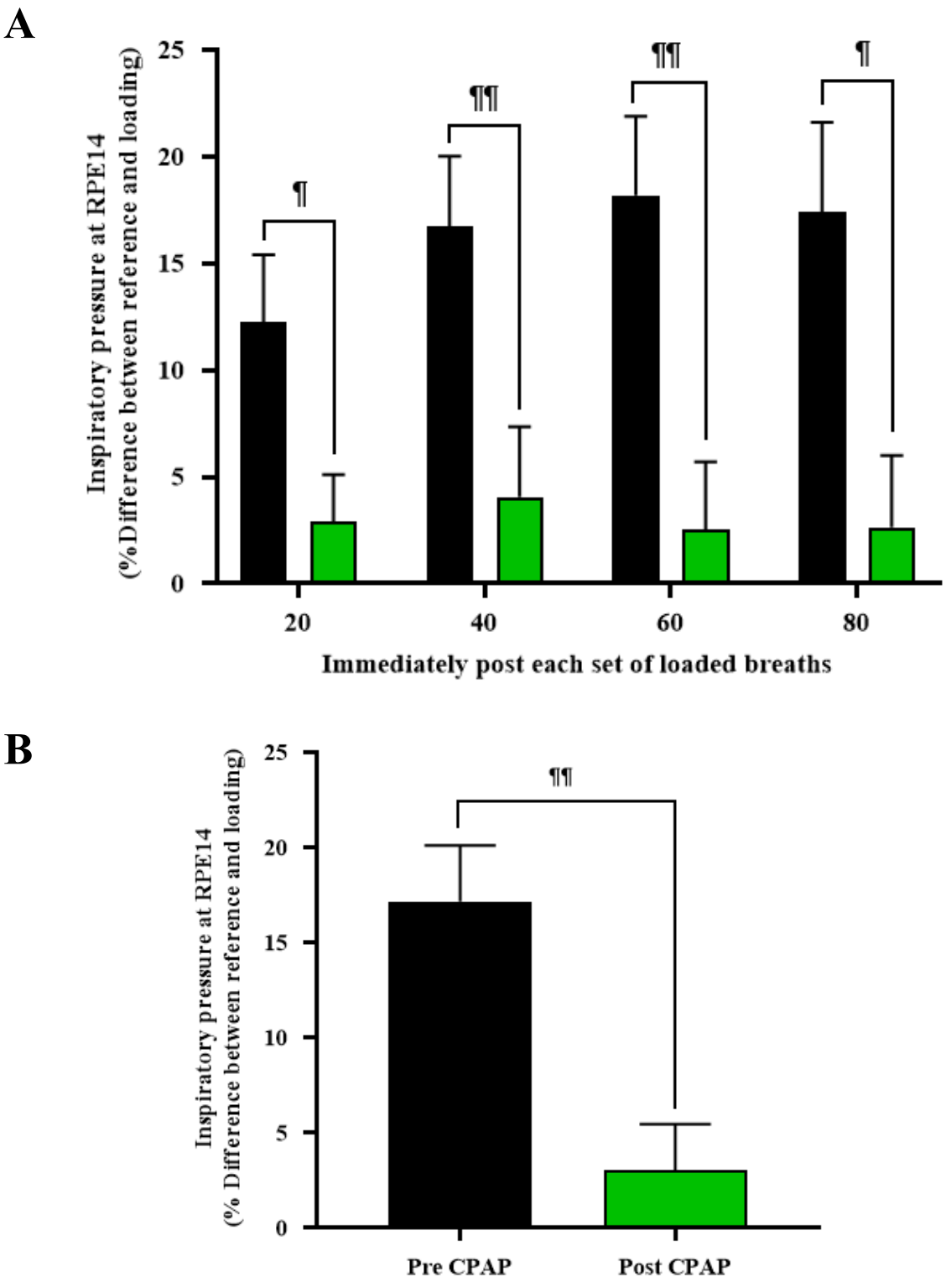

Figure 6.4.10. Inspiratory pressure at RPE of 14 (percentage difference between IP_{14} measured at the reference point and after repeated bouts of loading) **(A)**. Inspiratory pressure at RPE of 14 (mean percentage difference between IP_{14} reference and loading) **(B)**. Data is expressed as Mean ± SE. ■ = Pre-CPAP group (n = 15); ■ = Post-CPAP group (n = 15). ¶ = $p < .05$, ¶¶ = $p < .01$ vs. Pre-CPAP group.

6.5 Discussion

The present study examined the inspiratory pressures and electromyography activity of the inspiratory muscles recorded at RPE14 before and after repeated bouts of loading in healthy Trained and Untrained individuals and patients with newly diagnosed OSA. We further examined the inspiratory pressures generated at RPE14 before and after loading in OSA patients treated with CPAP therapy. The experiment was conducted to determine whether effort is perceived differently in OSA patients compared with Trained and Untrained individuals. The effort sensation paradigm adopted in the current study was not externally controlled (such as holding a weight or tension for a set duration) rather our participants were instructed to match the inspiratory pressures generated at a set effort of 14 before and after repeated bouts of loading at 50% PI_{max}. There are five major outcomes to be drawn from the experiment. First, newly diagnosed OSA patients were shown to generate relative inspiratory pressures that were similar to healthy individuals at the reference point (Figure 6.4.1 A). Second, the normalised Intercostal EMG amplitude was significantly increased during loading in healthy and OSA participants (Figure 6.4.4). Third, repeated bouts of inspiratory loading at 50% PI_{max} resulted in higher relative inspiratory pressures being generated at RPE14 compared with the reference point in OSA patients (Figure 6.4.1 B). Fourth, the normalised Trapezius EMG amplitude was significantly increased at RPE14 following loading in OSA patients compared with healthy individuals (Figure 6.4.6 C). Fifth, the relative inspiratory pressures generated at the reference point was significantly increased after 3-months of CPAP therapy in OSA patients (Figure 6.4.8 A).

We firstly examined the inspiratory pressures generated at a clamped effort of 14, which corresponded to the verbal anchors of 'somewhat hard' to 'hard/heavy' on the Borg scale, to determine the reference point of effort perception during unloaded conditions. Previous research have demonstrated that patients with neuromuscular weakness display a heightened neuromuscular output that is perceived as an increased respiratory effort before completing the loading protocol consisting of maximal voluntary ventilation tests (Bégin et al., 1982; Spinelli et al., 1992). Therefore, we expected patients with newly diagnosed OSA to demonstrate a heightened effort perception of the inspiratory muscles that reflects higher relative pressures being generated at the reference point. Our study demonstrated patients with newly diagnosed OSA generated relative pressures (% PI_{max}) that were similar to those observed in healthy individuals at the reference point ($p > .05$; Figure 6.4.1 A). However, the

inspiratory muscle recruitment at a fixed effort perception was revealed to be heightened in OSA patients compared with healthy individuals. The relative Intercostal EMG amplitude (% RMS) was found to be similar between the groups at the reference point ($p > .05$; Figure 6.4.6 A). Whilst, the normalised Trapezius EMG amplitude (% RMS) was revealed to be significantly increased in OSA patients at the reference point compared with Trained individuals ($p < .05$; Figure 6.4.6 B).

These outcomes suggest that OSA patients utilise a lower assumed capacity of their primary inspiratory muscles (possibly due to weakness) that is compensated by recruiting additional respiratory and accessory muscles to support inspiratory efforts at the reference point (Sharp, Druz, and Kondragunta, 1986). We expected the primary inspiratory muscles to work at a higher percentage of their capacity at the reference point in OSA patients (before CPAP treatment). However, the total motor command sent to the primary respiratory muscles could possibly have been too small at RPE14 to be effective given the weakened respiratory muscles in OSA patients. Consequently, these patients would become too sensitive to breathe against resistances and require higher inspiratory efforts, which could potentially result in respiratory sensations such as breathing discomfort and dyspnoea during wakefulness. However, the advantage of the respiratory system being more sensitive (lower motor command at RPE) in OSA patients would be an earlier respiratory arousal and awakening following an obstructive event during sleep. This concept is further supported by the significant correlation we observed between AHI (OSA severity) and the inspiratory pressures generated at the reference point in OSA patients before CPAP therapy ($r_s = -.567$, $p = .022$). From this finding we can suggest patients with severe OSA generate lower inspiratory pressures at the reference point (before CPAP therapy) due to potentially respiratory muscle weakness and the lower motor command at RPE14.

We then examined the inspiratory pressures generated at the reference point in OSA patients after receiving CPAP treatment. The present study revealed that OSA patients generate significantly higher relative pressures at the reference point following 3 months of CPAP treatment ($p < .05$; Figure 6.4.8 A; Table 6.6) that could be perceived as an increased total motor command sent to the respiratory muscles at RPE14. These findings can be interpreted as the system adjusting to the respiratory muscle weakness by increasing the motor command to the expected level after CPAP therapy. Consequently, the respiratory system may become

less sensitive, however, this would enable OSA patients to produce greater forces with less effort required. Interestingly, the relationship between AHI and inspiratory pressures generated at the reference point was not observed following CPAP treatment ($p > .05$) which provides some evidence to suggest CPAP therapy leads to a normalisation of AHI and possibly changes to the motor command at rest.

The next phase of our investigation was to examine the Intercostal and Trapezius EMG amplitudes during the first and fourth set of the inspiratory muscle loading protocol to determine whether signs of fatigue would be observed. The relative Intercostal EMG amplitude was found to significantly increase during the repeated bouts of loading at 50% PI_{max} in our tested populations ($p < .05$; Figure 6.4.4). Whereas, the relative Trapezius EMG amplitude demonstrated a slight increase with loading in OSA patients ($p < .05$; Table 6.3; Figure 6.4.5). Patients with OSA were shown to utilise a significantly higher proportion of their maximal RMS for both Intercostal and Trapezius muscles during loading compared with Trained individuals ($p < .05$). The findings of the present study suggest that repeated loading at 50% PI_{max} leads to an increased recruitment of the intercostal muscles in all of our tested populations. However, the accessory muscles were also found to be strongly recruited during loading in OSA patients which reflects a protective strategy adopted to prevent excessive fatigue of the primary inspiratory muscles (Roussos et al., 1979; Yokoba et al., 2003). Previous studies have reported a significant increase in the activity of both diaphragm and intercostal muscles during resistive loading which suggests the ribcage muscle may be preferentially fatigued in healthy participants (Hershenson et al., 1989; Ward et al., 1988). Whilst, the chest wall and trunk muscles are shown to contribute to the generation of inspiratory pressures during resistive loading in healthy individuals (Cala, Edyvean, & Engel, 1992; Hawkes et al., 2007). The altered recruitment strategy could also be adopted in order to reduce the level of discomfort, dyspnoea, and stress/strain on the respiratory system during inspiratory loading. Therefore, based upon our outcomes we can infer that repeated loading of the inspiratory muscles at 50% PI_{max} leads to a predominant activation of the intercostal muscles with different levels of assistance received from the accessory muscles in healthy individuals and OSA patients.

The inspiratory pressures and corresponding EMG amplitudes for both Intercostal and Trapezius muscles were then measured at RPE14 after each completed bout of loading. We

expected OSA patients to demonstrate a greater increase in the relative pressures generated and the Intercostal and Trapezius EMG amplitudes (% RMS) at RPE14 to remain constant following loading. Whereas, healthy individuals were expected to show a lesser increase in the relative pressures generated and the respective EMG amplitudes for the Intercostal and Trapezius muscles (% RMS) to remain unchanged at RPE14 following loading. These hypotheses were based upon the concept that loading the respiratory system may produce a higher motor output (at a set RPE) which would result in a desensitisation (loss of sensitivity) to possible obstructions / resistances. Our study revealed the relative pressures generated at RPE14 were found to be increased following repeated bouts of inspiratory loading at 50% PI_{max} in Untrained individuals and OSA patients ($p < .05$; Figure 6.4.1 B). The relative Intercostal EMG amplitude was revealed to be unchanged at RPE14 following loading in all groups ($p > .05$; Figure 6.4.6 C; Table 6.5). However, the normalised Trapezius EMG amplitude was found to be significantly increased at RPE14 following loading in OSA patients and Untrained individuals compared with Trained individuals (Figure 6.4.6 B; $p < .05$).

The present study revealed the inspiratory effort perception was differentially affected in OSA patients compared with healthy Trained and Untrained individuals. Patients with newly diagnosed OSA were shown to utilise a significantly greater percentage of their accessory muscles to supplement the primary respiratory muscles when generating inspiratory pressures at RPE14 following loading (compared with the reference point). These outcomes support an integrated motor output that is based upon the sensory feedback derived from a multitude of afferent proprioceptive receptors (located within the airways, thorax, lungs, and muscles) in addition to a complex central regulation to mediate the sense of respiratory effort (Scano, Innocenti-Bruni, & Stendardi, 2010). The matching of inspiratory effort with the expected level is dictated by the increasing the central (voluntary) motor command to the respiratory muscles (Chen, Eldridge, & Wagner, 1992; Gandevia, 1982; O'Donnell et al., 2007). However, afferent proprioceptive information seems to play a crucial role in continuously updating the central nervous system with regards to the appropriate matching of effort with tension/forces generated by the fatigued respiratory muscles for the prevailing motor drive (Killian et al., 1984; Von Leupoldt & Dahme, 2005; Zechman & Wiley, 1986). This concept is supported by the electromyography activity of the inspiratory and accessory muscles recorded at RPE14 in the present study. We have shown that OSA patients utilise the Trapezius muscles to a significantly greater extent during loading which presumably reflects

the accessory muscle supporting inspiratory efforts when the capacity of the inspiratory muscles are diminished due to weakness/fatigue (Dempsey et al., 2006; Orozco-Levi et al., 1995; Yokoba et al., 2003). The strategy could also be seen as the respiratory system attempting to redistribute the workload / demand of breathing onto the accessory muscles in order to minimise breathing effort and discomfort (Mead et al., 2014). In contrast, Trained individuals predominantly activate the intercostal muscles and to a lesser extent the accessory muscles at RPE14 following loading which suggests the primary inspiratory muscles are mainly responsible for meeting the demands of the respiratory system.

The experiment further demonstrated the relative pressures generated at RPE14 following loading were shown to operate at the same level as the reference point following CPAP therapy ($p > .05$; Figure 6.4.8 A & B). Therefore, the alteration of inspiratory effort is only observed at rest following CPAP treatment in OSA patients. Based upon these findings we can presume there is no change in the total motor command observed following loading in OSA patients treated with CPAP therapy given the functional capacity of the muscles are being utilised to their maximal limits.

The neuromuscular efficiency was further examined to determine the inspiratory pressures generated in relation to the Intercostal and Trapezius EMG amplitudes measured at RPE14 following loading. Our study revealed the neuromuscular efficiency of both Intercostal and Trapezius muscles were shown to increase following loading in Trained individuals compared with Untrained and OSA groups ($p < .05$; Figures 6.4.7 B & C). The gradual increase in the neuromuscular efficiency of the Intercostal and Trapezius muscles observed at RPE14 following loading was potentially a consequence of greater inspiratory forces being produced relative to the EMG activity measured. Whereas, the neuromuscular efficiency was unchanged in Untrained and OSA groups following loading relative to the EMG activity recorded. These results demonstrate lower inspiratory forces were potentially generated by the respiratory system (relative to the muscle activity). These findings provide further evidence that fatigue impairs the inspiratory effort perception at RPE14.

While this study provides new insight into the perceptual response to fatiguing exercise in Trained, Untrained, and OSA groups, several limitations warrant discussion. Firstly, we did not conduct sleep studies in our healthy individuals to exclude the possibility of OSA due to

the limited resources available. However, sleep-related questionnaires were completed and classified healthy individuals as normal according to normative data (i.e. no excessive sleepiness and normal sleep quality) (Buysse et al., 1989; Johns, 1991). Secondly, we found the recruited OSA patients were considerably older and heavier than both Trained and Untrained groups. Future studies should attempt to recruit older and/or overweight participants with no diagnosed OSA to address whether age and weight differences exist.. Thirdly, the forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were found to be considerably lower in OSA patients compared with healthy individuals. It's unclear whether any of the reported differences found in OSA patients may compromise the performance of the respiratory system and consequently influence effort perception. Finally, whilst surface EMG is an advantageous tool for measuring the amplitude of inspiratory muscles during loading in a non-invasive manner, the technique is limited by factors such as; cross-talk from adjacent muscles, cardiac artefacts, skin-electrode contact (e.g. sweating), and thickness of subcutaneous fat (Reaz, Hussain, & Mohd-Yasin, 2006). In order to reduce the influence of these factors we followed standardised positions for electrode placement and the influence of electrocardiography was minimised by recording EMG amplitudes from the right side of the body. We also followed standardised procedures by normalising the EMG amplitudes according to the maximal RMS (% RMS) (Orozco-Levi et al., 1995; Yokoba et al., 2003). By following standardised protocols suggested by previous studies we are confident the reported factors did not influence our results.

In summary, we have shown that newly diagnosed OSA patients generate relative inspiratory pressures that were similar to those observed in Trained and Untrained individuals at the reference point. The normalised Intercostal EMG amplitude was significantly increased during loading in healthy and OSA groups. Repeated bouts of inspiratory loading at 50% PI_{max} resulted in higher relative inspiratory pressures generated at RPE14 in OSA patients compared with Trained and Untrained individuals. The normalised EMG amplitude of the Trapezius muscle was significantly increased at RPE14 following loading in OSA patients compared with Trained individuals. The relative inspiratory pressures generated at the reference point was significantly increased following CPAP therapy in OSA patients. The results of the present study provide further evidence that newly diagnosed OSA patients demonstrate a heightened effort perception and activation of the inspiratory muscles before and after loading. CPAP therapy was shown to upregulate the perception of inspiratory effort at rest.

CHAPTER SEVEN

General Discussion

The final chapter discusses and integrates the main findings presented in the experimental chapters of this thesis. The thesis firstly demonstrated the ventilatory response to hypoxia and hypercapnia in newly diagnosed OSA patients compared with Trained and Untrained individuals. We investigated the contribution of cardiovascular fitness and body characteristics to the potential alterations observed in OSA. Later, we examined the ventilatory response to hypoxia and hypercapnia in OSA patients after receiving 3 months of CPAP treatment. Next, the perception of effort and activity of the quadriceps muscles was assessed before and after loading in Trained, Untrained, and Obstructive Sleep Apnoea groups. Thereafter, the perception of effort and activity of the inspiratory muscles was examined before and after loading in Trained, Untrained, and Obstructive Sleep Apnoea groups. We later examined the perception of effort before and after loading in OSA patients after receiving 3 months of CPAP treatment. Finally, we discuss the applied implications of the research and consider some directions for future research.

7.1 Ventilatory response to hypoxia and hypercapnia and the effects of Continuous Positive Airway Pressure treatment (Chapter 4).

The purpose of the study was to investigate the ventilatory response to hypoxia and hypercapnia during wakefulness in patients with newly diagnosed OSA and healthy individuals (Trained and Untrained groups). We investigated the contribution of cardiovascular fitness and body characteristics to the potential alterations observed in OSA. The ventilatory response to hypoxia and hypercapnia was later re-assessed in OSA patients to determine whether 3 months of CPAP therapy modifies chemosensitivity independent of changes to body characteristics. The current study used techniques reported by Earing et al., (2014) to gain a differentiation of central and peripheral chemosensitivity. The protocol used mixed hyperoxic / hypercapnic (25% O_2 / 6% CO_2) and hypoxic (13% O_2) gases to evaluate the sensitivity of the central and peripheral chemoreceptors. The combined response of central and peripheral chemoreceptors was assessed using the hypoxic / hypercapnic (13% O_2 / 6% CO_2) gas mixture. The ventilatory response to hypoxia and hypercapnia was examined by calculating the change in minute ventilation for each gas mixture from the baseline ventilation.

Patients with newly-diagnosed OSA were shown to have a significantly lower ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2) gas mixtures compared with Trained individuals (all $p <$ .05). Furthermore, Untrained individuals demonstrated a significantly lower ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2) gas mixtures compared with Trained individuals (all $p < .05$; **Figure 4.2)**. Whilst no differences were found in the ventilatory responses to the series of gas mixtures between Untrained and OSA groups. Regression analyses demonstrated that body characteristics such as BMI, FVC, neck and hip circumference explained 17.7 % of the variance in the central response ($p < .05$; **Table 4.4**). It was also found that BMI and VO_{2max} explained 27.6 % of the variance in the peripheral response ($p < .01$). Whilst, body characteristics such as BMI, FVC, and hip circumference explained 25.2 % of the variance in the combined central and peripheral response ($p < .01$). Alterations of the central response to CO_2 were explained by body characteristics (i.e. having a higher BMI, hip and neck circumference leads to a reduced central sensitivity). Whist, the altered peripheral response to O_2 is explained by BMI and cardiovascular fitness (i.e. higher VO_{2max} results in an increased peripheral chemosensitivity). Therefore, body characteristics and cardiovascular fitness explain the differential alterations observed in central and peripheral chemosensitivity.

We further examined possible correlations between the physical characteristics and ventilatory response to each gas mixture. We observed a negative correlation between the ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2) and waist circumference (r_s = -.276, p = .041; **Table 4.2)**. Furthermore, we found positive correlations between the ventilatory response to hypoxia with FEV_1 (r_s = .365, p = .006) and VO_{2max} (r_s = .526, p = .001). Finally, we observed positive correlations between the ventilatory response to hypoxia hypercapnia (13% O_2 / 6% CO_2) with chest circumference (r_s = -.414, p = .002) and FEV_1 (r_s = .435, p = .001). However, the severity of OSA (AHI) was not correlated with the ventilatory response to hypoxia and hypercapnia. These outcomes provide further support to demonstrate that cardiovascular fitness is related to peripheral chemosensitivity and body characteristics is associated with central chemosensitivity.

Our findings in healthy individuals were not consistent with previous studies that have shown an attenuated ventilatory response to hypoxia and/or hypercapnia in endurance-trained

individuals (Byrne-Quinn et al., 1971; Scoggin et al., 1978). The conflicting results may be attributed to methodological differences and inter-individual variability amongst the tested participants. Nevertheless, there is evidence to suggest that physical fitness is related to the hypoxic ventilatory response. Therefore, we can postulate that endurance training leads to changes in the sensitivity of peripheral chemoreceptors to hypoxia. Next, we investigated the effects of 3-months of CPAP therapy on ventilatory responses to hypoxia and hypercapnia in OSA patients. After 3-months of CPAP therapy no significant differences were observed in the ventilatory response to hyperoxia hypercapnia (25% O_2 / 6% CO_2), hypoxia (13% O_2), and hypoxia hypercapnia (13% O_2 / 6% CO_2) gases (all $p > .05$; **Figure 4.3)**. Furthermore, no correlations were observed between the physical characteristics (including AHI) and ventilatory response to hypoxic and hypercapnic gas mixtures **(Table 4.3)**. On the whole, our findings are in line with previous studies that have reported a diminished ventilatory response to hypoxia and/or hypercapnia that remains unchanged following CPAP therapy in OSA patients (Berthon-Jones & Sullivan, 1984; Gold et al., 1993; Osanai et al., 1999). The reduced sensitivity of the central chemoreceptors observed in the present study could be attributed to a leptin deficiency and/or resistance that has been previously linked to animal models of obesity and respiratory disorders such as OHS and OSA (Fitzpatrick, 2002; O'Donnell et al., 2000; Redolfi et al., 2006; Tankersley et al., 1998). However, an adaptation of the central and peripheral chemoreceptor sensitivity may have also occurred after chronic exposure to intermittent hypoxia during sleep in OSA patients. The underlying ventilatory instability was shown to be unchanged following 3-months of CPAP treatment in OSA patients. Furthermore, body characteristics (BMI, FVC, hip circumference) and cardiovascular fitness partially contributed to the alterations observed in central and peripheral chemosensitivity.

7.2 Perception of effort and activity of the skeletal muscles before and after loading (Chapter 5).

The experiment was conducted to determine whether effort was perceived differently before and after skeletal muscle loading in OSA patients compared with Trained and Untrained individuals. We designed an intensity-matched loading protocol to study the skeletal muscle response based upon the novel inspiratory loading protocol reported by Earing et al., (2014). The skeletal muscle loading protocol consisted of five sets of twenty isometric contractions at a resistance equivalent to 50% of participants maximal voluntary capacity (MVC) using a custom-made isometric chair. The effort sensation paradigm was not externally controlled

(such as holding a weight or tension for a set duration) rather our participants were instructed to match the isometric force generated at an RPE of 14 before and after loading **(Appendix V)**. Electromyography recordings were simultaneously measured to examine the activity of the quadriceps (Vastus Medialis and Lateralis muscles) before and after the loading protocol.

Firstly, the maximal strength of the quadriceps muscles was measured at rest. The maximal isometric force (MVC) was found to be significantly lower in OSA patients compared with Trained and Untrained individuals ($p < .05$; **Table 5.1)**. The lower maximal strength of the quadriceps muscles in OSA patients is presumably attributed to their extremely sedentary lifestyles and potentially a consequence of the inflammation they experience during sleep (Anzueto et al., 1994; Barreiro et al., 2007). Participants were then instructed to produce a series of isometric forces at a clamped effort of 14, which corresponded to the verbal anchors of 'somewhat hard' to 'hard/heavy' on the Borg scale, to determine the reference point of effort perception during unloaded conditions. The relative forces produced at the reference point (% MVC) were shown to be significantly higher in newly diagnosed OSA patients compared with Trained and Untrained individuals ($p < .05$; **Figure 5.4.1 A)**. However, the normalized EMG amplitudes for both Vastus Medialis and Lateralis muscles (% RMS) were shown to be similar between the groups at rest ($p > .05$; **Figure 5.4.6 A)**. These findings suggested that OSA patients utilise a greater percentage of their capacity to generate isometric forces at the reference point compared with healthy individuals which indicates an augmented effort sensitivity at rest. Our findings provide compelling evidence that effort is perceived differently for the skeletal muscles at the reference point (before loading) and following loading in OSA patients compared with healthy Trained and Untrained individuals.

Next, we examined the normalised EMG amplitudes during the first and fourth set of the skeletal muscle loading protocol to determine whether signs of fatigue would be observed. The Vastus Medialis and Lateralis EMG amplitudes were found to significantly increase during loading in healthy individuals and OSA groups ($p < .05$; **Figures 5.4.4 & 5.4.5).** Our findings are in line with previous studies that suggest repeated loading of the skeletal muscles at 50% MVC leads to an altered neural strategy (i.e. increased recruitment of the motor units and firing rates) which provides support to imply the locomotor muscles were fatigued in our tested populations (Bigland-Ritchie, Furbush & Woods, 1986; Maton & Gamet, 1989; Smilios et al., 2010).

Participants were then asked to generate an isometric force at RPE14 after each bout of loading. This allowed us to determine whether loading would alter the effort perception and activation of the skeletal muscles with comparison to the reference point. We expected OSA patients to demonstrate a greater loss of isometric force production and the EMG amplitudes to remain constant in response to loading. Whereas, healthy individuals were expected to show a lesser decline in the isometric force production and the respective EMG amplitudes to remain constant / unchanged at RPE 14 following loading. Repeated loading of the quadriceps muscles at 50% MVC was shown to result in a decline of isometric forces being generated at RPE14 in OSA patients compared with the reference point ($p < .05$; **Table 5.2; Figure 5.4.1 A)**. Consequently, the percentage difference between the isometric forces generated at RPE14 was significantly greater in OSA patients compared with Trained individuals ($p < .05$; **Figure 5.4.3)**. In contrast, healthy individuals did not reveal a significant change in the isometric forces generated at RPE14 following loading ($p > .05$). We can therefore conclude that repeated loading of the skeletal muscles at 50% MVC impairs the isometric force production (at a fixed perception of effort) in OSA patients more than healthy individuals. From these findings we can also presume the afferent input from the locomotor muscles was not used to correct/adjust the force loss at RPE14 in OSA patients, rather effort perception was a product of corollary discharge discounting feedback from the periphery.

The quadriceps muscle activity measured at RPE 14 following loading was also differently altered in our tested populations. The outcomes of the isometric experiment were in contrast to our hypotheses for OSA patients. Patients with newly diagnosed OSA demonstrated a significantly increased Vastus Medialis and Lateralis EMG amplitude when generating isometric forces at RPE14 after loading ($p < .05$; Figures 5.4.6 B & C; Table 5.5). Whilst, Trained and Untrained individuals demonstrated minimal change in the Vastus Medialis and Lateralis EMG amplitude at RPE14 following loading ($p > .05$). These results were potentially attributed to an increased recruitment of additional motor units and firing rates that acts to counteract the fatigued quadriceps muscles in OSA patients (Bigland-Ritchie & Woods, 1984). Whereas, healthy Trained and Untrained individuals were shown to respond to loading by adopting the same neural strategy (i.e. muscle recruitment) at RPE14. We further examined the neuromuscular efficiency to determine the isometric force production in relation to the Vastus Medialis and Lateralis EMG amplitudes. The neuromuscular efficiency was shown to decline following loading in all of the tested populations **(Figures 5.4.7 B & C)** which suggests the fatigued muscle fibres were being increasingly recruited following

loading. However, there could be some indication that the decline is attributed to an alteration or failure of the muscle contractile properties (Miller et al., 1987). On the whole, these findings provide further evidence that fatigue hampers the isometric force production at RPE14 irrespective of the increasing EMG amplitudes observed in OSA patients. In summary, the collective results presented in Chapter 5 reveal an impaired perception of effort and heightened skeletal muscle activation before and after loading in OSA patients.

7.3 Perception of effort and activity of the inspiratory muscles before and after loading (Chapter 6).

The experiment was conducted to determine whether effort was perceived differently before and after inspiratory muscle loading in OSA patients compared with Trained and Untrained individuals. The novel inspiratory loading protocol reported by Earing et al., (2014) was performed in the current study. The inspiratory loading protocol consisted of five sets of twenty loaded breaths at a pressure equivalent to 50% of participant's maximal inspiratory pressure (PI_{max}). The effort sensation paradigm was not externally controlled (such as holding a weight or tension for a set duration) rather our participants were instructed to match the inspiratory pressure generated at an RPE of 14 before and after loading **(Appendix V)**. Electromyography recordings were simultaneously measured to examine the activity of the inspiratory muscles (Intercostal and Trapezius) before and after the loading protocol.

Firstly, the maximal strength of the inspiratory muscles was measured at rest. The maximal inspiratory pressure (PI_{max}) was found to be similar between healthy individuals and newly diagnosed OSA patients ($p > .05$; **Table 6.1)**. This could possibly be explained by the structure of the respiratory muscle system (i.e. shape of the ribcage) restricting/capping the inspiratory pressures generated in order to prevent an excessive expansion of the chest wall during maximal inspiratory efforts leading to muscle injury (De Troyer & Boriek, 2011). Following this, participants were instructed to produce a series of inspiratory pressures at an RPE of 14 (% PI_{max}), which corresponded to the verbal anchors of 'somewhat hard' to 'hard/heavy' on the Borg scale, to determine the reference point of effort perception during unloaded conditions. The inspiratory pressure generated at the reference point was revealed to be similar between the groups ($p > .05$; **Figure 6.4.1 A)**. The normalised Intercostal EMG amplitude (% RMS) was shown to be similar between the groups ($p > .05$; **Figure 6.4.6 A)**.

However, the normalised Trapezius EMG amplitude (% RMS) was shown to be significantly higher at the reference point in OSA patients compared with Trained individuals ($p < .05$). These findings demonstrated that OSA patients utilise a lower percentage of their capacity to generate inspiratory pressures with the primary respiratory muscles which is compensated by recruiting additional accessory muscles. We can also presume the respiratory muscles generate lower motor command at RPE14 in OSA patients before CPAP therapy which potentially reflect the muscular adaptations that occur after experiencing years of repeated obstructive events (i.e. loading) during sleep in OSA patients. Following CPAP treatment, OSA patients were shown to produce significantly higher relative pressures at the reference point which most likely reflects an increased motor command generated at RPE14 **(Figure 6.4.8 A; Table 6.6)**. These findings demonstrate compelling evidence that inspiratory effort perception is upregulated at the reference point after receiving CPAP treatment in OSA patients.

Next, we examined the normalised EMG amplitudes during the first and fourth set of the inspiratory loading protocol to determine whether signs of fatigue would be observed. The normalised Intercostal EMG amplitudes were found to be increased during loading in all of our tested populations ($p < .05$) **(Figure 6.4.4)**. Whereas, the normalised Trapezius EMG amplitude only slightly increased in Trained individuals ($p < .05$; **Figure 6.4.5)**. Our findings suggested that repeated loading at 50% PI_{max} leads to an increased recruitment of the inspiratory muscles in our tested populations. However, the accessory muscles were found to be increasingly activated during the first and fourth set of loading in OSA patients and to some extent Untrained individuals which possibly reflects a protective strategy adopted to balance the work of breathing and minimise the fatigue development within these populations (Hershenson et al., 1989; Orozco-Levi et al., 1995; Yokoba et al., 2003).

Participants were then asked to generate an inspiratory pressure at RPE14 after each set of loading. This allowed us to determine whether loading would alter the effort perception and activation of the inspiratory muscles with comparison to reference values. Repeated loading of the inspiratory muscles at 50% PI_{max} resulted in significant higher inspiratory pressures generated at RPE14 for all t tested populations ($p < .05$; **Table 6.2)**. Consequently, the matching of inspiratory pressures at RPE14 were greater in OSA patients compared with Trained individuals ($p < .05$; **Figures 6.4.3 A & B)**. In contrast, healthy individuals produced

a better matching of inspiratory pressures at RPE14. From these findings we can postulate that an integrated motor output was generated based upon the central motor command (motor drive) and the afferent input received from the multitude of lung, muscle, and pressure receptors to correct/adjust the pressure at RPE14 (Scano et al., 2010). There is some supporting evidence which indicates that obese OSA patients have an impaired load compensation (ventilatory effort) to inspiratory loading which influences the mechanical control of ventilation by predisposing these individuals to hypercapnia (Greenberg & Scharf, 1993; Lopata & Onal, 1982). Therefore, based upon these outcomes, we can conclude that repeated loading of the inspiratory muscles at 50% PI_{max} heightens the effort perception of the inspiratory muscles at RPE14 in OSA patients compared with healthy individuals.

The inspiratory muscle activity measured at RPE14 following loading was also differently altered in our tested populations. The outcomes of the inspiratory loading experiment were in contrast to our hypotheses for OSA patients. Newly diagnosed OSA patients were shown to respond to repeated bouts of loading with an increased EMG amplitude of the Trapezius muscle at RPE14 compared with Trained individuals ($p < .05$; **Figure 6.4.6 B)**. Furthermore, Untrained individuals demonstrated a significantly increased Trapezius EMG amplitude at RPE14 following loading compared with Trained individuals ($p < .05$). However, the Intercostal EMG amplitude was shown to be unchanged following loading ($p > .05$; **Figure 6.4.6 C; Table 6.5)**. These findings demonstrated that OSA patients respond to loading by increasingly recruiting the accessory muscles to compensate for the fatigued primary respiratory muscles. The strategy of muscle recruitment potentially reflects the accessory muscles supporting inspiratory efforts when the capacity of the inspiratory muscles are diminished due to weakness/fatigue (Dempsey et al., 2006; Orozco-Levi et al., 1995; Yokoba et al., 2003). Whereas, Trained individuals were primarily reliant upon the intercostal muscles as a power source during inspiratory loading with less assistance received from the accessory muscles.

After inspiratory muscle loading, the neuromuscular efficiency of both Intercostal and Trapezius muscles was significantly increased in response to loading in Trained individuals which indicates an increased force production relative to inspiratory muscle activity ($p < .05$; **Figures 6.4.7 B & C)**. Whereas, the neuromuscular efficiency was unchanged in Untrained and OSA groups following loading relative to the EMG activity recorded. These results

demonstrated lower inspiratory forces were potentially generated by the weakened respiratory muscles in these populations. These findings provide further evidence that fatigue differentially alters the inspiratory effort perception at RPE14 in OSA patients.

The experiment further demonstrated the relative pressures generated at RPE14 following loading were shown to operate at the same level as the reference point following CPAP therapy in OSA patients **(*p* > .05; Figure 6.4.8 A & B)**. Therefore, the alteration of inspiratory effort was only observed at rest (reference point) following CPAP treatment in OSA patients. Based upon these findings we can presume there was no change in the total motor command observed following loading in OSA patients treated with CPAP therapy given the capacity of the muscles were being utilised to their maximal limits. Therefore, the collective results presented in Chapter 6 demonstrate that repeated loading of the inspiratory muscles at 50% PI_{max} heightens the perception of effort and inspiratory muscle activity in OSA patients compared with healthy individuals. CPAP treatment upregulates the inspiratory effort at rest in OSA patients.

7.4 Applied implications

The thesis adds to the existing literature by providing an in-depth investigation into the pathophysiological mechanisms of OSA with future outlooks of effective treatment options for patients. The outcomes of the thesis provide further understanding of the perceptual and muscular differences that are unique to patients with OSA. We have demonstrated that newly diagnosed OSA patients have an attenuated sensitivity of the central and peripheral chemoreceptors (i.e. ventilatory response to hypoxia and hypercapnia) during wakefulness which remains unchanged following 3 months of CPAP therapy. Behavioural strategies that focus upon diet modification, regular physical exercise, and lifestyle changes (e.g. reduce alcohol intake) should be emphasised by clinicians to prevent the weight gain observed in OSA patients treated with CPAP treatment. There is limited knowledge of the long-term effects of CPAP treatment and the associated mechanisms involved the chemical drives in OSA patients. However, it has been postulated the adrenosympathetic response to stress may be improved after 1-year of CPAP treatment in OSA patients due to an attenuated hypoxic stimulation of the respiratory network (Hedner et al., 1995).

We have also revealed that OSA patients demonstrate a heightened perception of effort and recruitment of the respiratory and skeletal muscles before and after loading. In addition, the

thesis highlights the effectiveness of CPAP treatment for upregulating the perception of inspiratory effort at rest. These findings provide support for the recommendation of Inspiratory Muscle Training (IMT) alongside usual CPAP treatment for OSA patients to improve the overall efficiency of the inspiratory muscles (i.e. increase Intercostal muscle activity and decrease the reliance of Accessory muscles). Previous research has demonstrated that CPAP treatment has the ability to reverse structural and functional abnormalities of the genioglossal muscle in OSA patients (Carrera et al., 1999; Deegan, Nolan, and McNicholas, 1996; Strohl and Redline, 1988). Whilst, IMT has the ability to improve overall efficiency of the respiratory muscles (i.e. strength, endurance, and exercise tolerance) and attenuate dyspnoea in healthy and clinical populations (Larson et al., 1988; Lisboa et al., 1997; Lotters et al., 2002; Verges et al., 2009). The findings of this thesis provide evidence for the recommendation of both CPAP treatment and IMT to OSA patients in order to attenuate the frequency of obstructive events, improve the efficiency of the respiratory muscles, and alleviate perceived exertion during activities of daily living. Our findings also support the application of IMT as a future intervention to improve exercise performance by altering exertional dyspnoea and fatigue in physically inactive/sedentary populations (Illi et al., 2012).

7.5 Suggestions for future research

Future research is required to determine whether central and/or peripheral chemosensitivity is improved following long-term CPAP therapy (12 months) in OSA patients using the novel methodology adopted in this thesis. These outcomes would contribute to the limited knowledge of long-term effects of CPAP treatment on the chemical drives in OSA patients. The importance of weight loss for altering the sensitivity of the chemical drives cannot be emphasised enough given the high prevalence of obesity in the OSA population and the significant correlations observed in the current study between central chemosensitivity and anthropometric measures (e.g. BMI). Another direction for future studies would be to incorporate Inspiratory Muscle Training (IMT) alongside traditional CPAP treatment for the personalised management plan of OSA patients. It would be interesting to examine the combined impact of CPAP and IMT on the perceptual response to loading and respiratory/skeletal muscle efficiency in OSA patients and other conditions known to be affected by fatigue (e.g. Cancer, Multiple Sclerosis, COPD). It would also be interesting to examine how weight loss in obese OSA patients influences the inspiratory effort perception and activation of the respiratory muscles following loading. These findings would expand

upon the knowledge of how obesity influences work of breathing and perceptions of effort when the respiratory muscles are loaded.

7.6 Conclusions

The studies reported in the thesis extend upon the previous knowledge regarding the attenuated central and peripheral chemosensitivity in patients with newly diagnosed OSA which is also found to be unchanged following 3 months of CPAP therapy. We have demonstrated for the first time that OSA patients display an impaired perception of effort and heightened muscle recruitment before and after loading of the inspiratory and skeletal muscles. These outcomes indicate OSA patients have a generalised neuromuscular impairment that is observed across a number of respiratory muscles and modified to some extent following CPAP treatment.

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APPENDIX I

Claire Griffith - Mcgeever
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Invitation to take part in a research project.

PROJECT TITLE: Perception of effort and patterns of muscle recruitment during fatiguing inspiratory and limb muscle exercise in patients with Obstructive Sleep Apnoea (OSA).

Dear potential participant,

Would you like to take part in our study?

Having recently been diagnosed with a sleep disordered breathing condition we would like to invite you to take part in a research study examining some of the factors that contribute to the symptoms of Obstructive Sleep Apnoea (OSA). This will help us understand the condition and potentially help us to develop alternative treatment options for this condition.

Please find attached an information sheet, which provides detailed information about the requirements from you as a participant, the potential benefits and risks of taking part.

If after reading this, you would like to take part in the study, please contact me by email or phone using the contact details above. I look forward to hearing from you. If you do have any questions or concerns, please do not hesitate to contact via email or phone.

Kind regards,

Claire Griffith – McGeever BSc, MSc,
PhD Sport, Health, and Exercise Sciences Student

Version 2 **Project Id: 210056** 24/07/2017

Claire Griffith – Mcgeever (Patients GP's address)
School of Sport, Health and Exercise Sciences,
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Dear Dr. (GP name)

I am writing to you with regards to (Patient Name). He has expressed an interest in participating in our study titled **'Perception of effort and patterns of muscle recruitment during fatiguing inspiratory and limb muscle exercise in patients with Obstructive Sleep Apnoea (OSA)'**. The purpose of the study is to examine some of the factors that contribute to the symptoms of Obstructive Sleep Apnoea (OSA). These findings will allow us to understand whether patients with this condition have altered; respiratory muscle function, perception of breathing effort and/or respiratory control. This study has been reviewed by the ethics committee of the School of Sport, Health, and Exercise Sciences (Bangor University) and been approved by the North West Wales NHS Research Ethics Committee (REF).

These study procedures take place at the Pulmonary Function Department, Ysbyty Gwynedd. The study involves the patient having their lung function assessed (maximal inspiratory pressure, FVC and FEV1). The second protocol will assess the patients' perception of inspiratory effort, breathlessness, and respiratory muscle recruitment using electromyography (EMG) during a fatiguing breathing protocol. The protocol consists of five sets of twenty breaths at a resistance that is equivalent to 50% of their maximal inspiratory pressure using a hand-held, resistive breathing device (PowerBreathe KH2). The third protocol will assess patients' perception of limb effort, breathlessness, and muscle recruitment (using EMG) during a fatiguing isometric leg exercise task. The protocol consists of five sets of twenty efforts at a resistance that is equivalent to 50% of their maximal force against an immovable pad that is positioned just below the ankle.

The final test procedure will determine patients' respiratory sensitivity to moderate hypoxia and hypercapnia. The ventilatory response to three breathing mixtures made up of different levels of carbon dioxide and oxygen (25% O_2 / 6% CO_2; 13% O_2 and 13% O_2 / 6% CO_2) is assessed for 3-5 minutes. Throughout the protocol we will monitor patients' heart rate, oxygen saturation, and blood pressure. At set intervals, patients' will be asked to complete a questionnaire to measure their breathlessness.

Please feel free to contact me if you have any questions or require further information.

Kind regards,
Claire Griffith – McGeever BSc, MSc,
PhD Candidate, School of Sport, Health, and Exercise Sciences

APPENDIX II

PARTICIPANT INFORMATION SHEET

(VERSION 5.0 – 07/03/2017)

School of Sport, Health and Exercise Sciences
Bangor University,
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Title of study: Inspiratory effort perception, ventilatory control and awareness in athletes and sedentary individuals.

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Dr Christopher Earing [2]

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[1] School of Sport, Health and Exercise Sciences, Bangor University
[2] Pulmonary Function Department, Ysbyty Gwynedd, Bangor

Invitation to take part

You are being invited to take part in a research study as a healthy physically inactive or endurance trained male or female, aged between 18-45 years old. You will not be eligible to take part in the study if you have any of the following: presence of cardiovascular, respiratory, peripheral vascular or neuromuscular disease, type II diabetes, high blood pressure, sleeping disorder, diagnosed perforated ear drum, history of spontaneous pneumothorax, acute sinusitis, recent trauma to the ribcage, lower limb injury within the past month or current use of medication that affects breathing (i.e. opiate based painkillers). Females must be pre-menopausal and not pregnant. Before you decide to take part it is important for you to understand why the research is being conducted and what is required of you should you agree to be involved. Please take a moment to read through the following information to see if you would be interested in taking part. If you have any questions or would like further information please ask the primary investigator.

What is the purpose of the research?

Breathlessness during physical activity has been shown to reduce adherence to exercise in sedentary individuals, and potentially limit exercise performance in athletic populations. Although the cause is currently unclear, breathlessness may occur due to: an increase in breathing or whole body effort perception; the influence of an increase in waste products in the exercising muscle; fatigue of the respiratory muscles; or as a result of how well the body detects changes in oxygen and carbon dioxide levels. Therefore, the purpose of this study will be to assess breathlessness, effort perception and muscle recruitment during breathing and leg exercise tasks. In addition, we will assess how well the body detects blood oxygen and carbon dioxide levels using a controlled breathing task. The results of this research will help to develop our knowledge of the factors that give rise to breathlessness during physical activity.

Do I have to take part?

This is entirely your decision. If you decide to take part you will be given this information sheet to keep and be asked to read and sign a consent form during the first session. Even if you decide to take part in the study **you are free to withdraw at any time point without giving a reason** and this will not affect your relationship with the School of Sport, Health, and Exercise Sciences or any of the researchers involved. **Any information collected during the study will be treated confidentially.**

Study Procedures

Visit 1 – Health screen

In order to assess your health and eligibility for the study we will take measurements of your height, weight, neck, chest, waist and hip circumference, body composition and resting lung function. Additionally, you will be required to perform a 10-15 minute maximal exercise test on a cycle ergometer that will be used to measure your maximal oxygen uptake (aerobic fitness) and your peak power output.

Visit 2. Fatiguing breathing protocol

The second visit will assess your perception of effort, breathlessness, and muscle recruitment during a fatiguing breathing protocol. We will firstly measure the maximal force that you can produce whilst breathing in using a hand-held, resistive breathing device. We will also assess your muscle recruitment and effort perception while sitting and lying as you breathe against a slight resistance. You will then complete the fatiguing breathing protocol, which consists of five sets of twenty breaths at a resistance that is equivalent to 50% of your maximal force. By making you breathe very hard (i.e. against resistance), we will induce fatigue of the respiratory muscles. Between each set of twenty breaths, we will assess your effort perception, breathlessness, and respiratory muscle activity using a technique called electromyography (EMG). EMG involves having self-adhesive electrodes attached to the skin of your shoulder and ribs in order to measure the electrical activity of your muscles.

Visit 3. Muscle fatiguing task

The third visit will assess your effort perception, breathlessness and muscle activity during a fatiguing leg exercise task. The test will involve a measurement of the maximal force that you can produce with the quadriceps whilst pushing against an immovable pad that is positioned just below the ankle. You will then complete the fatiguing muscle task which consists of five sets of twenty efforts at a resistance that is equivalent to 50% of your maximal force. Between each set of twenty limb contractions, we will assess your effort perception and quadriceps muscle activity using EMG.

Visit 4: Ventilatory response to hypoxia and hypercapnia.

The fourth visit will employ a safe breathing protocol to determine the respiratory sensitivity to moderate hypoxia (lower levels of oxygen) and hypercapnia (higher levels of carbon dioxide). The test involves breathing with three gas mixtures that are made up of different levels of carbon dioxide and oxygen. Throughout the protocol we will monitor your heart rate, blood oxygen saturation, and blood pressure. At set intervals you will be asked to complete a questionnaire to measure your breathlessness.

What are the possible disadvantages and risks of taking part?

- **Time commitment:** You will be required to visit the School of Sport, Health and Exercise Sciences department on four separate occasions amounting to approximately 6 hours (4.5 testing hours).

- **Limited training before the testing sessions:** Your training should be limited to 1 hour of moderate intensity in the 24 hour period before testing sessions.

- **Maximal exercise testing:** Performing maximal aerobic exercise tests has minimal risk of inducing a cardiac event (6 per 10,000 tests) in healthy populations who possess a low-risk for cardiovascular disease. You will experience muscular fatigue during the maximal aerobic exercise test as it requires maximal effort.

- **Fatiguing breathing protocol:** You may experience breathlessness, breathing discomfort, and dizziness during the fatiguing breathing protocol.

- **Inspiratory loading task:** You may experience a slight increase in blood pressure during the protocol, but we will monitor this during the procedure.

- **Fatiguing muscle task:** Performing fatiguing muscle tasks has minimal risk of inducing an injury in healthy populations. You may experience a range of physiological symptoms such as, a general level of discomfort, physical exertion, and delayed onset muscle soreness in response to skeletal muscle fatigue.

- **Ventilatory response to hypoxia and hypercapnia:** During the breathing protocol, you may experience a slight headache, breathlessness, and dizziness as you will be exposed to higher levels of carbon dioxide and lower levels of oxygen for a short duration (3-5 minutes).

What are the possible benefits of taking part?
By participating in the study you will receive comprehensive information regarding health and fitness parameters including: resting blood pressure and heart rate, body composition, resting lung function, and aerobic fitness.

Will my taking part in this research be kept confidential?
Any personal information collected during the study will be kept confidential and anonymised by replacing your names with codes. All data will be stored on password protected computers and locked in filling cabinets. No personal information will be reported should this study be published.

Who is organising and funding the study?
The study is being organised by the named researchers and funded by Bangor University, Coleg Cymraeg Cenedlaethol, and a research grant from BCUHB charitable funds.

Who has reviewed the study?
Before the study commences, ethical approval has been sought from the School of Sport, Health, and Exercise Sciences (SSHES) Committee.

Who do I contact if I have a complaint?
If you have any complaints or comments regarding the study you can address / contact the Head of the School of Sport, Health and Exercise Sciences (Professor Tim Woodman) by phone: 01248 388256, or by email: t.woodman@bangor.ac.uk. Complaints or comments can be made at any period during your study participation.

Thank you very much for taking the time to read this information sheet.

Patient Information Sheet

(VERSION 3 – 24/07/2017)

School of Sport, Health and Exercise Sciences
Bangor University, George Building,
Bangor, Gwynedd, LL57 2PZ

RESEARCH PROJECT: Perception of effort and patterns of muscle recruitment during fatiguing inspiratory and limb muscle exercise in patients with Obstructive Sleep Apnoea (OSA).

Main Investigator

Miss Claire Griffith - McGeever [1]
(PhD Candidate)
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Telephone: +44(0)1248 388254

Supervisory team

Dr Julian Andrew Owen[1] (Lecturer of Exercise Physiology)
(j.owen@bangor.ac.uk)

Dr Hans-Peter Kubis[1] (Senior Lecturer of Physiology)

Dr Christopher Earing[2,3] (Respiratory Physiologist)

Miss Julia Roberts[2,3] (Clinical Physiologist)

Dr Damien McKeon[2,3] (Consultant in Respiratory and General Medicine)

[1] School of Sport, Health and Exercise Sciences, Bangor University
[2] Pulmonary Function Unit, Ysbyty Gwynedd, Bangor
[3] Sleep Unit, Ysbyty Gwynedd, Bangor

Purpose of study:

The purpose of this study is to examine some of the factors that contribute to the symptoms of Obstructive Sleep Apnoea (OSA). These findings will allow us to understand whether patients with this condition have altered function of the muscles required for breathing, the effort they feel when breathing or the way they control breathing.

Do I have to take part?

This is entirely your decision. If you decide to take part, please contact the main investigator, Claire by email or phone or if you are undecided, you can contact her for further information (*details at the front of this information sheet*).

Even if you do decide to take part in the study **you are free to withdraw at any time point without giving a reason** and this will not affect the standard of care you receive from the NHS or your relationship with the School of Sport, Health, and Exercise Sciences, Bangor University or any of the researchers involved.

What will happen if I decide to take part?

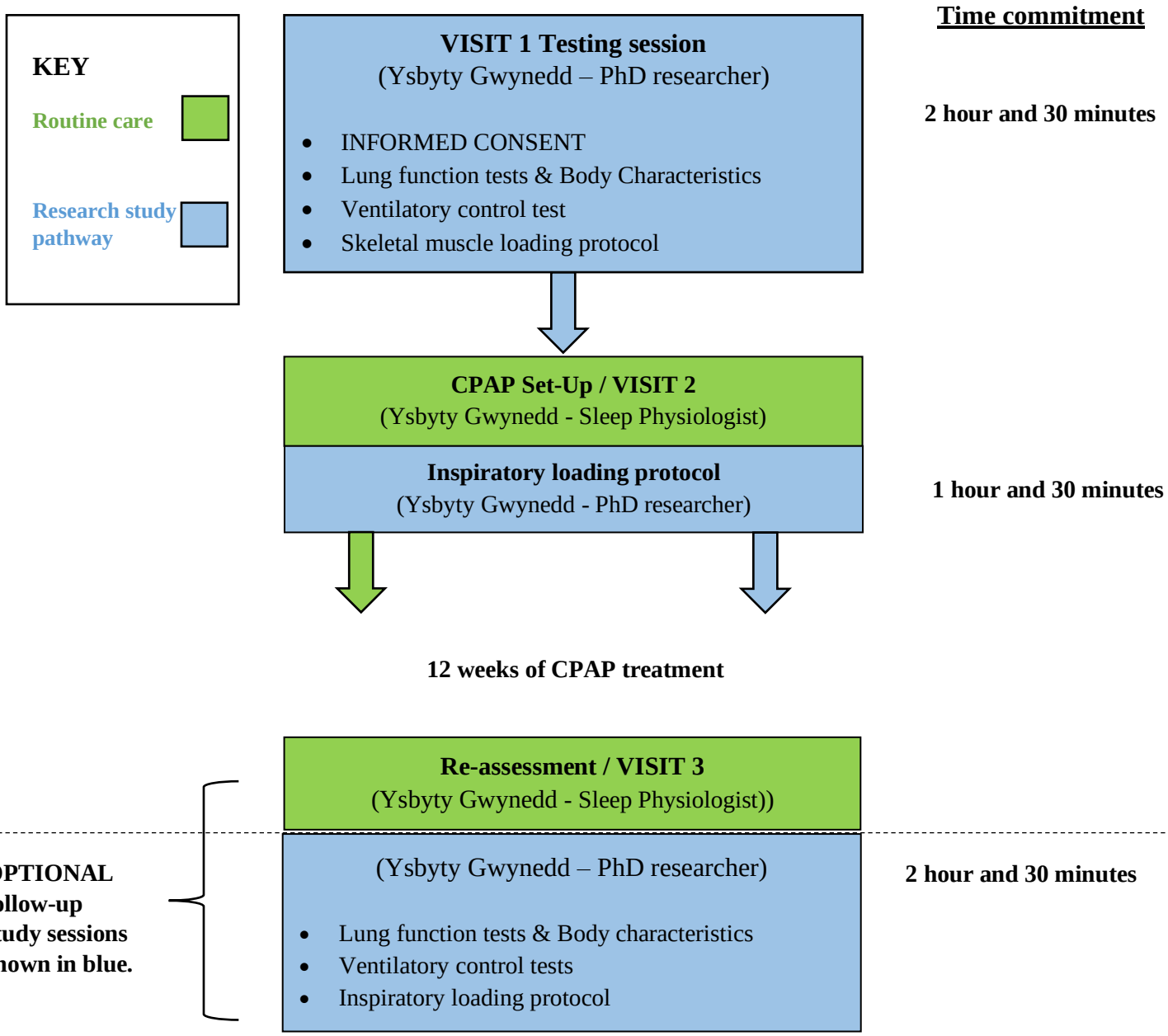
If you agree to take part, we will send your GP a letter to inform them of your interest in taking part in the study and you can discuss this with them if you wish to do so. We will contact you to arrange suitable dates for you to attend Ysbyty Gwynedd to participate in the study (*please see an overview of the visits and additional details in the next few pages*).

How many times will I be assessed?

In total, you will be required to take part in two separate assessments and this should take approximately 4 hours of your time. You will have received a letter from Ysbyty Gwynedd with an appointment for continuous positive airway (CPAP) treatment. Before this appointment, we would require you to visit the hospital for an assessment (*Health Screen, Ventilatory Control Test and Skeletal muscle fatigue task*). When you then attend Ysbyty Gwynedd for your CPAP appointment, we will also ask you to take part in a second assessment (*Inspiratory muscle fatigue task*).

After the final assessment, we will ask if you would like to repeat this process in about 4-8 weeks' time. These additional two assessments will again take approximately 4 hours of your time. However, it is entirely your decision and you do not have to agree to take part in these optional assessments.

OVERVIEW OF ASSESSMENTS



More information about the assessments in this research project

Health screen

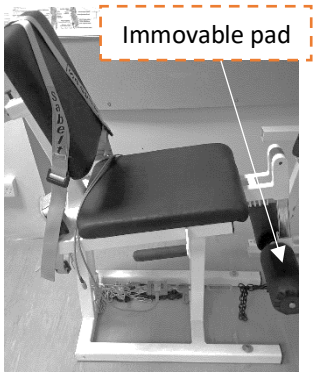
As part of the research study, we will assess aspects of your health such as measurements of your height and weight, and also your neck, chest, waist and hip circumference using a standard tape measure. We will also ask you to stand bare-foot on a weighing scale, which measures your body fat percentage. We will assess your resting lung function with a hand-held portable spirometer device. In addition, we will ask you to complete some additional questionnaires, which will assess your general feelings and emotions, anxiety, health and your awareness of sensations such as breathlessness.

Ventilatory control test.

Immediately after the health screen, we will measure the sensitivity of your breathing control. To do this, you will breathe three different gas mixtures with slightly different amounts of carbon dioxide and oxygen, via a facemask, for a short period of less than 5 minutes. This is a safe breathing protocol and is routinely used in research to assess how sensitively you control your breathing. Throughout the breathing test, we will record your heart rate, using a chest strap, and how much oxygen is in your blood, using a device attached to your finger. We will also record your blood pressure and you will be asked to complete a questionnaire to record your breathlessness after breathing each gas mixture.

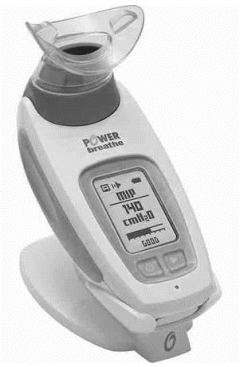
Skeletal muscle fatigue task

Immediately after the Ventilatory control test, we will assess your thigh-muscle (quadriceps) function during a leg exercise task. The test involves pushing against an immovable pad that is positioned just below the ankle whilst you are in a seated position. Using this equipment, we can record the forces produced during each push. At the start of the test, you will push maximally so that we can record your maximum force. After a rest, you will then push at 50% of your maximum force twenty times.



This set of exercises will be repeated a further five times with a rest between each set. In these rest periods, we will ask you to push against the pad and we will record how much effort you feel this requires. At the same time, we will measure you thigh-muscle activity using electromyography (EMG). EMG is a simple and painless technique, which involves placing two self-adhesive electrodes to the skin of your thigh (near the knee joint) in order to record the electrical muscle activity during exercise.

Inspiratory muscle fatigue task



During the following session, we will assess your respiratory muscle function during a breathing exercise task. This test involves breathing-in against a resistance using the device in the picture opposite. Firstly, we will measure the maximal force that you can produce whilst breathing-in. Initially, you will be required to carry out a series of breathing maneuvers whilst both seated and lying down.

You will then complete twenty repetitions at 50% of your maximum effort (in a seated position). This set of exercises will be repeated a further five times with a rest between each set. In these rest periods, we will ask you to breathe in against a slight resistance and we will record how much breathing effort you feel this requires. At the same time, we will measure you breathing-muscle activity using electromyography (EMG). This will involve placing two self-adhesive electrodes, on your shoulder and the other near the ribs.

Are there any special requirements?

If you are physically active, you should limit this to 1 hour of moderate exercise in the 24-hour period before each assessment. Please refrain from eating or consuming caffeine in the 8 hours before the ventilatory control test. You will also need bring loose fitting clothing (e.g. t-shirt, leggings, or shorts) and trainers with you for the skeletal muscle function test.

What are the possible benefits of taking part?

By participating in the study you will receive information regarding your resting blood pressure, resting heart rate, body composition (Body mass index and Body fat percentage), and resting lung function. However, the research team cannot guarantee that you will benefit from taking part in the study. Your participation in this study will also help us improve the knowledge and understanding of this condition.

What are the possible risks of taking part, and how do we reduce the risk?

- **Ventilatory control test & Inspiratory muscle fatigue task:** During these breathing assessments, your blood pressure may rise slightly and you may experience a slight headache, breathlessness, and dizziness. However, any symptoms you experience will be mild and will only last for a short duration (less than 5 minutes). In addition, we will monitor your levels of breathlessness throughout these protocols using a simple scale. During the breathing assessments, we will monitor your blood pressure and during the respiratory control test, we will also monitor your blood oxygen concentration. If either of these near our safe thresholds, we will stop the test.

- **Skeletal muscle fatigue task:** Performing fatiguing muscle tasks has minimal risk of inducing an injury. However, you may experience a range of physiological symptoms such as, a general level of discomfort, physical effort, and muscle soreness in response to the exercise. To reduce the risk of injury and aid recovery we will guide you through a progressive warm-up and cool-down before and after the test.
- **It is important to note that you will also be free to tell the investigator or to raise your hand during any assessment if you feel uncomfortable or you feel you would like to stop.**

Will my taking part in this research be kept confidential?

Any personal information collected during the study will be kept confidential and anonymised by replacing your name with a code. All data will be stored on password protected computers and any paper files will be locked in filling cabinets. No personal information will be reported should this study be published. No individuals (except your direct care team and researchers) will have access to the information and all information will be destroyed after five years.

Feedback on Conduct of Research:

If you have any feedback or comments about your experience as a participant in this study, you can contact Dr Anthony Blanchfield, Chair, SSHES Ethics Committee, School of Sport, Health and Exercise Sciences, Bangor University, Bangor LL57 2PZ. All information is treated in a strictly confidential manner. If you have any further questions or uncertainties, please contact Claire Griffith-Mcgeever by phone: 01248 388254, or by email: pep619:bangor.ac.uk. Comments can be made at any period during your study participation

Thank you very much for taking the time to read this information sheet. Please contact me if you are interested in participating in this research project or if you would like more information.

Miss Claire Griffith - McGeever [1]

Email: pep619@bangor.ac.uk

Telephone: +44(0)1248 388254

APPENDIX III

PARTICIPANT INFORMED CONSENT FORM: HEALTHY SUBJECTS

1	**Title of project**	Inspiratory effort perception, ventilatory control and awareness in athletes and sedentary individuals.
2	**Name and e-mail address(es) of all researcher(s)**	Claire Griffith - Mcgeever - (pep619@bangor.ac.uk) Matthew Gummer – (peu20b@bangor.ac.uk) Jiajung Huang – (peu926@bangor.ac.uk Dr Julian Owen - (j.owen@bangor.ac.uk) Dr Hans-Peter Kubis - (h.kubis@bangor.ac.uk) Dr Christopher Earing - (c.earing@bangor.ac.uk) Dr Damian McKeon - (d.mckeon@bangor.ac.uk)

Please tick boxes or initial

1. I confirm that I have read and understand the Information Sheet (V1 dated 07/03/2017) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. (i) Students:
   I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive or the outcome of my period of study or my standing with my supervisor or with other staff members of the School. ☐

   (ii) General members of the public:
   I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no impact on my relationship with SSHES or any of the researchers involved. ☐

3. I understand that I may register any complaint I might have about this experiment with Professor Tim Woodman, Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

4. I agree to take part in the above study. ☐

Name of Participant _____

Signature _____ Date _____

Name of Person taking consent_____

Signature _____ Date _____

WHEN COMPLETED - ONE COPY TO PARTICIPANT; ONE COPY TO FILE

Version 2 **15/05/17** **Project Id: 210056**

PARTICIPANT INFORMED CONSENT FORM

1	**Title of project**	Perception of effort and patterns of muscle recruitment during fatiguing inspiratory and limb muscle exercise in patients with Obstructive Sleep Apnoea (OSA).
2	**Name and e-mail addresses of all researchers**	Claire Griffith - Mcgeever - (pep619@bangor.ac.uk) Dr Julian Owen - (j.owen@bangor.ac.uk) Dr Hans-Peter Kubis - (h.kubis@bangor.ac.uk) Dr Christopher Earing - (c.earing@bangor.ac.uk) Dr Damian McKeon - (d.mckeon@bangor.ac.uk) Julia Roberts - (Julia.roberts3@wales.nhs.uk)

Please tick boxes

1 I confirm that I have read and understand the Information Sheet (V2 dated 15/05/17) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

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

3 I understand that by consenting I agree for my personal information that is of relevance to the study as highlighted in Study Information Sheet to be shared with the chief investigator (Miss Griffith-Mcgeever) for the purpose of the research study only. ☐

4 I understand that by consenting my General Practitioner will be informed of my decision to take part in this study. ☐

5. I understand that I may register any complaint I might have about this experiment with Professor Tim Woodman, Head of the School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

6. I agree to take part in the above study. ☐

Name of Participant _____

Signature _____ Date _____

Name of Person taking consent_____

Signature _____ Date _____

WHEN COMPLETED - ONE COPY TO PARTICIPANT; ONE COPY TO FILE

APPENDIX IV

Physiology Informed Consent and Medical Health Questionnaire (V 2.0 - 30/03/2017)

D NUMBER:	
NAME:	**D.O.B / AGE:**
ADDRESS:	**GENDER:**
POSTCODE:	**HEIGHT:**
TELEPHONE NO:	**WEIGHT:**
OCCUPATION:	**BMI:**

CURRENT HEALTH

Are you in good health (*If no, please explain*)? ☐ **YES** ☐ **NO**

……

Are you currently taking medication, supplements or vitamins (*If yes, please give particulars*)? ☐ **YES** ☐ **NO**

……..

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months (*If yes, please give particulars*)? ☐ **YES** ☐ **NO**

…….....

Within the past six months have you suffered from a serious illness, injury or accident (*If yes, please give particulars*)? ☐ **YES** ☐ **NO**

……

PERSONAL HISTORY: Currently, or in the past have you experienced any of the following, *(please tick and explain were relevant)*

Fractures/Trauma_____ Rheumatic Disease_____

Headaches _____Shortness of Breath _____ Persistent Infections_____

Asthma _____ Bronchitis _____COPD_____

Heart Disease _____Diabetes _____

Neuromuscular condition _____Hypertension _____

Bone / joint / tendon problems aggravated through exercise_____

Any injury within last month _____

Any other condition affecting lower limb function_____

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to take part in the experimental study if they:

- **Have a fever, cough or cold, or suffer from fainting spells or dizziness;**
- **Have recently suspended training due to a joint, muscle, tendon or ligament injury (past month);**
- **Have a known history of medical disorders, e.g. high blood pressure, heart or lung disease ($FEV_1/FVC < 0.70$ or $FEV_1 \geq 80\%$ predicted (Asthma / COPD).**
- **Have a diagnosis of perforated ear drum (or other middle ear pathology), history of spontaneous pneumothorax, acute sinusitis (until condition resolved) and recent trauma to the rib cage.**
- **Have a recent fracture of the lower extremity (within last 6-months).**
- **Are currently taking anti-diabetic treatment and / or medications known to alter respiratory drive (i.e. opiate based painkillers).**

Patient Signature ……..……. Date: ……

Print name **……**

Researcher Signature …….. Date: ……..…...

Print name **…….…….……..…….**

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month.

Please take the time to read and answer each question by ticking the box that best represents your response.

1. **During the past month**, what time have you usually gone to bed at night? _____

2. **During the past month**, how long has it usually taken you to fall asleep each night (min)?

_____

3. During the past month, what time have you usually gotten up in the morning? _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed) _____

5. During the past month, how often have you had trouble sleeping because you…	**Not during the past month**	**Less than once a week**	**Once or twice a week**	**Three or more times a week**
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				
How often during the past month have you had trouble sleeping because of this?				

	Very good	Fairly good	Fairly bad	Very bad
6. During the past month, how would you rate your sleep quality overall?				
	Not during the past month	**Less than once a week**	**Once or twice a week**	**Three or more times a week**
7. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?				
	Not during the past month	**Less than once a week**	**Once or twice a week**	**Three or more times a week**
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
	No problem at all	**Only a very slight problem**	**Somewhat of a problem**	**A very big problem**
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	No bed partner or room mate	**Partner / roommate in other room**	**Partner in same room but not same bed**	**Partner in same bed**
10. Do you have a bed partner or roommate?				
	Not during the past month	**Less than once a week**	**Once or twice a week**	**Three or more times a week**
If you have a roommate or bed partner, ask him / her how often in the past month you have had:				
a. Loud snoring				
b. Long pauses between breaths while asleep				
c. Legs twitching or jerking while you sleep				
d. Episodes of disorientation or confusion during sleep				
e. Other restlessness while you sleep, please describe:				

Epworth Sleepiness Scale (ESS)

Instructions: The following questions relate to how likely are you to doze off or fall asleep in the following situations; in contrast to just feeling tired. This refers to your usual way of life in recent times (over the past week). Read the **situation in the first column**, select your **response from the second column** and enter that **number in the third column.** Total all of the entries in the third column and enter the total in the last box.

SITUATION	RESPONSES	SCORE
Sitting and reading	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
Watching TV	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
Sitting inactive in a public place (e.g. meeting)	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
As a passenger in a car for an hour without a break	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
Lying down to rest in the afternoon when able	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
Sitting and talking to someone	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
Sitting quietly after a lunch without alcohol	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
In a car while stopped for a few minutes in traffic	0 = Would never doze 1 = Slight chance of dozing 2 = Moderate chance of dozing 3 = High chance of dozing	
TOTAL SCORE		

The Positive and Negative Affect Schedule (PANAS) Watson et al., 1988)

Instructions: This scale consists of a number of words that describe different feelings and emotions. **Read each item and indicate to what extent you have felt like this in the past few hours by recording the number (from the scale below) next to each word.**

Very Slightly or Not at All	A little	Moderately	Quite a Bit	Extremely
1	2	3	4	5

1. Interested		**11. Irritable**	
2. Distressed		**12. Alert**	
3. Excited		**13. Ashamed**	
4. Upset		**14. Inspired**	
5. Strong		**15. Nervous**	
6. Guilty		**16. Determined**	
7. Scared		**17. Attentive**	
8. Hostile		**18. Jittery**	
9. Enthusiastic		**19. Active**	
10. Proud		**20. Afraid**	

Watson, D., Clark, L. A., & Tellegan, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. Journal of Personality and Social Psychology, 54(6), 1063–1070.

Anxiety Sensitivity Index – 3

Instructions: Please **tick the box that best corresponds to how much you agree with each item**. If any items concern something that you have never experienced (e.g., fainting in public) answer on the basis of how you think you might feel if you had such an experience. Otherwise, answer all items on the basis of your own experience. **Tick only one box for each item and please answer all items.**

	Very Little	A Little	Some	Much	Very Much
1. It is important for me not to appear nervous					
2. When I cannot keep my mind on a task, I worry that I might be going crazy.					
It scares me when my heart beats rapidly.					
1. When my stomach is upset, I worry that I might be seriously ill.					
2. It scares me when I am unable to keep my mind on a task.					
3. When I tremble in the presence of others, I fear what people might think of me.					
4. When my chest feels tight, I get scared that I won't be able to breathe properly.					
5. When I feel pain in my chest, I worry that I am going to have a heart attack					
6. I worry that other people will notice my anxiety.					
7. When I feel "spacey" or spaced out I worry that I may be mentally ill.					
8. It scares me when I blush in front of people.					
9. When I notice my heart skipping a beat, I worry that there is something seriously wrong with me.					
10. When I begin to sweat in a social situation, I fear people will think negatively of me.					
11. When my thoughts seem to speed up, I worry that I might be going crazy.					
12. When my throat feels tight, I worry that I could choke to death.					
13. When I have trouble thinking clearly, I worry that there is something wrong with me.					
14. I think it would be horrible for me to faint in public.					
15. When my mind goes blank, I worry there is something terribly wrong with me.					

Taylor, S., Zvolensky, M.J., Cox, B.J., Deacon, B., Heimberg, R.G., Ledley, D.R., Abramowitz, J.S., Holaway, R.M., Sandin, B., Stewart, S.H. and Coles, M., 2007. Robust dimensions of anxiety sensitivity: development and initial validation of the Anxiety Sensitivity Index-3. Psychological assessment, 19(2), p.176.

Multidimensional Assessment of Interoceptive Awareness (Mehling et al. 2012)

Instructions: The following questions relate to your interoceptive body awareness. Below you will find a list of statements, Please indicate how often the statements apply to you generally in daily life.

Circle one number on each line	Never					Always
1. When I am tense I notice where the tension is located in my body	**0**	**1**	**2**	**3**	**4**	**5**
2. I notice when I am uncomfortable in my body	**0**	**1**	**2**	**3**	**4**	**5**
3. I notice where in my body I am comfortable.	**0**	**1**	**2**	**3**	**4**	**5**
4. I notice changes in my breathing e.g. whether it slows down or speeds up.	**0**	**1**	**2**	**3**	**4**	**5**
5. I do not notice (I ignore) physical tension or discomfort until they become more severe	**0**	**1**	**2**	**3**	**4**	**5**
6. I distract myself from sensations of discomfort.	**0**	**1**	**2**	**3**	**4**	**5**
7. When I feel pain or discomfort, I try to power through it.	**0**	**1**	**2**	**3**	**4**	**5**
8. When I feel physical pain, I become upset	**0**	**1**	**2**	**3**	**4**	**5**
9. I start to worry that something is wrong if I feel any discomfort.	**0**	**1**	**2**	**3**	**4**	**5**
10. I can notice an unpleasant body sensation without worrying about it	**0**	**1**	**2**	**3**	**4**	**5**
11. I can pay attention to my breath without being distracted by things happening around me	**0**	**1**	**2**	**3**	**4**	**5**
12. I can maintain awareness of my inner bodily sensations even when there is a lot going on around me	**0**	**1**	**2**	**3**	**4**	**5**
13. When I am in conversation with someone, I can pay attention to my posture.	**0**	**1**	**2**	**3**	**4**	**5**
14. I can return awareness to my body if I am distracted.	**0**	**1**	**2**	**3**	**4**	**5**
15. I can refocus my attention from thinking to sensing my body.	**0**	**1**	**2**	**3**	**4**	**5**
16. I can maintain awareness of my whole body even when a part of me is in pain or discomfort	**0**	**1**	**2**	**3**	**4**	**5**
17. I am able to consciously focus on my body as a whole.	**0**	**1**	**2**	**3**	**4**	**5**
18. I notice how my body changes when I am angry.	**0**	**1**	**2**	**3**	**4**	**5**
19. When something is wrong in my life I can feel it in my body.	**0**	**1**	**2**	**3**	**4**	**5**
20. I notice that my body feels different after a peaceful experience.	**0**	**1**	**2**	**3**	**4**	**5**
21. I notice that my breathing becomes free and easy when I feel comfortable	**0**	**1**	**2**	**3**	**4**	**5**
22. I notice how my body changes when I feel happy / joyful	**0**	**1**	**2**	**3**	**4**	**5**
23. When I feel overwhelmed I can find a calm place inside	**0**	**1**	**2**	**3**	**4**	**5**
24. When I bring awareness to my body I feel a sense of calm	**0**	**1**	**2**	**3**	**4**	**5**

25. I can use my breath to reduce tension	**0**	**1**	**2**	**3**	**4**	**5**
26. When I am caught up in thoughts, I can calm my mind by focusing on my body / breathing	**0**	**1**	**2**	**3**	**4**	**5**
27. I listen for information from my body about my emotional state.	**0**	**1**	**2**	**3**	**4**	**5**
28. When I am upset, I take time to explore how my body feels.	**0**	**1**	**2**	**3**	**4**	**5**
29. I listen to my body to inform me about what to do.	**0**	**1**	**2**	**3**	**4**	**5**
30. I am at home in my body	**0**	**1**	**2**	**3**	**4**	**5**
31. I feel my body is a safe place.	**0**	**1**	**2**	**3**	**4**	**5**
32. I trust my body sensations.	**0**	**1**	**2**	**3**	**4**	**5**

APPENDIX V

THE BORG RATING OF PERCEIVED EXERTION SCALE

6	**No Exertion at all**
7	**Extremely Light**
8	
9	**Very Light**
10	
11	**Light**
12	
13	**Somewhat Hard**
14	⟵ **IF_{14} or IP_{14}**
15	**Hard (Heavy)**
16	
17	**Very Hard**
18	
19	**Extremely Hard**
20	**Maximal exertion**

Borg RPE scale (1970, 1985, 1984, 1998)