THE LINK BETWEEN AGEING AND SLEEP DISRUPTION AND THEIR IMPACT ON CARDIORESPIRATORY CONTROL

By

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ABSTRACT

Research has suggested that a reduction in respiratory chemosensitivity with age could be linked with a greater occurrence of sleep disordered breathing (SDB) in elders. However, it remains unclear whether this reduction is mediated by the central or peripheral chemoreceptors. Study 1 used a novel approach with a multi frequency binary sequence test to demonstrate a reduction in central chemosensitivity with age. Ageing is also associated with a fragmentation of sleep and sleep loss. Studies 2 and 3 demonstrated attenuations in central chemosensitivity following three consecutive nights of sleep loss and a night of sleep fragmentation, respectively. These alterations in chemosensitivity were similar in nature to those shown in study 1. These comparable observations and a possible mutual mechanism for them, led to the novel conclusion that age associated sleep disruption may contribute to the changes in chemosensitivity seen in older individuals. These findings may have a bearing on the development of SDB in the older generation. Lastly, pulmonary hypertension can occur in tangent with SDB and research has suggested that its occurrence is greater in older individuals suffering from SDB. Study 4 demonstrates a possible mechanism for this association, in a greater hypoxic pulmonary vasoconstriction response in older individuals.

To my parents,

for their constant support and belief.

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Abbreviations

$[H^+]$ – Hydrogen ion

AHI – Apnoea-hypopnoea index

AHVR test – Acute hypoxic ventilatory response test

BMI – Body mass index

CBF – Cerebral blood flow

CBF_v – Cerebral blood flow velocity

COPD – Chronic obstructive pulmonary disease

CPAP – Continuous positive airway pressure

CSA – Central sleep apnoea

DEF – Dynamic end tidal forcing

ECG - Electrocardiography

EEG - Electroencephalography

EMG - Electromyography

EOG - Electrooculography

FMD – Flow mediated dilation

GABA - Gamma-aminobutyric acid

HCVR – Hypercapnic ventilatory response

HIF-1 – Hypoxic inducible factor

HPV – Hypoxic pulmonary vasoconstriction

HVR – Hypoxic ventilatory response

MFBS – Multi-frequency binary sequence

NREM – none Rapid eye movement

NTS - Nucleus of the Solitary Tract

OSA – Obstructive sleep apnoea

$PaCO_2$ - Arterial partial pressure of carbon dioxide

PaO_2 - Arterial partial pressure of oxygen

PCO_2 - Partial pressure of carbon dioxide

P_{crit} – Critical pressure

PET scan - Positron emission tomography

$P_{ET}CO_2$ – End tidal partial pressure of carbon dioxide

$P_{ET}O_2$ – End tidal partial pressure of oxygen

PO_2 - Partial pressure of oxygen

Pre-BötC - Pre-Bötzinger complex

$\dot{Q}$ - Cardiac output

RAR – Rapidly adapting receptors

REM – Rapid eye movement

ROS – Reactive oxygen species

RTN - Retrotrapezoid nucleus

RVLM - Rostral ventrolateral medulla

SaO_2 – Oxygen saturation

SAR – Slowly adapting receptors

SDB – Sleep disordered breathing

SPAP – Systolic pulmonary artery pressure

SWS – Slow wave sleep

TCD – Transcranial Doppler Ultrasound

VRG - Ventral respiratory group

CHAPTER 1.

INTRODUCTION

1.1 Respiratory Control

Human breathing can be controlled by voluntary or involuntary input. Voluntary input describes the behavioural control humans have over the ventilatory pattern. We can voluntarily increase or decrease our breathing rate inducing a hyperventilatory or hypoventilatory response, respectively. We are also able to pause ventilation to allow us to talk, eat, play musical instruments and also breath-hold for prolonged periods. All of the above are termed volitional breathing. Observations from positron emission tomography (PET scan) during volitional breathing show that the voluntary control of breathing arises from the higher cortical regions of the brain (Colebatch *et al.*, 1991; Fink *et al.*, 1996). One particular region of the brain that is used in volitional breathing is the higher motor cortex. Evidence for this comes from a more direct approach, through transcranial magnetic stimulation, showing that the higher motor cortex regions are influential in diaphragm contraction (Maskill *et al.*, 1991). Despite the voluntary control humans appear to have over ventilation, involuntary pathways of respiratory control eventually take over, causing us to resume normal spontaneous ventilation. The breakpoint of breath-holding is an example of the involuntary pathways taking control (Parkes, 2006). Additional evidence of the intervention by involuntary aspects of respiratory control is in the disruption caused when a hypercapnic stimulus is administered during voluntarily controlled rhythmic breathing (Corfield *et al.*, 1999).

The involuntary control of respiration arises from the generation of a respiratory rhythm by neurons located in a region of the medulla oblongata called the ventral respiratory column (Alheid & McCrimmon, 2008; Feldman *et al.*, 2013). One particular region that is suggested

as key to the generation of respiratory rhythm is the pre-Bötzinger complex (Pre-BötC). Research using selective destruction of Pre-BötC neurons with toxins, resulting in the progressive deterioration of the breathing pattern lends weight to this hypothesis (Gray *et al.*, 2001). However, because of the progressive, sometimes across a number of days, deterioration of the breathing pattern following Pre-BötC neuron inhibition and the survival of the animal for more than a week, it could be suggested, although unlikely, that secondary elements could be involved in breathing rhythm generation. However, the same research group has more recently used ligand transfection to cause an immediate inhibition of specific Pre-BötC neurons in rats (Tan *et al.*, 2008). Following transfection a subsequent and immediate termination of ventilation in these animals occurs. This evidence has confirmed the Pre-BötC as the origin of the respiratory pattern generation involved in inspiration. The pattern is described as an inspiratory pattern because of the passive nature of expiration at rest brought about by the elastic re-coil of the lungs causing a return to their original positions. However, during times of increased metabolic rate, for example during exercise, an active expiration occurs. The active expiration is controlled by the recruitment of abdominal and internal intercostals muscles (Aliverti *et al.*, 1997). Due to this, a second site of respiratory pattern generation was sought. This second site of respiratory rhythm control was shown to be the retrotrapezoid nucleus (RTN) and was responsible for this state dependent expiratory rhythm (Janczewski & Feldman, 2006).

Both these intrinsic regions of rhythmogenesis project to separate regions of the ventral respiratory group (VRG). From these regions efferent motor neurons innervate the respiratory muscles involved in inspiration and expiration. The Pre-BötC projects to the rostral VRG that is itself projected to the phrenic nucleus. The phrenic nucleus relays

efferent information via the phrenic nerve to the inspiratory muscles; the diaphragm and external intercostals (Alheid & McCrimmon, 2008) to bring about inspiration. When active expiration is required neural information for the RTN is projected to the caudal VRG innervating the expiratory muscles, abdominals and internal intercostals, via bulbospinal neurons (Alheid & McCrimmon, 2008). Both the Pre-BötC and RTN also have important projections to upper airway muscles important for the patency of the upper airway; tongue and larynx (Jordan & White, 2008). Modulation of the respiratory patterns is necessary to insure adequate control of these efferent pathways to the respiratory and upper airway muscles.

1.1.1 Modulators of Respiratory Rhythm

While respiratory rhythm is projected out to the respiratory muscles a number of neural feedback and feed-forward mechanisms relay afferent activity to the respiratory pattern generators to modulate the overall ventilatory output. Such influences can arise from suprapontine centres of the brain (Shea, 1996; Horn & Waldrop, 1998). The hypothalamus is one particular area involved in the suprapontine effect on respiratory control. An example of such higher cortical influence is the hyperpnoea that occurs in rabbits when the hypothalamus is stimulated (Duan *et al.*, 1996). This response is said to be characteristic of the 'flight or fight' response.

Hypothalamic sensory input regarding core temperature also has an effect on ventilation, whereby hyperthermia results in an augmentation in ventilation (Mortola & Maskrey, 2011; Zila & Calkovska, 2011).

An additional example of modulation by suprapontine regions is the increased phrenic nerve activity observed during cortically stimulated locomotion in paralyzed cats (Eldridge *et al.*, 1981); an observation that could be perceived as evidence of a central command contribution to the control of breathing during exercise (Forster *et al.*, 2011).

Neural information related to arousal and emotional state originating from the hypothalamus has an effect on ventilatory output (Shea, 1996). For more information on the effect of arousal state on respiratory control see section 1.2.3.

In addition to cortical modulation, afferent input from muscles, lungs and airways innervate at the brainstem to influence respiratory rhythm. Examples of such reflex influences on respiratory output include lung and airway reflexes. Three main categories of receptors are involved in lung and airway reflexes, slowly adapting (SAR) and rapidly adapting (RAR) stretch receptors and bronchopulmonary C fibers (Kubin *et al.*, 2006). SARs within the lung are stimulated during inflation to inhibit inspiratory drive, protecting against over inflation of the lungs and during deflation that leads to the initiation of inspiratory activity, this is termed the Hering-Breuer reflex (Widdicombe, 2006). RARs are involved in an additional reflex termed the cough reflex. RARs act as irritant receptors that detect foreign bodies such as noxious gas, inhaled dust, cold air, etc and in response they stimulate a large diaphragmatic inhalation and subsequent active expiration or cough in an attempt to expel the foreign elements (Kubin *et al.*, 2006). Bronchopulmonary C fibers respond to a number of different stimuli including chemical stimuli, temperature and mechanical stretch (Kubin *et al.*, 2006) and have similar effect on respiratory control as both SAR and RAR (Lee & Pisarri, 2001).

Lastly, an important afferent feedback loop that also modulates the respiratory pattern is present in the form of the chemical feedback loop. The chemical control of breathing is vital in maintaining the homeostasis of the human body by keeping the arterial partial pressures of arterial carbon dioxide and oxygen ($PaCO_2$ and PaO_2) at desired levels through the alteration of ventilation. Specific chemical receptors called chemoreceptors detect changes in PCO_2 and PO_2 and feedback to the ventilatory respiratory column. There are two groups of respiratory chemoreceptors, the peripheral and the central chemoreceptors, see sections 1.1.2 and 1.1.3 respectively. It is this chemical control of respiration that is the particular area of interest in this thesis.

An augmentation in $PaCO_2$ results in a substantial increase in minute ventilation, or a hypercapnic ventilatory response (HCVR), in order to expel the excessive CO_2. Whereas reductions in PaO_2 result in an immediate increase in ventilation or a hypoxic ventilatory response (HVR), in order to maintain O_2 homeostasis. High levels of PaO_2 (hyperoxia) will result in the opposite effect, a reduction in ventilation in order to return PaO_2 to normal levels. However, during normal $PaCO_2$ levels (normocapnia) PaO_2 must fall to very low levels (≈65 mmHg) before ventilation is finally stimulated. In contrast, $PaCO_2$ need only deviate by 2-3 mmHg to stimulate a HCVR (Haldane & Priestley, 1905). The reason for the differences in HCVR and HVR thresholds are twofold. Firstly, the relationship between PO_2 and haemoglobin O_2 saturation (O_2 saturation curve) is such that mild reductions in atmospheric PO_2 have little effect on the affinity of haemoglobin to O_2. Secondly, the regulation of $PaCO_2$ plays a key role in the maintenance of acid-base homeostasis. A change in the acid-base balance can affect numerous physiological functions in the human body such as important

enzymatic chemical reactions. Therefore, it is imperative the $PaCO_2$ and thus the acid base balance are maintained at homeostatic levels.

1.1.2 Peripheral Chemoreceptors

The peripheral chemoreceptors were first discovered in the 1930's situated within the carotid body at the bifurcation of the carotid artery (Heymans & Bouckaert, 1930). Further research demonstrated that the carotid bodies are integral in sensing PaO_2 and lead to an immediate augmentation in ventilation once PaO_2 has reached levels within the proximity of 60 mmHg. The unique role of the carotid bodies to sense O_2 and stimulate ventilation is evident when the carotid body is removed or denervated and the subsequent ablation of HVR (Chalmers *et al.*, 1967; Bouverot *et al.*, 1973). Additional research also discovered that the carotid bodies were sensitive to changes in $PaCO_2$ and changes in pH (Gray, 1968; Buckler *et al.*, 1991). The ability of the carotid bodies to 'taste' the arterial blood means that they are very quickly stimulated by alterations in $PaCO_2$ and PO_2. This in part led to the suggestion that the CO_2 sensitive carotid bodies are responsible for an initially fast ventilatory response characteristic of the HCVR (Band *et al.*, 1970; Gelfand & Lambertsen, 1973). However, despite the initial increase in ventilation during hypercapnic exposure the contribution of the peripheral chemoreceptors to the overall HCVR is said to be 20 – 40% (Heeringa *et al.*, 1979; Pedersen *et al.*, 1999b). A more substantial contribution to the HCVR occurs after 60 seconds and contributes approximately 60 – 80% of the total HCVR (Bellville *et al.*, 1979; Pedersen *et al.*, 1999b). It is the central chemoreceptors that are responsible for the majority of the steady state HCVR.

1.1.3 Central Chemoreceptors

The existence of central chemoreceptive regions within the brain was first proposed following observed ventilatory hyperpnoea during brain perfusion of acidic cerebrospinal fluid (Leusen, 1953). An area of particular attention for central chemoreception was identified as the ventral medullary surface (Mitchell *et al.*, 1963). It was later evident that the concentration of hydrogen ions ($[H^+]$) and PCO_2 within the brain interstitial fluid were the source of stimulus for the central chemoreceptors (Fencl *et al.*, 1966).

Hypoventilation, increased metabolism and increased inspired PCO_2 will increase $PaCO_2$ via pressure gradients between the tissue and the blood. The PCO_2 within the interstitial fluid will increase due to either CO_2 crossing the blood brain barrier or through greater CO_2 production within the brain. Once CO_2 has accumulated within the interstitial fluid it is ionized via the carbonate buffer system, see equation 1 below. Through mass action $[H^+]$ increases and the central chemoreceptors detect the decreased pH, resulting in a stimulation of ventilation. Conversely, a reduction in interstitial PCO_2, via hyperventilation or reduced CO_2 production, lowers the $[H^+]$ and suppresses ventilation.

$$CO_2 + H_2O \quad \leftrightarrow \quad H_2CO_3 \quad \leftrightarrow \quad HCO_3^- + H^+ \qquad \textbf{(1)}$$

Where H_2CO_3 is carbonic acid and its formation is catalysed by carbonic anhydrase. The formation of bicarbonate (HCO_3^-) occurs because of proton dissociation.

With this understanding of H^+ influence on central chemoreceptors a number of techniques have been adopted to cause focal acidosis within the brain stem in the search of the specific sites of central chemoreception. Particular central chemoreceptive areas discovered using such techniques include the RTN (Li *et al.*, 1999), the rostral and caudal medullary raphe

(Nattie & Li, 2001; Hodges *et al.*, 2004), the nucleus of the solitary tract (NTS) (Nattie & Li, 2002), the Pre-BötC (Krause *et al.*, 2009), and the locus coeruleus (Biancardi *et al.*, 2008). The more rostral hypothalamic orexin neurons have also been shown to be chemoreceptive (Williams *et al.*, 2007). However, there is still debate as to whether all of these proposed sites of chemoreception are in fact respiratory chemoreceptive sites. In a recent review by Guyenet *et al.* (2013), the authors highlighted the integral importance of in vivo research in comparison to in vitro methods, such as focal acidification of brain slices. While in vitro methods may provide integral information about the properties of specific neurons, the nature of these methods compromises the entire network homeostasis and the projection of the indentified chemoreceptive neurons to the respiratory centres of the brain is assumed. Due to these weaknesses the observations in an in vitro preparation may not be replicated by in vivo studies. One example of this comparison between in vitro and in vivo results, put forth by Guyenet and colleagues, is the activation of the locus coeruleus in vitro with focal acidification (Hartzler *et al.*, 2008) and the minor respiratory response reported following the activation of locus coeruleus neurons in vivo (Elam *et al.*, 1981).

Guyenet prescribed that current evidence demonstrates the RTN region to be the strongest candidate for the site of central respiratory chemoreception. In vitro evidence shows activation of these neurons with CO_2 (Sato *et al.*, 1992; Teppema *et al.*, 1994), these observations are supported by data from Guyenet's group showing in vivo activation of RTN neurons by CO_2 (Guyenet *et al.*, 2005) and using a more modern technique of optogentics the same group showed the activation of RTN regions in vivo resulted in the marked stimulation of breathing (Abbott *et al.*, 2009). Additional evidence shows the reduction of respiratory chemoreflex responses following selective ablation of the RTN region in vivo

(Takakura *et al.*, 2008) and the total abolishment the chemoreflex in selective RTN knockout mice (Ramanantsoa *et al.*, 2011). These observations, coupled with those of the stimulation studies, are strong evidence for the chemoreceptive properties of the RTN and the possibility of it being the sole central chemoreceptive region.

However, additional in vivo evidence has also shown a markedly reduced respiratory chemoreflex following the ablation of additional neurons, such as the locus coeruleus and orexin neurons (Nattie, 2011). Orexin neurons have also been recently suggested to contribute to the HCVR in an arousal state dependent manner, whereby they promote respiratory drive during the wakefulness but not during sleep (Nattie & Li, 2010). It is argued that the orexin neurons may play a facilitative role for chemosensitive regions during wakefulness, instead of holding intrinsic chemosensitive abilities in detecting changes in pH/CO_2. The topic of central chemoreceptive regions is still widely debated and further research is warranted in the identification of central chemoreceptive sites and their roles in the HCVR response.

The prime role of the central chemoreceptive sites is ventilatory modulation to control $PaCO_2$ and brain pH. In addition to this role it has been shown that the possibly chemosensitive raphe neurons and the chemosensitive region of the locus coeruleus have innervations to airway related vagal neurons and the hypoglossal nucleus, respectively. Stimulation of these central chemosensitive regions can affect the tone of airway muscles directly affect airway calibre (Kc & Martin, 2010). These observations show that central chemoreceptors are also important in the modulation of upper airway tone.

1.1.4 Influence of Cerebral Blood Flow on Central Chemoreception

The mass balance equation for CO_2 within the brain was devised by Read and Leigh (1967) and Berkenbosch *et al.* (1989). The equation states that tissue PCO_2 is determined by, the metabolism of the brain, $PaCO_2$, blood flow density, the slope of the CO_2 dissociation curve, the Haldane principle and the parameter that locates brain tissue PCO_2 between $PaCO_2$ and venous PCO_2. The sensitivity of the vascular response to changes in blood gas levels can directly influence the blood flow density and the tissue-to-arterial PCO_2 gradient, thus effecting the brain tissue PCO_2 (Ainslie & Duffin, 2009). The indirect effect that the cerebrovascular bed has on brain pH serves as a protective mechanism against excessive brain acidity or alkalinity. An example of this protection is the vasodilatation that occurs during exposure to hypercapnia, this in turn increases the blood flow through the cerebral circulation (Poulin et al., 1996). The increase in blood flow ensures the excess $PaCO_2$ is 'washed out' from the brain in order to maintain its pH. The opposite effect occurs in the presence of hypocapnic levels (Ide et al., 2003). The vessels constrict so that the limited PCO_2 within the circulation remains, aiding the maintenance of metabolic balance. Similar occurrences are present during alterations in PaO_2, where hypoxia causes vasodilation in order to increase the PO_2 delivery to the brain and prevent hypoxic brain injury (Poulin et al., 1996). Hyperoxia has also been shown to effect cerebral perfusion causing a vasoconstrictive response in cerebral blood vessels and thus reducing cerebral blood flow (CBF) (Watson et al., 2000; Tajima et al., 2014). The hyperoxic vasoconstriction of cerebral blood vessels has been suggested to be caused, in part, by the inhibition of nitric oxide production (Zhilyaev et al., 2003). The functional role of hyperoxic cerebral vasoconstriction remains unclear, but it is possible that it contributes to the redistribution of blood flow from highly to less

oxygenated regions. However, recent data from focal cerebral ischemic mice models demonstrated an improvement of CBF in ischemic regions, without any reduction in normoxic regions (Shin et al., 2007), suggesting no such redistribution. An additional suggestion for the role of hyperoxic vasoconstriction is protection against ROS accumulation during exposure to high O_2 levels. However, this is questionable following the demonstration of attenuated production of ROS following hyperoxic administration in focal cerebral ischemia (Liu et al., 2006). It would seem that the vasoactive response of the cerebral blood vessels to hyperoxia is dependent on the conditions of vessels at the time of administration (Shin et al., 2007). This state dependent response will have an effect on the functionality of the vasoactive response to hyperoxia. Therefore the hyperoxic vasoconstriction of normoxic cerebral vessels could still serve as a protective mechanism against ROS accumulation and possible oxygen toxicity.

Alterations in cerebral vascular reactivity to $PaCO_2$ will affect the magnitude and speed of the HCVR because of the effect it can have on amount of CO_2 crossing the blood brain barrier and thus the H^+ within the interstitial fluid. Such an effect on ventilation can be observed with the pharmacological manipulation of CBF with indomethacin. Indomethacin acts as an inhibitor of cerebrovascular reactivity to hypercapnia (St Lawrence *et al.*, 2002). Indomethacin appears to have little direct effect on ventilation (Ainslie & Duffin, 2009), making it an ideal research tool for assessing the effect of CBF on ventilation. When cerebrovascular reactivity to hypercapnia is diminished with indomethacin, the observed ventilatory response to hypercapnia is much greater (Fan *et al.*, 2010). These observations provided clear evidence of the indirect effects cerebral perfusion can have on the central ventilatory control.

1.1.5 Chemosensitivity

The chemical control of the respiratory system can be described as a classical negative feedback loop, shown in figure 1.1. The feedback loop works to maintain blood gas homeostasis by way of correcting fluctuations in PaO_2 and $PaCO_2$. To do this the chemoreceptors detect blood gases and relay afferent feedback to the central respiratory control region. Changes in ventilation are then made via efferent feedback to the respiratory muscles to restore the blood gases to original values. Additional afferent feedback in the presence of the SARs and RARs relay neural information to the central control regions, further modulating respiratory control.

Due to a delay between the changes in blood gases at the lung and the detection of such changes by the chemoreceptors, a perfect and immediate correction of any alteration in $PaCO_2$ or PaO_2 does not occur. This postponement is caused by a circulatory delay determined by cardiac output and the peripheral and cerebral circulations. Thus, changes in ventilation brought about via the detection of changes in blood gases at the chemoreceptors will ultimately happen after subsequent changes in blood gases have occurred at the lungs. Due to this offset in the correction time of the chemical feedback loop, oscillations in ventilation will come about before the original error in blood gas levels is amended.

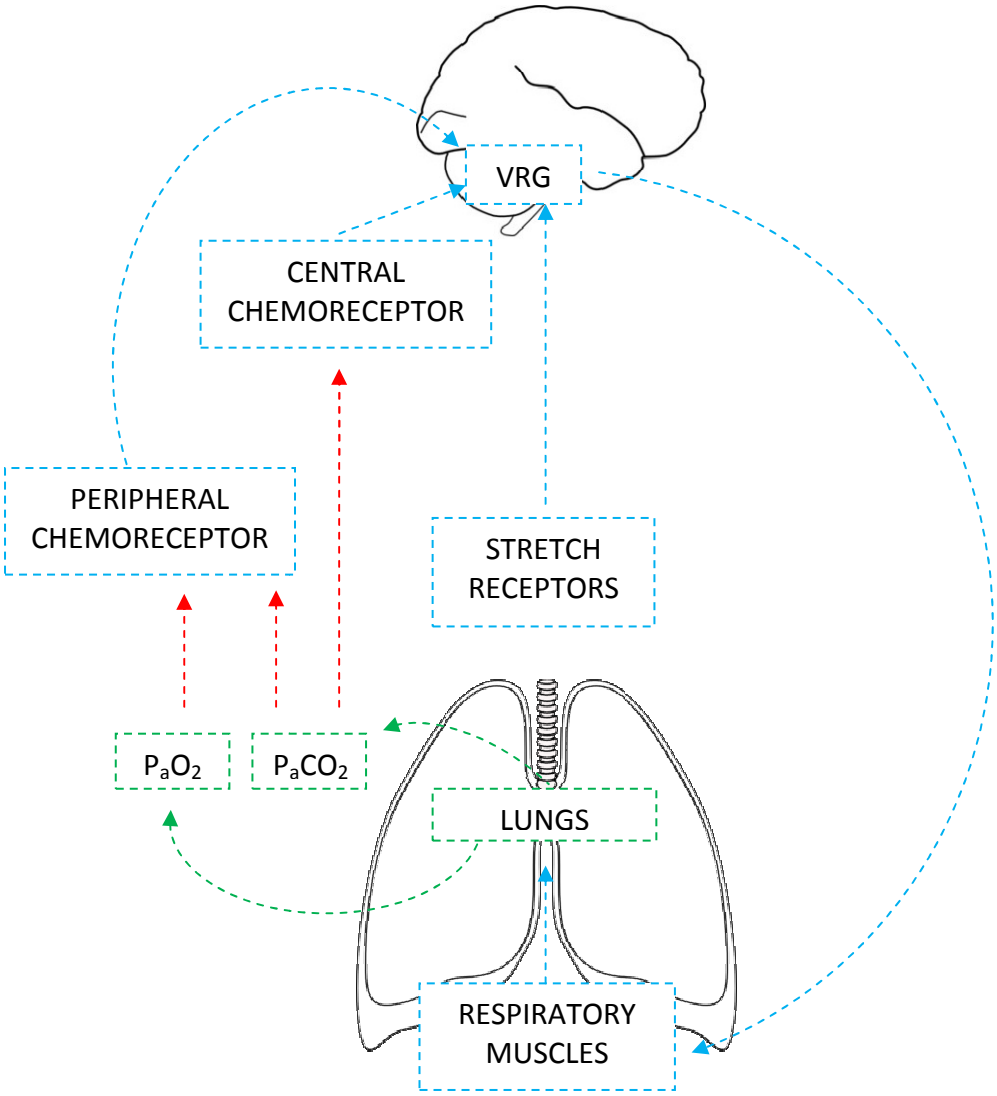

Figure 1.1 A schematic of the feedback loop governing ventilatory control. The **green** broken lines represent the plant gain effectors; gas exchange at the lung that directly affect $PaCO_2$ and PaO_2. The **blue** broken lines represent the regions affecting controller gain from the sensory stretch to chemoreceptors that provide afferent feedback to the ventral respiratory group (VRG) to the eventual efferent output translated into alterations in ventilation. Finally, the **red** broken lines show the circulatory delay, the delivery of blood to the carotid bodies and brain stem through the peripheral and cerebral circulations.

The frequency and magnitude of these oscillations in breathing depend upon the sensitivity of the feedback loop or chemosensitivity. Chemosensitivity can also be referred to as loop gain, an engineering term used in control theory (Khoo, 2000). Loop gain is formed of two additional system sensitivities, controller and plant gain. In terms of the respiratory system controller gain refers to the magnitude of the respiratory chemoreflex (i.e. chemoreceptor detection, the afferent feedback, central processing and the efferent feedback to the respiratory muscles). Plant gain describes the change in arterial PCO_2 and PO_2 for a given change in ventilation.

According to control theory, a mathematical branch of engineering, loop gain is the product of plant and controller gain. The sensitivity of a feedback control system has a direct effect on its functional stability (Khoo, 2000). A graphical representation of the relationship between controller and plant gain for CO_2 ventilatory response is shown in figure 1.2. Plant gain can be shown as a hyperbolic curve and the controller gain is represented by a slope, assumed to be linear. The intersection of the controller slope and the hyperbolic curve represents the operating point and the point at which the ventilatory response slope intercepts the x-axis is termed the apnoeic threshold or B value. The CO_2 reserve is represented by the level between the apnoeic threshold and the operating point.

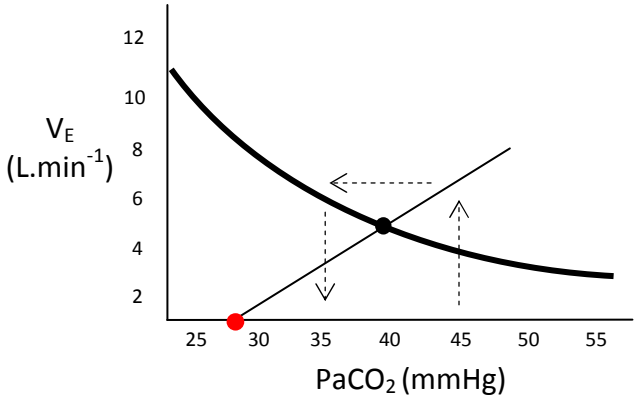

Figure 1.2. Representation of plant gain (hyperbolic curve) and controller gain (linear slope). Where by an increase in ventilation (V_E) on the hyperbola results in a subsequent decrease in $PaCO_2$. The black dot shows a set point. A deviation from this set point, by 5 mmHg here, results in an increase in ventilation, brought about by the controller gain, to reduce the augmented $PaCO_2$. The part of the linear slope below the hyperbola represents the CO_2 reserve. The reserve is the level of PCO_2 between the set point and the apnoeic threshold (red dot).

The loop gain of a system has a direct impact on its functional stability. This stability can be depicted by an oscillatory waveform showing the ventilatory responses to a change in $PaCO_2$ or PaO_2 from homeostatic levels, as shown in Figure 1.3. A high loop gain, considered an unstable system, will result in an exaggerated ventilatory response to a stimulus, characterised by large oscillations in ventilation termed periodic breathing, shown by the broken line in figure 1.3 (Wellman *et al.*, 2008). When faced with increased $PaCO_2$ the resulting ventilatory response is large, such a large ventilatory output leads to a lower $PaCO_2$ that then stimulates a large reduction in ventilation. The reduced ventilation then leads to increased $PaCO_2$ and the cycle continues. Conversely, the lower the loop gain, the more stable the system is considered to be. A stable loop gain can be displayed by small oscillations in ventilation that quickly resolve the original error that stimulated the ventilatory response and do not result in any additional errors occurring, shown by the solid

line in figure 1.3. However, if the loop gain is too low then it is possible that the resulting ventilatory output of the system may not be sufficient to correct the original error and it could persist. A feedback control system with an optimal stability would be a control system with a sensitivity that yields the near perfect output correction for a given error/stimulus, thus resulting in a fast and stable return to homeostasis levels. In this thesis the sensitivity of the respiratory system to changes in either $PaCO_2$ or PaO_2 will be termed chemosensitivity.

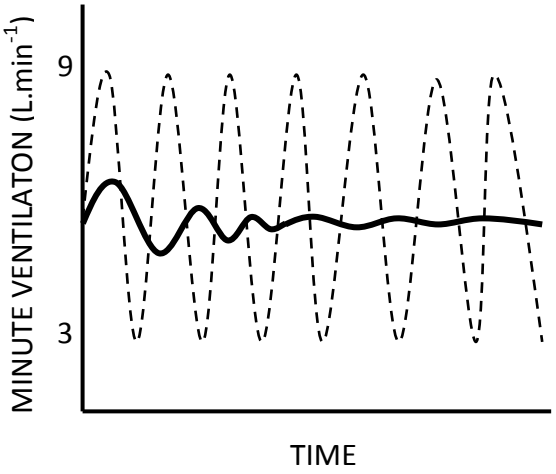

Figure 1.3 Graph of oscillatory lines representing two loop gains or chemosensitive responses to a disturbance in arterial gases. These responses lead to a stimulation of ventilation at time = 0. The solid line shows a low loop gain and more stable system and the broken line is a higher loop gain response and less stable system. Figure adapted from Wellman *et al.* (2008).

1.1.6 Nucleus of the Solitary Tract

The NTS is the common site of innervation for afferent neurons relaying feedback information from the sensory organs relevant to respiratory control (Alheid & McCrimmon, 2008). The NTS projects to the RTN (Takakura *et al.*, 2006) which then innervates with the VRG to modulate ventilation. The NTS also has innervations to the rostral ventrolateral medulla (RVLM) that has important influences on sympathetic nerve activity; see below for more information.

Bonham *et al.* (1993) demonstrated that the NTS is a key relaying region involved in the Hering-Breuer reflex. Bonham and colleagues showed afferent feedback arising from SAR

involved in the Hering-Breuer reflex operates via the NTS through both stimulation and inhibition of secondary neurons and subsequent reflex propagation and attenuation respectively. RAR and Bronchopulmonary C fibers have also been shown to have neural projections to NTS using antidromic mapping techniques (Davis *et al.*, 1983; Kubin *et al.*, 1991).

The peripheral chemoreceptors also innervate the NTS via the carotid sinus nerve. Research has confirmed this both anatomically, with histochemistry (Finley & Katz, 1992) and experimentally with the inhibition of the NTS region during carotid body stimulation that resulted in the reduction of the typical phrenic nerve excitation (Chitravanshi *et al.*, 1994).

In addition to the convergence of respiratory related afferent feedback on the NTS additional feedback reflexes involved in cardiovascular control involve the NTS region. The baroreceptors within the carotid bodies relay neural information relating to vascular pressure important in the baroreflex (Ciriello, 1983; Donoghue *et al.*, 1984). Group III and IV muscle afferents also project neural information regarding muscle metabolites and mechanical stretch to the NTS (Potts *et al.*, 1999), providing a mechanistic influence for cardiorespiratory control during exercise. The input of these cardiovascular controlling mechanisms to the NTS highlights an additional purpose of NTS, the relaying of neural information to the RVLM to modulate sympathetic nerve activity and thus blood pressure control (Guyenet, 2006). The convergence of both respiratory and cardiovascular neural networks at the NTS results in an interaction between the two systems. This interplay between the two is evident in the effects that respiratory reflexes have on cardiovascular parameters. In addition to augmented ventilatory output, peripheral chemoreceptor

stimulation also results in tachycardia and augmented blood pressure, as a result of augmented sympathetic outflow (Guyenet, 2000). Airway reflexes can also result in vasodilatation, via bronchopulmonary C fibers and tachycardia and brachycardia during the cough and Hering-Breuer reflexes, respectively (Kubin *et al.*, 2006).

Another example of cardiorespiratory integration within the brainstem is the impact of central chemoreception on cardiovascular control (Dean & Nattie, 2010; Guyenet *et al.*, 2010). In addition to a potent HCVR, central acidosis also results in augmented blood pressure via increased sympathetic activation. The proposed mechanism for the activation of sympathetic nervous discharge is via central chemoreceptive projections to the RVLM and NTS (Guyenet *et al.*, 2010).

1.1.7 Chemoreceptor interaction

As previously described the chemoreceptors interact to modulate ventilatory output. This interaction is evident both between the central chemoreceptive regions, and between the central chemoreceptors as a whole and the peripheral chemoreceptors.

One example of the interaction between the two forms of chemoreceptors, peripheral and central, is the interaction between the two ventilatory responses, hypoxic and hypercapnic. The PaO_2 has a manipulative effect on the response to hypercapnia, with hypoxia and hyperoxia resulting in the augmenting and dampening of the HCVR, respectively. By measuring carotid body afferent discharge previous research has shown that O_2 and CO_2 interact at the level carotid bodies (Lahiri & DeLaney, 1975).

Furthermore and more recent research with the use of acetazolamide has shown that the multiplicative interaction does also occur between chemoreceptors (Teppema *et al.*, 2010). So in addition to the interaction between O_2 and CO_2 within the carotid bodies, the chemoreceptors themselves also interact during CO_2 exposure. The nature of the peripheral-central interaction is still a heavily debated topic, evident in the recent Journal of Physiology cross-talk (Duffin & Mateika, 2013; Teppema & Smith, 2013; Wilson & Day, 2013). Research is divided in the investigation of the type of interactional relationship between the two chemoreceptors, resulting in three viewpoints arising; a hyper-additive, an additive and a hypo-additive interaction.

An additive relationship suggests a simple summation of the two chemoreceptor responses to stimuli. An additive response is defined by a greater magnitude of response but no change in the sensitivity of the response. The majority of research suggesting such a chemoreceptor interaction has been conducted in humans (Clement *et al.*, 1992; St Croix *et al.*, 1996; Cui *et al.*, 2012). There are methodological limitations in the use of human participants for such investigation. The inability to directly measure afferent discharge from each chemoreceptor in humans means that assumptions of the peripheral and central contributing elements of ventilatory responses are made. Changes in blood gases will also affect additional systemic factors such as, sympathetic activity, baroreflex activation and other modulating respiratory reflexes. The persistence of these factors when assessing chemoreceptor interaction in humans could confound the results obtained.

Further evidence against an additive interaction can be gained from a critical review of Cui *et al.* (2012). In this study the authors used three tests to expose participants to differing

central and peripheral stimuli. In two tests voluntary hyperventilation was used to create lower central PCO_2, immediately following this hyperventilation and thus during the low central PCO_2, participants were exposed to normoxic hypercapnia in test 1 and hypoxic hypercapnia in test 2. These secondary exposures were used to manipulate PCO_2 and PO_2 at the level of the peripheral chemoreceptors during central hypocapnia. In a final test an initial period of hypercapnia was implemented to create high central PCO_2, following which a phase of hypoxic hypercapnia was completed to expose the peripheral chemoreceptors to both low PO_2 and high PCO_2. The protocols are depicted in figure 1.4. While this experimental design was robust, the authors' interpretation of findings could be considered flawed. In making their comparison between the data obtained for each test Cui and colleagues failed to account for the multiplicative relationship of O_2 and CO_2 that occurs at the carotid bodies. If this relationship had not been ignored it could be suggested a hyper-additive interaction would have been interpreted (Teppema & Smith, 2013).

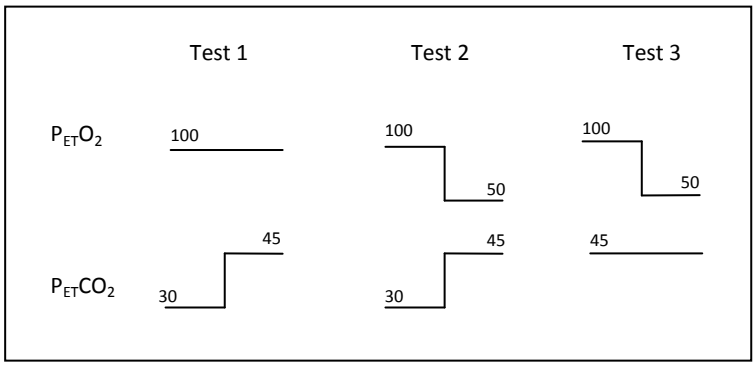

Figure 1.4 Diagrammatic representation of the three tests used by Cui *et al.* (2012). During test 1 central PCO_2 was low and peripheral PCO_2 was high and PO_2 was normoxic. Test 2 had a similarly low central PCO_2 coupled with a peripherally low PO_2 and high PCO_2. Test 3 consisted of a high central PCO_2 together with a peripherally low PO_2 and high PCO_2.

A hyper-additive or multiplicative relationship is where the stimulation of one chemoreceptor results in the augmentation of the other chemoreceptor response and vice

versa. Recent evidence in dogs showed increased and deceased ventilatory slopes during respective activation (hypoxic, normocapnic perfusate) and inhibition (hyperoxic, hypocapnic perfusate) of a unilateral isolated carotid body, providing strong evidence for a hyper-additive interaction (Blain *et al.*, 2010). Further experiments in goats (Pan *et al.*, 1998) show a reduction in the HCVR slope following denervation of the carotid bodies, further suggesting a hyperadditive interaction. Similar data have been demonstrated in humans (Dahan *et al.*, 2007). Patients show reduced central chemoreceptor sensitivity to hypercapnia following carotid body removal, lending evidence to a multiplicative interaction. However, other researchers argue that the complete removal of the carotid bodies not only ablates the peripheral chemoreceptor but also the baroreceptors, a source of sympathetic modulation that could affect ventilatory control and confound the observations.

Earlier evidence for a hyper-additive interaction has been reported by Robbins (1988). Using the differences in temporal dynamics of the two chemoreceptors, in a similar manner to Cui *et al.* (2012), Robbins reported a more than additive relationship between the two chemoreceptors. However, attempts to replicate these findings have failed (St Croix *et al.*, 1996).

Lastly, a hypo-additive effect relates to the activation of a chemoreceptor resulting in a consequential inhibition of the other chemoreceptor response. Unlike Blain *et al.* (2010), an animal preparation set up by Day and Wilson (2009) isolated both carotid bodes in conjunction with a separately perfused brainstem. During reduced brainstem PCO_2 phrenic nerve sensitivity was increased during carotid body infusion with high CO_2 concentrations, thus demonstrating a hypo-additive interaction. However, others would argue that the

decerebrate preparation used for the dual perfusion studies will abolish the possibly facilitative orexin chemoreceptors (Nattie, 2011) and other descending inputs from the cortex that may affect respiratory output.

Although the nature of the relationship is still heavily debated the interaction of the chemoreceptors should not be ignored. It is because of this interaction and the separated contributions of chemoreceptors to the hypercapnic ventilatory response that it is important not only to assess total chemosensitivity but also the separate chemoreceptor sensitivities.

Further interaction between the chemoreceptors could possibly occur at a central chemoreceptive level. The general assumption is an additive relationship between the numerous central chemoreceptive sites (Nattie & Li, 2010). However, evidence for a more complex interaction between the chemoreceptive sites is demonstrated by the lack of a simple additive interaction during simultaneous stimulation of the caudal medullary raphe and RTN and stimulation of the RTN and caudal medullary raphe alone (Dias *et al.*, 2008). During these separate stimulations it appears that the caudal medullary raphe does not respond to activation by acidosis unless the RTN is simultaneously stimulated. These results demonstrate the possibility of a more complex synergy between the central chemoreceptive sites, with the caudal medullary raphe being a modulator. More recent findings also suggest this modulation role in central chemoreception by the caudal medullary raphe (da Silva *et al.*, 2012).

1.2 Methods of CO_2 Chemosensitivity Assessment

CO_2 chemosensitivity is a primary outcome measure in the work presented in this thesis. Therefore what will follow is a description of the common methods that are used in research to assess overall CO_2 chemosensitivity, the separation of central and peripheral chemosensitivity and the apnoeic or ventilatory thresholds.

A common method of quantifying chemosensitivity is via the assessment of the ventilatory response slopes to changes in $PaCO_2$. Additional parameters assessed include the ventilatory recruitment thresholds and apnoeic thresholds. The most popular methods of assessment of CO_2 chemosensitivity are steady state and progressive re-breathing methods.

1.2.1 Steady state method

Steady state exposures involve distinct steps of hypercapnia lasting a number of minutes. During steady state steps inspired CO_2 is increased to reach desired hypercapnic level. CO_2 is released from pressurised canisters, via experimenter or computer controlled solenoids, which is then mixed into the inspired air to produce a greater concentration of inspired CO_2. The hypercapnic ventilatory responsiveness to the stimuli is assessed once a steady state of ventilation is reached. In order to quantify a realistic slope of the HCVR it is suggested that a number of differing levels of hypercapnia be used to insure a linear response is produced (Mohan *et al.*, 1999). However, most steady state tests will assume this linearity of hypercapnic ventilatory response and test just two levels of PCO_2; eucapnia (1 or 2 mmHg above normal $PaCO_2$ levels) and a level of hypercapnia. This assumption could be considered a limitation of the steady state method.

The use of a high inspired PCO_2 to rapidly increase $PaCO_2$ from normal levels results in the maintenance of PCO_2 gradients between the tissues and measured end-tidal partial pressures of CO_2 ($P_{ET}CO_2$) at the mouth. Due to the differences in these gradients the measured $P_{ET}CO_2$ is said to be only an estimate of the PCO_2 detected by the chemoreceptors, particularly the central chemoreceptors. In order to estimate CO_2 apnoeic thresholds data is extrapolated from steady state values of ventilation to the CO_2 x-axis intercept.

A method of dynamic end-tidal forcing (DEF) is the most preferred method of gas control during steady state methods. The DEF system is described in greater detail in the Chapter 2.

1.2.2 Re-breathing method

Progressive re-breathing methods employ a re-breathing bag to administer the hypercapnic stimulus. Re-breathing bags usually contain a composition of mild percentages of CO_2 and a balance of O_2. The high levels of CO_2 within the bag provide an initial mild increase in $PaCO_2$ as participants inspire from the bag. This is necessary to create equilibrium in the PCO_2 gradients between tissue, mixed venous and end-tidal PCO_2. Once equilibrium is reached participants will then also expire into the re-breathing bag as well as inspire. Their natural metabolism causes a progressive increase in PCO_2 within the bag. The initial equality in PCO_2 gradients is said to ensure that during the progressive exposure to hypercapnia these gradients remain minimal (Berkenbosch *et al.*, 1989). Therefore, the measurement of $P_{ET}CO_2$ is considered an accurate measurement of PCO_2 sensed by the chemoreceptors; assuming that CO_2 production at the brain is similar to the rest of the body. An additional assumption made by this method is a linear increase in CBF during the re-breathing test, meaning that the wash-out of $PaCO_2$ within the cerebral circulation is constant and does not alter the

invariable tissue – arterial PCO_2 gradient. However, the observed variability in measured middle cerebral artery blood flow during a re-breathing test would suggest this assumption to be wrong (Battisti-Charbonney *et al.*, 2011). Although, it could be argued that because the measurement of CBF was made at middle cerebral artery it is not a direct measurement of the blood flow past the central chemoreceptors, and that a measurement of a more posterior cerebral artery would give a more accurate representation. Also, recent research has shown regional differences in cerebrovascular reactivity to CO_2 between the middle cerebral and posterior cerebral arteries, with the middle cerebral artery being reported to have greater CO_2 reactivity than the posterior cerebral artery (Skow *et al.*, 2013). Thus, when relating cerebrovascular reactivity and the effects it can have on the perfusion of specific brain regions, the selection of cerebral vessel with which to assess reactivity is an important consideration that should be taken into account.

The most common form of re-breathing test is the "*Read re-breathing test*" (Read, 1967). A recently revised re-breathing method, developed by Duffin has been recently employed in a number of studies (Mohan & Duffin, 1997). The "*modified re-breathing*" has two main differences to the Read's re-breathing. Firstly, PaO_2 is controlled throughout the test, either at a specific hypoxic or hyperoxic level. Secondly, prior to the initiation of hypercapnia participants are asked to voluntarily hyperventilate for 5 minutes, so that $PaCO_2$ levels become hypocapnic (20 – 25 mmHg). By introducing a hypocapnic $PaCO_2$ level prior to the start of hypercapnic re-breathing, participants are below their ventilatory recruitment threshold. Therefore the point at which ventilation begins to increase can be considered the ventilatory recruitment threshold. These two additions mean that the modified re-breathing is better controlled and provides the additional information of ventilatory recruitment

threshold. However, the possible confounding effects of volitional modulation of respiratory control during participant controlled hyperventilation could affect the assessed HCVR response and should not be ignored.

Comparisons of these two methods, steady state and re-breathing, have been previously recorded (Berkenbosch *et al.*, 1989; Mohan *et al.*, 1999). Differing results occur, particularly if using a Read re-breathing test, whereby the steady state test results in lower calculated chemosensitivities in comparison. It is thought that differences in the effect of the CBF response to CO_2 could account for these between test variations (Berkenbosch *et al.*, 1989).

Also, it has been proposed that because of the maintenance of the tissue-blood gradient during steady state, that the steady state test assesses the ventilatory sensitivity in conjunction with the cerebral circulation responsiveness to changes in $P_{ET}CO_2$. Whereas, because of the abolishment of the tissue-blood gradient during the re-breathing tests that the measurement measures solely the chemoreceptor sensitivity (Duffin, 2011).

1.2.3 Mouth occlusion pressure method

An additional indirect measurement of ventilatory drive is to measure mouth occlusion pressure during the exposure to a respiratory stimulus, usually delivered via a re-breathing method. The occlusion of the mouth is achieved by the inflation of a small balloon within the mouth piece. A transducer is then used measure the resultant pressure of the participant's ventilatory effort against the closed airway; the greater the pressure the greater the ventilatory effort. Although primarily a measure of neuromuscular drive, this technique has been used to infer chemosensitivity. The technique is limited to just 0.1 seconds of occlusion. Therefore, the timing of the occlusion within a breath could affect the estimated

ventilatory effort. Additionally, the ventilatory effort could be confounded by the strength of the participant's respiratory muscles (Fitzgerald *et al.*, 1976).

1.2.4 Separation of central and peripheral chemosensitivity

Further complication with the assessment of chemosensitivity arises when researchers attempt to distinguish the contribution of peripheral and central chemosensitivity to total CO_2 chemosensitivity in humans.

There are two main methods of separating the two chemoreceptors, the most popular of which is to ablate or stimulate the peripheral chemoreceptors. A background of hyperoxia is a common tool used to supposedly silence the peripheral chemoreceptors (Miller *et al.*, 1974). High levels of PO_2 have been used in both steady state and re-breathing methods so that solely the central chemoreceptor response to CO_2 can be measured. However, research is sceptical on the peripheral chemoreceptor silencing capabilities of hyperoxia. Evidence has demonstrated that the carotid body afferent nerves in anaesthetised cats are still active during hyperoxic exposure (Fitzgerald & Parks, 1971; Lahiri & DeLaney, 1975) and in humans the peripheral element of the ventilatory response to hypercapnia remains during hyperoxic hypercapnia (Dahan *et al.*, 1990; Pedersen *et al.*, 1999b).

The use of hyperoxia itself could have effects on ventilation via the mass balance equation for CO_2, which could confound results obtained during hyperoxic chemosensitivity tests. One parameter of the mass balance equation is the Haldane effect. During hyperoxia tissue PCO_2 will increase due to the reverse Haldane effect and lead to the stimulation of the central chemoreceptors and augmented ventilation (Becker *et al.*, 1996). Therefore, using background hyperoxia during HCVR measurements could confound the tests.

An additional parameter of the mass balance equation is cerebral blood flow. Hyperoxia results in vasoconstriction of the cerebrovascular bed (Watson *et al.*, 2000) causing a reduction in CBF. Such attenuation in CBF results in an increased tissue PCO_2, which affects the magnitude of central chemoreception and overall ventilatory output. This effect could contribute to the paradoxical hyperoxic stimulated hyperventilation (Becker *et al.*, 1996). However, a more recent suggestion points to a possible role of the hyperoxic stimulated accumulation of reactive oxygen species (ROS). The increased levels of ROS are thought to stimulate regions of central chemoreception within the medulla leading to a increase in ventilation (Dean *et al.*, 2004). The hyperventilation observed during hyperoxic breathing could confound chemosensitivity tests, where hyperoxia is used to diminish the peripheral chemoreceptor output and infer central chemosensitivity. Such an effect would lead to the overestimation of central chemosensitivity.

Further complications with the use of hyperoxia in hypercapnic respiratory tests arise when considering the interaction of the two chemoreceptors. A potential multiplicative interaction between peripheral and central chemoreceptors would suggest that a background of hyperoxia would affect the central chemoreceptive response to CO_2 and thus confound the measured central chemosensitivity.

An additional method of separation takes advantage of the differences in response time constants of the two chemoreceptors to model ventilatory responses to pseudo-random exposures of hypercapnia differing in length of exposure (Pedersen *et al.*, 1999b). The ventilatory responses are then modelled using a two-compartment mathematical model. By using exponential curve peeling and the response times of the chemoreceptors the model

assesses the contributing sensitivities of central and peripheral chemoreceptors to the hypercapnic chemosensitivity. Using this model, total chemosensitivity is derived by the sum of central and peripheral contributions. Pedersen *et al.* (1999b) developed this test, termed a multi-frequency binary sequence (MFBS test). Pedersen and colleagues also demonstrated, using the MFBS test, that a peripheral element of chemosensitivity is still present during hyperoxic hypercapnic exposure. This finding is further evidence against the ability of hyperoxia to "silence" the peripheral chemoreceptors, thus suggesting it cannot be used to distinguish solely central chemosensitivity. In this thesis the multi-frequency binary sequence test is adopted to assess both the total and the separate peripheral and central chemosensitivities.

1.3 Ageing and respiratory function

The ageing process is associated with a reduction in respiratory function (Janssens *et al.*, 1999; Lalley, 2013). It is thought that the maximal ability of the respiratory system is reached at the age of 30, following which, the ability of the respiratory system declines with age (Lalley, 2013). The most common decrements in respiratory function arise from the mechanics of inspiration and expiration. A reduction in the elastic recoil of the lung, reduction in the compliance of the chest wall and a weakening of the respiratory muscles, all reduce the capacity to inspire and expire air (Lalley, 2013).

In addition to the decline in mechanical aspects of ventilation, ageing is also associated with an increasing mismatch between ventilation and perfusion of the lung (Sorbini *et al.*, 1968) and a decline in pulmonary diffusion capacity (McGrath & Thomson, 1959). These effects of age contribute to the lowering of PaO_2 with age, sometimes reported as low as 75 mmHg

(Cardus *et al.*, 1997). Despite the decline in PaO_2 with age there appears to be no change in $PaCO_2$ level (Wahba, 1983).

The ventilatory responsiveness to changes in PaO_2 and $PaCO_2$ is an additional area of respiratory physiology affected by age. Chapter 3 will provide an in depth review of the literature relating to this area.

1.4 Sleep

The historical perspective of sleep was that it was a passive state and wakefulness was seen as the active state. Technical advancements in the twentieth century and development of the discipline of sleep medicine have now lead to the assumption that sleep is in actual fact a very active state. Hans Berger was a pioneer in the study of electrical activity in the brain. In 1924 he observed, with the use of electroencephalography (EEG), clear differences in the electrical activity of brain between awake and sleeping states (Haas, 2003). Using this technique of EEG we now know that sleep is a very active state made up of a number of stages and it dynamically moves from one stage to another as sleep progresses (Kryger *et al.*, 2011). These stages can be categorized into two forms, rapid eye movement (REM) and non rapid eye movement (NREM) sleep (Kryger *et al.*, 2011). REM sleep, discovered by Nathaniel Kleitman in 1957 (Dement & Kleitman, 1957), is characterised by high frequency brain waves very similar to wakefulness but the voltage remains low. The metabolism of the brain during REM sleep is also very similar to wakefulness (Siegel, 2003). The most vivid dreaming occurs during REM sleep (Siegel, 2003) and in order to prevent us acting out these dreams the motor neurons are inhibited during REM sleep by the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA) (Chase & Morales, 1990). Despite the inhibition of

motor neurons the muscles involved in eye motion are still excitable during REM sleep, therefore the eyes roll under the eyelids, hence the term rapid eye movement sleep. Conversely, NREM sleep is characterised by the high amplitude and low frequency brain waves. NREM sleep can be broken down into a number of stages of sleep. Initially, a characteristic "light sleep" stage or stage 1 takes place, that then progresses into a second stage of sleep, stage 2 sleep. A further important stage of NREM sleep is termed slow wave sleep (SWS), referred to as stage 3 and/or 4 of sleep (Kryger *et al.*, 2011). A normal night's sleep is characterised by the cycling in and out of these stages of sleep, with NREM sleep being a dominant characteristic of the first half of the night and REM sleep making up the majority of the second half (Kryger *et al.*, 2011).

The sleep wake cycle itself is governed by the biological clock or the circadian rhythm. The circadian rhythm is characterised by 24 hour biological oscillations that occur in parallel with the cycle of night and day. There is extensive research showing numerous circadian controlled processes across the body from metabolism, hormonal secretion and gene expression (Huang *et al.*, 2011; Lowrey & Takahashi, 2011). The key central neural pacemaker of circadian rhythms is the suprachiasmatic nucleus region within the hypothalamus (Moore, 1983).

The physiological governess of circadian rhythms is a complex process and beyond the scope of this thesis. However, it is integral in the sleep-wake cycle. The sleep-wake cycle is the most empirical of these circadian controlled processes, with a complex decrease and increase in physiological variables associated with the onset of sleep and the bringing about of wakefulness (Fuller *et al.*, 2006). Examples of these oscillating variables include body

temperature, melatonin, serotonin and cortisol. Disruption of the biological body clock is evident in shift workers and been suggested to play a role in associated metabolic disorders, cardiovascular disease and cancer incidence (Knutsson, 2003; Davis & Mirick, 2006).

How we sleep has been revealed by numerous investigations. However, the function of sleep is much more elusive. One thing is clear, the neglect of sleep can result in dire consequences to our health and mortality, see the next section for more information.

The investigation of the function of sleep has been approached in a dual manner with functional purposes being attributed to the two stages of sleep; NREM sleep and REM sleep (Siegel, 2005). The majority of these theories suggest a function of sleep that is involved in neurophysiological repair and psychological consolidation. SWS has been associated with a greater protein synthesis within the brain (Nakanishi *et al.*, 1997) suggesting that NREM sleep plays a role in assisting neurogenesis. This suggestion lends weight to an additional proposed function of NREM sleep, memory consolidation (Fogel *et al.*, 2012). However, REM sleep has also been suggested to play a role in memory consolidation. A recent review suggests differences in the type of memory reinforcement by each stage of sleep. The consolidation of hippocampal dependent declarative memories, long term memory of knowledge, has been suggested to be benefited by NREM/SWS sleep. Whereas, REM sleep aids the enhancements of procedural and emotional memories (Ackermann & Rasch, 2014).

An additional psychological function of REM sleep that has been suggested is the regulation of emotions (Siegel, 2005). Such a suggestion has arisen following the observed anti-depressive effects of REM sleep deprivation (Vogel *et al.*, 1980). However, more recent evidence shows a decrease in depressive symptoms following selective deprivation of SWS

(Landsness *et al.*, 2011) suggesting another possible dual process regulation of emotions via both NREM and REM sleep.

One hypothesis concerning the function of sleep relates to body mass and metabolism (Siegel, 2005). When considering body mass and sleep duration, one can see that smaller animals sleep much longer than larger animals. For example a mouse sleeps approximately 14 hours per day, whereas an elephant, colossal in comparison to the mouse, sleeps just 3 hours.

The postulated reason for the association between body size and sleep duration is metabolic rate and the resultant greater levels of ROS. ROS are reactive molecules that are released as a result of metabolism via the oxygen transport chain. Smaller animals tend to have a greater metabolic rate than larger animals and as a consequence a greater generation of ROS (Siegel, 2005). ROS have been shown by numerous studies to have a dual role in body, causing cellular damage through oxidative stress and facilitating physiological function via signalling pathways (Murphy *et al.*, 2011).

Studies attempting to investigate the association of sleep length and ROS production, have shown that sleep deprivation leads to a greater level of ROS generation in peripheral tissues and subcortical regions such as the hypothalamus and brainstem (Eiland *et al.*, 2002; Ramanathan *et al.*, 2002). Other higher regions of the brain such as the cerebral cortex do not demonstrate the same observations (Ramanathan *et al.*, 2002; Gopalakrishnan *et al.*, 2004). Siegel (2005) suggests that the differences in these findings relate to the levels of ROS generation in the brain regions. Brain regions with the larger levels of ROS generation, such

the hypothalamus, are protected by sleep through that attenuation of ROS damage and facilitation of protein synthesis and repair.

The function of sleep is still a much clouded question. The majority of evidence has focused on central brain elements, in the facilitation of memory building and protection against oxidative stress within the brain. However, sleep loss has additional residual effects on peripheral body function in healthy individuals and in disease states.

1.4.1 Sleep loss and health and disease

Our attitude to sleep has changed in the last century with research suggesting we are sleeping less than our ancestors (Webb & Agnew, 1975; Bonnet & Arand, 1995). However, despite this reduction in sleep over time the American Time Use Survey of 2012 reveals that on average sleep still consumes 39% of our entire day, making it the single most time consuming task of the 24 hour day. The amount of time spent asleep suggests a fundamental importance of sleep in our everyday lives. Nevertheless, considering the number of hours taken up by sleep it is unsurprising that it is being sacrificed for extended wakefulness so further time can be devoted to productivity and entertainment. However, the progressive reduction in sleep length with the ages can have a bearing on our health status.

Findings from epidemiological research suggest that there is a greater risk of all causes of mortality in individuals who on average sleep less than seven to eight hours a night (Ferrie *et al.*, 2007; Cappuccio *et al.*, 2010b). Particular risk factors of mortality include increased occurrence of cardiovascular disease, metabolic disorders and obesity (Spiegel *et al.*, 2005; Knutson & Van Cauter, 2008; Cappuccio *et al.*, 2010a; Cappuccio *et al.*, 2011). Also, sleep loss results in cognitive dysfunction that can indirectly contribute to the greater level mortality

(Kronholm *et al.*, 2009). Past experimental investigations have suggested possible mechanisms for the increase in these mortality risk factors as a result of reduced sleep length. Increased insulin resistance and vascular dysfunction following periods of sleep loss in otherwise healthy participants have been demonstrated in experimental trials (Spiegel *et al.*, 1999; Takase *et al.*, 2004b; Sauvet *et al.*, 2010).

Experimental data relevant to the research presented in this thesis have previously demonstrated a reduction in respiratory control following total deprivation of sleep (Cooper & Phillips, 1982; Schiffman *et al.*, 1983; White *et al.*, 1983). However, more recent research proposed otherwise, observing no such alterations in respiratory regulation following the deprivation of sleep (Phillips *et al.*, 1987; Ballard *et al.*, 1990; Spengler & Shea, 2000). More information about this is presented in chapter 4.

An additional study investigating the effect of sleep disruption, in the form of sleep fragmentation, has suggested a reduction in respiratory chemosensitivity to hypercapnia (Liu *et al.*, 2011), whereas early studies found no such change (Bowes *et al.*, 1980; Espinoza *et al.*, 1991b). This topic will be covered in detail in Chapter 5.

Alterations in respiratory control as a consequence of sleep disruption could have a bearing on respiratory disorders that are associated with a form of sleep disruption. Sleep disordered breathing (SDB) is a condition associated with repetitive arousals from sleep throughout the night. This chronic sleep disruption leads to daytime somnolence and has a debilitative effect on the everyday lives of sufferers. SDB patients have a greater likelihood of experiencing road traffic accidents as a result of the effects of chronic loss of sleep (Turkington *et al.*, 2001).

Whilst the condition of SDB causes a disruption of sleep, it appears that sleep loss itself can have a exacerbating effect on the severity of SDB (Guilleminault *et al.*, 1984; Canet *et al.*, 1989; Persson & Svanborg, 1996; Thomas *et al.*, 1996). These observations suggest that a viscous cycle of cause and effect occurs in the condition of SDB, whereby recurrent fragmentation and loss of sleep caused by SDB, could contribute in part to the progression of the condition itself that eventually leads to further sleep disruption.

Of these studies, two investigated the effect in infants (Canet *et al.*, 1989; Thomas *et al.*, 1996), the earliest study tested elderly individuals with a mean age of 73.3 years who suffered from mild SDB (Guilleminault *et al.*, 1984) and a single study investigated moderate SDB sufferers of varying ages (Persson & Svanborg, 1996). All studies investigated the effect of total sleep deprivation over 1 night. The participants in these studies served as their own controls meaning there was no need for a healthy control population. However, a different study using such a control population has suggested that there is no change in nocturnal respiratory event characteristics following 24 hours of sleep deprivation in either patients or controls (Desai *et al.*, 2003). The main difference between the study conducted by Desai *et al.* (2003) and the other early studies is that they attempted to control sleeping lengths prior to sleep deprivation and control periods, with activity monitoring equipment. Despite this attempted control, participants were said to be mildly sleep deprived prior to their experimental nights. Baseline nights under experimental conditions prior to the actual deprivation or control nights would possibly have prevented this from occurring. Nevertheless, the authors deemed the possible effect of this mild sleep loss as negligible to the observed results.

An obvious suggested mechanism for the observed increase in SDB severity following sleep deprivation is the possible effect it has on respiratory control. Respiratory control has been suggested by some previous research to be affected by sleep deprivation, as has been described earlier and as will be described in greater detail in chapter 4. However, only one of the studies investigating the effect of sleep deprivation on SDB has assessed respiratory chemosensitivity (Thomas *et al.*, 1996). The authors observed an increase in peripheral chemosensitivity in infants, but made no attempt to assess central chemosensitivity. Furthermore, as the study was conducted in 3 month old infants, sleep deprivation was promoted for as long as possible until the child fell asleep. This protocol could be considered less controlled than that of studies conducted in adults. Although, this is still important research concerning possible causes of sudden infant death syndrome, it is difficult to interpret these findings for an adult population.

An additional noteworthy point about these studies is that they all involved total sleep deprivation for a single night. The loss of sleep through reduced sleep length is a more representative form of sleep deprivation to what is observed in the common lifestyle of individuals in our modern society. A study in this thesis (chapter 4) will attempt to assess if this form of sleep pattern can result in alterations in respiratory control that could lead to respiratory events during sleep.

An additional condition that causes sleep disruption and could also be affected by alterations in respiratory control as a result of sleep disruption is COPD. COPD is characterised by the narrowing and obstruction of the airways within the lung that results in breathlessness and chronic hypoxemia in patients. During the night COPD patients experience similar symptoms

to SDB that causes gross sleep fragmentation that can affect the overall quality of life in these patients (Nunes *et al.*, 2009; Scharf *et al.*, 2011). Alterations in respiratory control, as a result of poor sleep, in these patients could affect the daytime and nocturnal respiratory control, possibly worsening the condition of COPD and contributing to a decreasing patient quality of life.

1.4.2 Sleep and ageing

All aspects of sleep are affected by advancing age. These include, the architecture of the sleep stages, the quality of the period of sleep and the overall duration of the sleep. Two large meta-analysis studies have compiled a large body of research investigating the alterations in sleep with age (Ohayon *et al.*, 2004; Redline *et al.*, 2004). Ohayon *et al.* (2004) selected 65 studies, amounting to 2391 participants within the age range of 19-102 years. They found that the time spent in NREM sleep was increased with age, characterised by increases in stages 1 and 2. No change in SWS percentage was reported. However, more recent studies have shown a reduction in both SWS duration and wave amplitude with age (Dijk *et al.*, 2010; Carrier *et al.*, 2011). REM sleep was also affected by age. At an early age REM sleep makes up nearly 25% of total sleep length. The percentage of time in REM progressively decreases with age to an approximate level of 11% at the age of 60 (Van Cauter *et al.*, 2000).

Ohayon and colleagues also found a marked increase in the number of subconscious awakenings after sleep onset in older individuals, highlighting the prevalence of sleep fragmentation in older individuals. Additionally and likely a consequence of the increased

level of subconscious awakenings, an inverse linear relationship between total sleep length and age was found by the authors.

Recently, researchers have suggested that these alterations in sleep with age have an impact of the cognitive ability of older individuals (Pace-Schott & Hobson, 2002; Kondratova & Kondratov, 2012). One particular change with age that has been suggested to impact cognition and brain health in older individuals is a progressive disruption in the circadian rhythm with age (Kondratova & Kondratov, 2012). This disruption has been suggested to be caused by a reduction in electrical activity arising from the suprachiasmatic nucleus (Hofman & Swaab, 1994). This neural degradation of the central regions of circardian rhythm could be a cause of reduced amplitude in oscillatory patterns involved in the sleep-wake cycle, such as body temperature and melatonin (Kondratova & Kondratov, 2012). The disruption of circadian rhythm control of physiological functions could also be the chief mechanism behind the aged associated alterations in sleep architecture, quality and duration.

As well as affecting cognitive function in older individuals, these alterations in sleep with age could also have consequences on the overall health in this population, just as has been demonstrated in younger individuals who sleep less.

1.4.3 Sleep and respiratory control

Cortical projections to respiratory motor neurons and central respiratory areas within the brain stem, results in an overall wakefulness drive to breathe. At sleep onset the activation of ventrolateral preoptic neurons leads to the inhibition of cortical arousal. Additional suppressions of arousal occur because of the stimulation of inhibitory GABA receptors leading to the inhibition of arousal neurons and sleep onset (McGinty & Szymusiak, 2000).

These GABA receptors are also situated within the respiratory control network and their stimulation at sleep onset leads to the suppression of respiratory controlling mechanisms (Finnimore *et al.*, 1995). A further reduction in ventilation is brought about by the reduction in muscular tone during sleep (Worsnop *et al.*, 1998) that reduces respiratory muscle activity during the night. All these arousal state alterations contribute to the overall reduction in minute ventilation, that causes a small decrease in oxygen saturation and mild increase in $PaCO_2$ to a higher operating point on the hyperbolic curve, see figure 1.5. The mild reduction in oxygen saturation during sleep will have little effect on respiratory control because it does not reach a threshold necessary for HVR stimulation. However, the small increase in $PaCO_2$ plays a key role in the control of ventilation during sleep through the respiratory chemoreflex. Additionally, an increase in the operating point will mean that plant gain becomes higher. Thus, a small increase in ventilation at the higher operating point will yield a greater change in $PaCO_2$, thus increasing the likelihood of reaching the apnoeic threshold. A dampening of the chemoreflex to CO_2 during sleep would serve as a protective mechanism against reaching the apnoeic threshold, by way of increasing the CO_2 reserve, see figure 1.5.

Such a change in the chemoreflex control of ventilation does occur during sleep, with previous research showing both the HCVR and HVR to be attenuated. These reductions are shown to be sleep state dependent, with the most pronounced reductions in HCVR and HVR shown to occur during REM sleep (Douglas *et al.*, 1982a; Douglas *et al.*, 1982b). Interestingly, the cerebral vascular reactivity to both hypoxia and hypercapnia is also reduced (Meadows *et al.*, 2004; Meadows *et al.*, 2005), suggesting that the reduction in HCVR during sleep is independent of CBF influences on central ventilatory output.

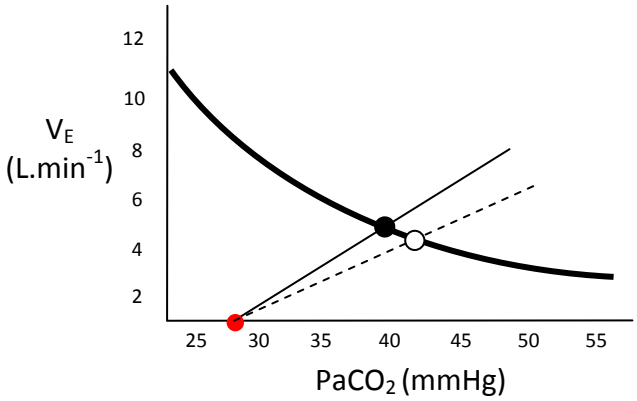

Figure 1.5 Graph showing the changes in respiratory control during sleep. The solid linear line shows the normal respiratory controller slope during wakefulness. The black dot represents the operating point during wakefulness. During sleep chemosensitivity is reduced (dotted line) and the operating point resides at a higher $PaCO_2$ level (white dot). The lower chemosensitivity increases CO_2 reserve, serving to protect the system from increased plant gain, brought on by the greater operating point, and greater likelihood of reaching the apnoeic threshold.

One hypothesis for the changes in chemosensitivity from wakefulness to sleep is the arousal state dependent nature of orexin neurons (Kuwaki *et al.*, 2010; Nattie & Li, 2010). Orexin, also known as hypocretin, is a neurotransmitter involved in the regulation of arousal, appetite and wakefulness (Sakurai, 2007). Orexin producing neurons originate from the lateral hypothalamus but have been shown, by way of immunohistochemical staining, to have numerous afferent and efferent projections to regions throughout the brain (Peyron *et al.*, 1998), meaning that the contributions of these neurons are diverse.

In terms of arousal, orexin has been shown to play a key role in the maintenance of wakefulness, as is clearly demonstrated in the condition of narcolepsy. Narcoleptic patients suffer from the inability to maintain a wakeful state and their lives are characterised by the intermittent and spontaneous occurrences of REM and NREM sleep periods. It was later discovered that the condition was caused by a deficiency in orexin. Orexin deficient mice and

dogs demonstrated the interruption of wakefulness with REM and NREM sleep, and greater fragmentation of sleep across a 24-hour period, both strong characteristics of the human condition of narcolepsy (Chemelli *et al.*, 1999; Lin *et al.*, 1999). A year later this orexin deficiency was confirmed in the human disease state when orexin levels were seen to be virtually undetectable in the cerebrospinal fluid of narcoleptic patients (Nishino *et al.*, 2000).

As previously described these orexin neurons have been shown to respond to changes in CO_2/pH and possibly have a facilitative role in central chemoreception (Williams *et al.*, 2007; Nattie, 2011). These more recent studies, lead by Eugene Nattie's research group, have emerged to reveal a state dependent role of orexin neurons in the modulation of ventilatory control and in particular central chemoreception. The microdialysis of orexin antagonists onto the RTN region is reported to result in a 30% reduction in the ventilatory response to hypercapnia during wakefulness. This attenuation of orexin inhibition that is seen during wakefulness is markedly reduced when repeated during sleep (Dias *et al.*, 2009). The same effect is observed in the rostral medullary raphe (Dias *et al.*, 2010). These findings lead to the suggestion that orexin neurons play a governing role in the wakefulness drive to breathe. If this were the case, orexin inhibition would result in the reduction of normal resting ventilation levels. However, this has been shown not to be true (Dias *et al.*, 2010).

The removal of the wakefulness drive to breathe during sleep reveals an apnoeic threshold. This can be shown by mechanically ventilating healthy sleeping individuals so that $PaCO_2$ returns to normal waking levels (Dempsey, 2005). At this level of $PaCO_2$ ventilation is terminated by a removal of central respiratory drive, this is termed the apnoeic threshold.

The apnoeic threshold is a key mechanism for the development of apnoeas during sleep, as discussed further in greater detail in section 1.6.3.

As chemoreception plays such a dominant role in the control of breathing during sleep, alterations in chemosensitivity and its pathophysiologcal role in the development of SDB have been researched extensively. However in order to describe how chemosensitivity could contribute to the development of SDB one must first describe SDB in detail.

1.5 Sleep Disordered Breathing (SDB)

SDB or what is commonly referred to as sleep apnoea, is a breathing disorder whereby breathing repeatedly attenuates and/or ceases during sleep. These reductions (hypopnoeas) and cessations (apnoeas) in breathing can be a mediator for cardiovascular disease and numerous other additional disorders. Furthermore, the cyclic hypopnoeas and apnoeas result in repetitive arousals from sleep causing chronic sleep fragmentation and debilitative daytime sleepiness. There are two main types of SDB, obstructive sleep apnoea (OSA) and central sleep apnoea (CSA).

OSA is characterised by a repetitive termination in ventilation during sleep caused by the collapse of the pharyngeal airway. Each obstruction is coupled with a continuation in the drive to breathe, causing forced inspirations of increasing force to occur against the closed airway.

Although CSA shares a number of similarities with OSA one key difference is the causal factor mediating a central apnoea. Instead of breathing being prevented by an obstruction in the airway, a central apnoea is instead characterised by a lack of ventilatory drive from the

respiratory regions within the brain. However, this does not rule out the possibility of an obstruction occurring in conjunction with an abolishment of the drive to breathe. This is because as explained previously the central regions governing the respiratory drive to breathe also have innervations to regions involved in neuromuscular control of the upper airway muscles. Termination of central respiratory drive will also lessen to drive to the airway muscles making the airway much susceptible to collapse.

Each hypopnoea and apnoeic episode causes hypoxia and progressive hypercapnia. The intermittent nature of sleep apnoea exposes patients to chronic intermittent hypoxia and hypercapnia. Apnoeas are characterised by a minimum of ten seconds of ventilatory cessation (Levitzky, 2008; Punjabi, 2008) until the eventual stimulated airway re-opening or re-initiation of ventilatory drive. Furthermore, forced inspirations against the closed airway result in progressive negative intrathoracic pressure to occur with each attempted inspiration. These characteristic features of SDB, are the mediators for the development of cardiovascular and metabolic diseases in tangent with SDB (Somers *et al.*, 2008).

The severity of sleep apnoea can be represented as an apnoea-hypopnoea index (AHI), indicating on average the number of hypopnoeas and apnoeas one suffers during an hour of sleep per night. The different levels of AHI severity are shown in table 1.1.

Severity of Condition	**AHI**
Mild	5-15
Moderate	15-30
Severe	>30

Table 1.1. Showing the severity levels of apnoea-hypopnoea index (AHI); the average number of apnoeas and hypopnoeas (Kryger *et al.*, 2011).

1.5.1 Prevalence

Epidemiological studies in numerous countries have shown that a large number of the people suffer from OSA, pointing to a globally prevalent condition (Young *et al.*, 1993; Bixler *et al.*, 2001; Ip *et al.*, 2001; Kim *et al.*, 2004; Udwadia *et al.*, 2004). Results from these studies suggest that 1 in 5 of all adults experience a mild form of OSA (AHI≥5), and 1 in 15 suffer from moderate to severe OSA (AHI≥15). An average of the data collected from population based studies also shows that 4% of males and 2% of females suffer from OSA. Additional research by Young *et al.* (1997) suggest that 85% of those suffering OSA remain undiagnosed. Thus suggesting that the figures of prevalence presented above represent a fraction of the true number of OSA suffers. The Wisconsin sleep cohort (Young *et al.*, 1993), a longitudinal study investigating various factors of OSA over an 8-year period, shows a progressive worsening of the condition of OSA with time. Data demonstrated that either the condition itself or additional external factors could play a role in its further development. One relating factor to the prevalence and progression of OSA highlighted by The Wisconsin sleep cohort was age. The prevalence of CSA is also very strongly related with advancing age, with research estimating that the prevalence of mild central apnoea for men in the age groups 20-44, 45-64 and 65-100 were 0%, 1.7% and 12% respectively (Bixler *et al.*, 1998; Bixler *et al.*, 2001).

1.5.2 Ageing and SDB

As previously mentioned ageing is associated with an increased prevalence of sleep disorders. The suggested prevalence of SDB among people aged over 60 years is within the region of 45 - 62% (Ancoli-Israel & Ayalon, 2006). This is a staggering number considering

that the number of undiagnosed sufferers in this particular population could be considered to be even greater than that suggested by Young *et al.* (1997). The reasoning behind this suggestion is because symptoms of SDB such as fatigue, daytime sleepiness and hypertension are themselves conditions associated with advancing age. Therefore the link between these symptoms and SDB are often ignored by the sufferer and even potentially by clinicians. This statement is also true for heart failure patients who suffer from sleep apnoea. A recent study revealed that out of a cohort of nearly 30,000 heart failure patients only 2% were screened for sleep apnoea, when the prevalence of sleep apnoea in heart failure patients is approximately 50% (Javaheri *et al.*, 2011).

Furthermore, the elderly are an already vulnerable population open to greater risk from disease. Adding an increased likelihood of the development of SDB with age puts this population at an even greater risk of developing other conditions and diseases that can be associated with SDB.

1.6 Causes of Apnoeas during Sleep

The pathophysiology of obstructive and central apnoeas is inherently different. Areas of mechanical and neural control of the upper airway are key elements in the development of obstructive apnoeas. Conversely, aspects of respiratory control and its stability are dominant reasons for central apnoea development. Therefore, the pathophysiology of obstructive and central apnoeas will be discussed separately.

1.6.1 Obstructive apnoeas

Upper airway anatomy and the control of its patency are key features in the development of obstructive apnoeas. The upper airway is comprised of twenty muscles arranged to provide a rigid structure called the pharynx. The patency and calibre of the upper airway is governed by a complex interaction of numerous factors that can influence the vulnerability of the airway to collapse. These factors include the changing pressures within the upper airway during ventilation, the anatomical structure of the airway influencing its collapsibility and the neural input to the airway that determines its behaviour and tone. Furthermore, extrinsic factors can influence these interactions to further increase the susceptibility of the individuals to airway collapse.

Mathematical models are often used to describe the behaviour of the upper airway. An example of such a mathematical model used to commonly describe the upper airway is the Starling Resistor model (Gold & Schwartz, 1996), shown in Figure 1.6. However, it should be noted that an opposing view has been voiced regarding the generalisation of the Starling Resistor to all SDB patients (Butler *et al.*, 2013). However, this argument is beyond the scope of this thesis. In the Starling Resistor model, two rigid tubes bracket a collapsible tube that is surrounded by a region of external pressure or extraluminal pressure. The critical pressure (P_{crit}), is the external pressure at which a collapse of the airway occurs. When the pressure within the mid region or intraluminal pressure falls below P_{crit} the mid region collapses. The two rigid tubes on either side of the collapsible segment represent the upstream (nasal and mouth) and downstream (tracheal) areas of the upper airway. It is the pressures of these two areas that govern the intraluminal pressure.

For inspiration to occur downstream pressure must fall below atmospheric pressure. If, during inspiration, downstream pressure falls below P_{crit} a limitation to flow is induced by the narrowing of the airway. Schwartz and colleagues defined a limitation to flow as the initial rise in airflow through the airway, which then reaches a plateau of maximal flow despite any further increases in inspiratory effort. However, a complete collapse of the airway does not occur immediately (Schwartz *et al.*, 1988; Smith *et al.*, 1988). A complete collapse does not occur under these circumstances because the flow or ventilation through the collapsible region is governed by the gradient between upstream pressure and P_{crit}. Therefore, if upstream pressure is greater than downstream pressure flow will continue to occur. Ventilation under these circumstances can be calculated by equation 2, assuming that downstream pressure remains below P_{crit}.

$$V_E = \frac{P_{UP} - P_{CRIT}}{R_{UP}} \quad \textbf{(2)}$$

Where V_E is ventilation, P_{UP} and P_{CRIT} represent upstream and critical pressure, respectively and R_{UP} is the upstream resistance. Commonly, manifestations of hypopnoeas and/or snoring during sleep present under these circumstances of pressure changes. The upstream pressure becomes further attenuated as ventilation decreases further, due to the increase in airway resistance. As upstream pressure decreases to below the P_{crit} value, causing a positive P_{crit}, a complete occlusion of the airway occurs. Airway occlusions can be prevented by maintaining the upstream pressure above P_{crit} values. The most common treatment for OSA, Continuous Positive Airway Pressure (CPAP), augments the upstream pressure by applying a positive pressure to the nasal and/or mouth region of the patient thus preventing a positive P_{crit} from occurring.

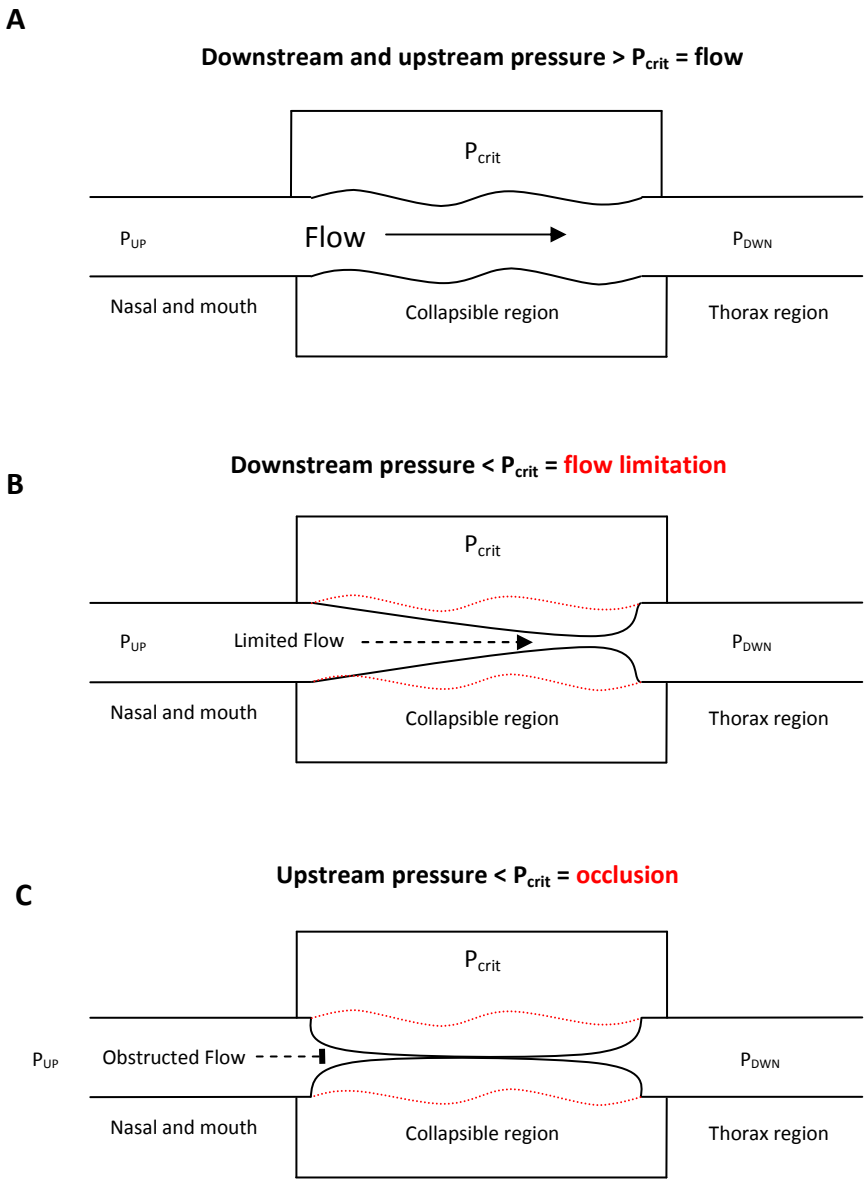

Figure 1.6 The Starling Resistor model. A) the airway is fully open allowing unobstructed inspiration to occur. B) the downstream pressure (P_{dwn}) reduced to a level lower than Critical pressure (Pcrit) causing flow limitation. C) A full obstruction to flow occurs when upstream pressures (P_{up}) reduce to below Pcrit values.

1.6.2 Factors affecting the P_{crit} value

P_{crit} values are a determining characteristic feature between healthy individuals and those suffering with SDB. A negative P_{crit} value will maintain airway calibre and airflow and a positive P_{crit} will cause a collapse of the airway. As has been described above, positive P_{crit} values can occur when upstream pressures fall below P_{crit}. This can be demonstrated by mechanically applying a negative nasal pressure, via nasal suction, in healthy sleeping individuals. In this instance upstream pressure falls below P_{crit} causing airway occlusion to be induced (Schwartz *et al.*, 1988). However, this clearly does not occur in OSA patients. In these patients, it is the increase in the P_{crit} value towards positive values, which increases the likelihood of apnoea development. P_{crit} is augmented in SDB patients through alterations in "passive" anatomical and/or "active" neuromuscular features.

The patency of the airway is controlled by neuromuscular input and is modulated by a variety of sensory inputs. At sleep onset the neural control of airway patency is markedly reduced, resulting in a lessening of airway tone. A reduced airway tone effectively increases the P_{crit} making the airway more susceptible to narrowing and collapse during inspiration (Patil *et al.*, 2007; McGinley *et al.*, 2008). The presence of this neuromuscular input during wakefulness prevents a positive P_{crit} from occurring during wakefulness, even in SDB patients (Dempsey *et al.*, 2010). In healthy individuals there are a number of neuromuscular reflexes that maintain the airway tone during sleep and prevent the collapse of the airway. One particular reflex prevents the narrowing of the airway during inspiration, such as in Figure 1.4B. In the occurrence of a narrowing, the displacement of the airway stimulates

mechanoreceptors located within the larynx resulting in a reflex contraction of upper airway dilator muscles to reduce airway collapsibility (Dempsey *et al.*, 2010). Furthermore, during inspiration, negative pressure causes a reflex excitation of pharyngeal airway dilator muscles to ward off airway narrowing. Additionally, negative pressures cause an inhibition of diaphragmatic activity to reduce resultant negative pressure during inspiration (Harms *et al.*, 1996). Lastly, chemical stimuli can result in the reflex activation and inhibition of upper airway muscles. Chemoreceptors have afferent nerve input to the muscles of the upper airway that can influence the tone of the upper airway muscles and thus airway patency (Bruce *et al.*, 1982; Mifflin, 1990; Kc & Martin, 2010). Previous research has also shown that hypercapnia and hypoxia result in reflex activation of the hypoglossal and laryngeal muscles to increase airway patency. Hypocapnia will result in the opposite effect, an inhibition of airway muscles (Weiner *et al.*, 1982; Lo *et al.*, 2006).

In healthy individuals these reflexes work to reduce airway compliance in order to prevent airway narrowing and collapse. However, reflex sensitivity of this kind has been shown to be blunted in OSA patients (Fogel *et al.*, 2001; Nguyen *et al.*, 2005; McGinley *et al.*, 2008; Chin *et al.*, 2012), rendering the airway more susceptible to increasing extraluminal pressures and collapse. At sleep onset, OSA patients experience a greater decrease in sensory input to the upper airway muscles, which immediately puts patients at greater risk of apnoea development (Fogel *et al.*, 2001). Additionally, neuromuscular airway reflexes have been shown to be dampened in OSA patients when faced with an airway obstruction (Nguyen *et al.*, 2005; McGinley *et al.*, 2008). Lastly and more important to the topic of this thesis, the reflex ventilatory responses to airway obstruction appear to be greater in less severe OSA patients with mild AHI when compared to patients with a greater AHI (Chin *et al.*, 2012). The

research by Chin *et al.* (2012) suggests a reduction in chemoreflex responses to airway occlusion results in a greater risk from airway collapse through reduced chemosensitive reflex actions.

Interestingly, the impairment of sensory feedback has also been shown to be regenerated slightly following treatment with CPAP (Kimoff *et al.*, 2001). The improvement of sensory function following treatment suggests a debilitate decline in function arising from the condition of sleep apnoea itself.

An example of mechanical or "passive," alterations, to the upper airway that will affect P_{crit} is an increase in anatomical loading. A chief example of increased intraluminal loading in OSA patients is obesity. Large deposits of adipose tissue surrounding the airway region of obese patients subject the airway to greater extraluminal pressure, decreasing the P_{crit} value to levels more susceptible to airway collapse. It is unsurprising then that the most prominent factor in the development of obstructive apnoeas is obesity (Young *et al.*, 2004), with an estimated 60% of middle aged OSA sufferers being classed as obese (Punjabi, 2008).

An additional anatomical risk factor for obstructive apnoeas is the presence of structural abnormalities within the upper airway. An enlargement of upper airway structures is characteristic in many sleep apnoea patients. These enlargements reduce the calibre of the upper airway (Schwab, 2003), causing greater airway resistance and increase the likelihood of obstruction occurring under reduced muscular tone during sleep (Schwab *et al.*, 2003).

1.6.3 Central apnoeas

An important aspect in the development of central apnoeas is the apnoeic threshold. When $PaCO_2$ moves beyond the apnoeic threshold, it will cause a central apnoea in order to cause an accumulation of CO_2 and return $PaCO_2$ back to normal levels. As previously described reductions in $PaCO_2$ are brought about by increases in ventilation. Large increases in ventilation to a stimulus result in an expiration of CO_2 reserve, reducing $PaCO_2$ to an apnoeic threshold. The most common stimulant of the hyperventilation is high chemosensitivity, or loop gain, response to changes in blood gases during sleep that result in periodic breathing. see figure 1.7 for explanation of how high chemosensitivity can increase the likelihood of a central apnoea occurring during sleep.

Sub-conscious arousals from sleep provide additional occurrence of hyperventilation that may bring about an apnoea. In the occurrence of an arousal mediated hyperventilatory response that will destabilise the system, particularly a system with a high chemosensitivity. The destabilisation will lead to oscillatory ventilation (periodic breathing) and eventually the crossing of the apnoeic threshold. Subsequent arousals are a common feature at the nadir of apnoeas, and therefore can result in cyclic central apnoeic events (Younes, 2004).

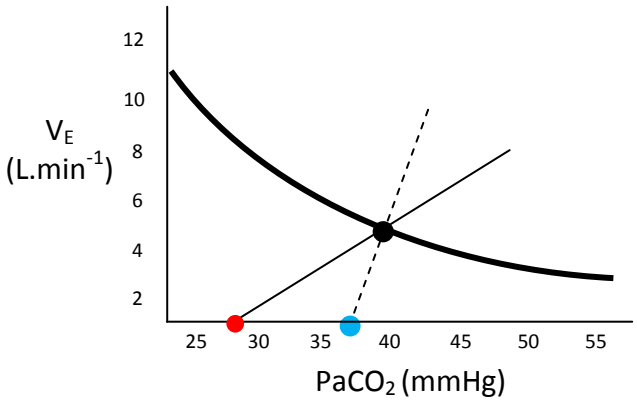

Figure 1.7 Graphical representation of an example of how high chemosensitivity can increase the likelihood of a central apnoea occurring. A greater chemosensitivity (dotted line) in comparison to normal (solid line) will result in greater ventilatory response for a given increase in PCO_2, which will lead to a greater resultant decline in $PaCO_2$ (due to plant gain). This coupled with the higher apnoeic threshold as a result of greater controller sensitivity (blue dot), significantly increases the probability of a central apnoea occurring.

1.6.4 The role of low chemosensitivity in apnoea development

High chemosensitivity is the most common form of apnoea development in sleep apnoea patients. Although low chemosensitivity by definition means a more stable loop gain, there are a number of mechanisms with which low chemosensitivity could lead to apnoea development.

One such mechanism is through the reduced ventilatory response to CO_2 (controller gain) and an increase in normal $PaCO_2$ (operating point). These two changes together can lead to a greater chance of central apnoeas occurring because of an increased plant gain and apnoeic threshold, see figure 1.8. Although CO_2 reserve will also increase serving, as it does during sleep, as a protection against crossing the apnoeic threshold. The degree of the decline in controller gain will determine the level of the protection given by the CO_2 reserve.

Nevertheless, a greater operating point will lead to the increased occurrence of hypopnoea during sleep, upon removal of the wakefulness drive to breathe (Kryger *et al.*, 2011).

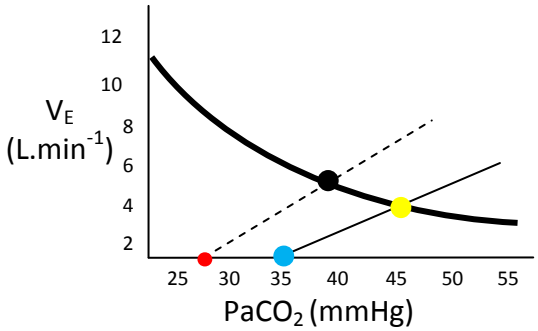

Figure 1.8 Example of how a reduction in chemosensitivity (solid line) from the norm (dotted line) could possibly result in an apnoeic event. An increased operating point (yellow dot) will increase the chances of hypopnoea occurrence. Also, such an increase will affect the plant gain, such that it will cause greater changes in $PaCO_2$ that could cross the higher apnoeic threshold. Adapted from Kryger *et al.* (2011) Figure 100-5.

A second mechanism for which low chemosensitivity may influence the development of apnoeic events is through the reduction in efferent feedback to the upper airway dilator muscles. Such a reduction could lead to a greater airway resistance and thus increased chance of obstructive hypopnoeas and/or apnoeas occurring.

Lastly, reduced chemosensitivity could also influence the severity of an apnoeic event through the reduction in the arousal threshold in response to hypercapnia during an apnoea. This would lead to the lengthening of each cessation in ventilation and greater levels of hypoxemia and hypercapnia during each apnoea. These consequences could possibly result in the development of secondary conditions as a result of the increased levels of hypoxia.

1.6.5 Causes of apnoeas in older individuals

Although it is a cemented fact that obesity is a strong mediator of obstructive apnoeas, the increased prevalence of sleep apnoea in older populations is reported to be independent of obesity levels (Bixler *et al.*, 2001; Tishler *et al.*, 2003). Additional mechanisms are at play in the age associated rise in the prevalence of OSA. Such mechanisms include an age-related reduction in upper airway muscle dilator tone (Crow & Ship, 1996), which makes the airway more susceptible to increased extraluminal pressures with age (Kirkness *et al.*, 2008). Furthermore, an impairment of pharyngeal sensory activity with age (Pontoppidan & Beecher, 1960; Aviv *et al.*, 1994) is thought to reduce the protective upper airway reflexes that prevent airway collapse during sleep. All these could play a role in the increased risk of pharyngeal collapse during sleep in older individuals (Eikermann *et al.*, 2007).

Alterations in respiratory chemosensitivity with age could play a role in the development of apnoeas during sleep. Such alterations could decrease the reflex maintenance of airway tone that increases the risk of airway collapse, as descried previously. Additionally, reduced sensitivity to both hypercapnia and hypoxia could also possibly delay the reflex opening of the upper airway, therefore prolonging an apnoea and exposing the patient to further oxygen desaturation. Conversely, an increased chemosensitivity in older individuals increases the likelihood of periodic breathing, resulting in a central apnoea development. Equally, reductions in chemosensitivity in elders could result in central hypopnoeas (reductions in flow absent of airway narrowing) and possible central apnoeas (Wellman *et al.*, 2007). The age associated alterations in respiratory chemosensitivity will be discussed in greater detail in Chapter 4.

1.7 SDB and Cardiovascular disease

SDB has been shown to have a strong casual relationship with cardiovascular disease (Somers *et al.*, 2008). A wealth of research has investigated the mechanisms behind such a strong relationship. However, SDB is also strongly associated with a number of other co-morbidities such as obesity, type II diabetes and metabolic syndrome. The strong link with additional conditions creates difficulty when attempting to distinguish between mechanisms arising solely from SDB and those associated with the additional co-morbidities. Furthermore, because of the numerous mediators of cardiovascular diseases present in SDB alone, such as intermittent hypoxia and hypercapnia, negative intrathoracic pressures and sleep disturbances it is difficult to determine which mechanism or mechanisms contribute to the development of cardiovascular ailments and how. Therefore, a number of studies have exposed healthy participants to single mediators, such as intermittent hypoxia to investigate their contribution to the development of cardiovascular disease. What will follow is a brief description of the two forms of hypertension that have a high level of prevalence in SDB patients; systemic and pulmonary hypertension.

1.7.1 Systemic Hypertension

Hypertension is the most common disease associated with SDB. A study conducted by the Wisconsin sleep cohort (Peppard *et al.*, 2000), investigated the relationship between the severity of SDB and hypertension over a four year period. The authors showed a significant positive relationship between the occurrence of hypertension and the severity of SDB.

The intermittent hypoxia experienced each night is thought to be the strongest mediator of augmented blood pressure in sleep apnoea patients. The process in which episodic hypoxia

mediates an augmentation in blood pressure is through the stimulation of sympathetic nerve activity. The hypoxia and hypercapnia that occur during an apnoea, act on the peripheral chemoreceptors stimulating sympathetic outflow via the NTS (Prabhakar *et al.*, 2005). Sympathetic nervous activity causes vasoconstriction of peripheral circulation and thus a progressive augmentation of blood pressure during each apnoeic episode (Somers *et al.*, 1995). The repetitive exposure of the chemoreceptors to hypoxia causes a sensitization to hypoxia and a continuation of increased sympathetic activity in rats (Greenberg *et al.*, 1999; Prabhakar *et al.*, 2005) and in humans (Cutler *et al.*, 2004). The sensitisation is mirrored in an increase level of resting sympathetic nerve activity in awake sleep apnoea patients (Carlson *et al.*, 1993; Narkiewicz *et al.*, 1998). The removal of chronic intermittent hypoxia through six months of treatment with CPAP has been shown to attenuate the sympathetic activity in patients in comparison to those not receiving treatment (Narkiewicz *et al.*, 1999). However, the investigators did not observe any attenuation in blood pressure in treated patients. This finding was also seen by Hedner *et al.* (1995). Treatment by CPAP has shown mixed results over the past. A recent meta-analysis of studies investigating the effect of CPAP treatment on blood pressure found an overall reduction in diastolic and systolic blood pressure following treatment with CPAP. However, the reported reductions in blood pressure were very small, with an average reduction of blood pressure within the region of 1.9 – 2.6 mmHg (Fava *et al.*, 2013). Possible reasons for the small decreases in blood pressure and the null findings by other previous studies following treatment include the use of normotensive participants and the possible presence of more long-term mediators of hypertension such as atherosclerosis and vascular re-modelling.

An additional cause of hypertension in sleep apnoea patients is the presence of endothelial dysfunction (Kato *et al.*, 2000b). A reduction in nitric oxide bioavailability in sleep apnoea patients has been shown to be one mechanism behind the reduction in endothelial function (Ohike *et al.*, 2005). The reduction in nitric oxide production can lead to vasoconstriction, platelet aggregation, smooth muscle cell proliferation and leukocyte adhesion which can all lead to the development of hypertension (Davignon & Ganz, 2004). Intermittent hypoxia is once again suggested to be a key cause of vascular dysfunction in SDB patients. Intermittent hypoxia is characterised by a repetitive de-oxygenation and re-oxygenation phases of the blood. This cycle of de-oxygenation and re-oxygenation leads to an increase in ROS release from the electron transport chain and eventual oxidative stress (Pialoux *et al.*, 2009; Pialoux *et al.*, 2011). Increased oxidative stress can directly affect the activity and production of nitric oxide (Davignon & Ganz, 2004; Pialoux *et al.*, 2011) thus reducing vascular function.

Short periods of treatment have been shown to improve vascular function, with research showing improvement after just one week and further improvement after a month of treatment with CPAP (Ip *et al.*, 2004; Ohike *et al.*, 2005). Reductions in oxidative stress after such a short period could account for augmentation of vascular function (Jelic *et al.*, 2008). An additional contributor to the quick recovery of vascular function could be the ability of CPAP to immediately attenuate sleep disruption and improve sleep quality (Issa & Sullivan, 1986). Total sleep deprivation studies have shown a decrease in vascular function in participating individuals (Takase *et al.*, 2004a; Sauvet *et al.*, 2010) suggesting that the sleep disruption experienced in SDB patients could contribute to the vascular dysfunction observed. However, these studies use a total 24 hour deprivation of sleep which is not representative of SDB sleep. An additional aim of this thesis is to investigate the effect of

shortened sleep length on vascular function. This will be discussed in more detail in Chapter 4.

1.7.2 Pulmonary hypertension

A number of studies have investigated the prevalence of pulmonary hypertension in patients suffering SDB. Atwood *et al.* (2004) reviewed the large body of research into the prevalence of pulmonary hypertension in sleep apnoea patients. They found that research showed a range of 17 – 53% of patients presented with pulmonary hypertension. Despite many of the studies in this analysis not controlling for the presence of co-existing cardiopulmonary conditions, studies by (Bady *et al.*, 2000) and (Sajkov *et al.*, 1994), that did control appropriately for these still found that 41% and 27% of patients presented with pulmonary hypertension, respectively. Atwood *et al.* (2004) also found age and obesity to be strong positive correlates with the magnitude of pulmonary hypertension. Treatment studies have further suggested a causal relationship between SDB and pulmonary hypertension. Arias *et al.* (2006) showed a significant reduction in systolic pulmonary pressure following 12 weeks of treatment with CPAP.

One clear mediator of the pulmonary hypertension in SDB patients is intermittent hypoxia (Zieliński, 2005), this is because, unlike in the systemic circulation, hypoxia stimulates a vasoconstriction of the pulmonary circulation. Hypoxic pulmonary vasoconstriction (HPV) is a unique characteristic of the pulmonary system where alveolar hypoxia results in constriction of the pulmonary vessels. An augmentation in the HPV with age could put older individuals suffering from SDB at greater risk from pulmonary hypertension. Chapter 6 in this thesis will investigate the effect of age on the magnitude of hypoxic pulmonary vasoconstriction.

1.7 Principal questions investigated this thesis

1. Does ageing affect the hypercapnic chemosensitivity response and what is the manner of this effect in terms of differential chemoreceptor gains?
2. Does ageing have any influence on the hypoxic ventilatory sensitivity?
3. Does the use of hyperoxia during the assessment of chemosensitivity in older individuals influence the findings?
4. Can sleep loss over consecutive days change respiratory CO_2 chemosensitivity and will it influence the breathing during sleep?
5. Does sleep fragmentation similar in nature to SDB effect CO_2 chemosensitivity and what is the nature of this effect?
6. Does sleep loss and/or sleep fragmentation effect the chemosensitivity in a similar way to changes observed in older individuals in this thesis?
7. Does ageing effect the hypoxic pulmonary vasoconstricition, predisposing older suffers of SDB to a greater risk of developing pulmonary hypertension?

CHAPTER 2.

GENERAL METHODS

A number of different methods were used in this thesis to obtain the measurements desired in each study. These methods will be described in detail in this chapter.

2.1 Gas control

In the studies conducted in this thesis end-tidal gases were manipulated in order to expose participants to different respiratory stimuli. To accurately control the exposure of respiratory stimuli a method of inspired gas control was adopted.

The feedback and correction nature of the respiratory control system creates obstacles for when attempting to investigate the effects of sustained gas stimuli. For example, during acute hypoxia, ventilation is increased with the purpose of increasing O_2 uptake to correct blood gas levels. Therefore, it is difficult to maintain a hypoxic stimulus for a prolonged period because the low O_2 levels are continuously corrected by the feedback loop in order to maintain O_2 homeostasis. An additional issue is the ability to control individual gases. For example, during hypoxic stimulated hyperventilation, $PaCO_2$ levels also decrease because of the augmentation in ventilatory output resulting in hypocapnia. This means that not one single variable can be investigated at any one time. Therefore, in order to create an accurately controlled respiratory stimulus these difficulties arising from the closed loop control must be overcome.

One method with which to alter the closed loop control of respiration is through alterations in inspired gas composition. To solve the problems in the hypoxic example above, continuous inspiration of a low O_2 gas mixture can be used to achieve a sustained hypoxic level. The addition of CO_2 to the inspired gas can also be used to avoid the subsequent hypocapnia brought about by the HVR.

It is important to measure the changes in blood gas levels so that the accuracy of inspired gas control can be monitored. However, directly monitoring blood gas levels continuously is both invasive and difficult. Therefore, an additional method of measurement is needed. The measurement of end expiration (end-tidal) partial pressures of O_2 and CO_2 is considered to be an accurate representation of alveolar O_2 and CO_2 partial pressures, which in healthy humans with normal ventilation-perfusion matching in the lungs is similar to arterial partial pressures. End-tidal partial pressures can therefore be used to assess the accuracy of inspired gas control.

Together, control of inspired gas composition and end-tidal monitoring can achieve sustained and measureable gas stimuli. However, this method can still present with large cross-subject variability, meaning that one particular inspired gas composition may create different stimulus conditions across participants. Furthermore, large breath–by–breath variability within participants will cause inaccuracies during sustained stimulus delivery.

Swanson and Bellville (1975) devised a system to overcome these limitations by accurately predicting inspired gas compositions to create a given stimuli in given participants. Also, a correction scheme to correct breath-by-breath deviations during gas delivery was created. End-tidal partial pressures were used to monitor and correct the composition of inspired gases. The system was called a dynamic end-tidal forcing (DEF) system.

Prior to the delivery of a gas stimulus, a prediction of the inspired gas compositions is made using a formula based on the circulation and respiratory systems (equation 3). During the actual delivery of gas stimuli, breath-by-breath alterations in inspired gas compositions can be made for correction of end-tidal values. Whereby, the actual end-tidal levels are compared to desired end-tidal levels to formulate the necessary corrections using equation

4. A DEF system minimizes breath-by-breath deviations from the desired partial pressures, thus maximising control. Swanson's initial DEF system was adapted a few years later to better achieve fast step changes in gas control (Robbins *et al.*, 1982). A DEF system was used throughout this thesis for all respiratory gas control and it is this adaptation to the prediction-correction scheme that was used.

2.1.1 Prediction

The prediction mathematical model is run offline prior to an experiment. This will produce a predicted set of inspired values for the production of desired end-tidal partial pressures at specific time points. The mass balance equations of CO_2 and O_2 at the lung are used as a starting point for the calculation of inspired values. The partial pressure of inspired gas (P_I) can be solved as follows:

$$P_I = \frac{\dot{V}_A P_A + V_L . {}^{dP_A}/_{dt} + \lambda \dot{Q}(Ca - C\bar{v})}{\dot{V}_A} \quad \textbf{(3)}$$

Where V_L is the mean lung volume; P_A is the partial pressure of the gas in question in the alveoli; $\dot{V}_A$ is the alveolar ventilation; $C\bar{v}$ is the mixed venous concentration of gas in the blood; $\dot{Q}$ is the blood flow through the lungs; and λ is a particular coefficient depending on the units. This equation is solved using a multi-compartment model of the circulation and a two-compartment model (representing central and a peripheral component) of respiratory control.

2.1.2 Correction

The correction factor is a feedback loop comparing the actual end-tidal partial pressures with the desired values. The correction to inspired partial pressures for each breath ($P_{I(n)}$) can be derived using the following formula:

$$P_{I(n)} = P_{I_{C(n)}} + g_p \cdot \left(P_{ET_{d(n-1)}} - P_{ET_{m(n-1)}}\right) + g_i \cdot \left(\sum_{j=1}^{n-1}(P_{ET_{d(j)}} - P_{ET_{m(j)}})\right) \quad \textbf{(4)}$$

Where P_{IC} is the predicted inspired partial pressure; P_{ET_d} is the desired end-tidal partial pressure; P_{ET_m} is the measured end-tidal partial pressure; g_p is the proportional gain and g_i is the integral gain. The proportional gain prevents breath by breath variation and the integral gain uses the sum of intra-breath differences to prevent drift and steady state error.

2.2 Gas delivery system

The delivery of inspired gases and the monitoring of respiratory parameters were conducted using the following apparatus. Participants breathed through a mouth piece whilst wearing a nose clip to prevent breathing through the nose. A clear plastic turbine (Interface Associates, Irvine, CA, USA) was connected to the mouth piece in order to measure tidal volume. A photodetector pick up surrounded the turbine and detected the turning of the impeller within. The speed and direction of the impeller's rotation represented the magnitude and direction of flow, respectively thus quantifying inspired and expired volumes. A pneumotach (Pneumo Hans Rudolph pneumotach amplifier 1, Shawnee, USA) was connected in series with the turbine to detect flow initiation and cessation. A pneumotach was preferred over a turbine for this use because the inertia of the turbine presented a delay in the detection of inspiration/expiration termination. The minimisation of the delay is important for the

accurate detection of the end of the breath, so that precise end-tidal values can be obtained. Gas samples were taken continuously through a catheter placed immediately after the mouth piece. Gas levels from the catheter were measured by a gas mass spectrometer (AirSpec 2000, Case Scientific, London, UK) sampling at a rate of 20 $mL.min^{-1}$. Information from the mass spectrometer, turbine and pneumotach were fed into a computer program, where current end tidal partial pressures were compared with desired partial pressures.

Once a new inspired gas composition was calculated a signal was sent to a pair of control boxes, each controlling two of four gases, O_2, CO_2, N_2 and compressed air. The control boxes used mass flow controllers for the adjustment of gases delivered for participant inspiration. Valves of the mass flow controllers were opened according to the voltage sent to the control boxes.

Gases were fed through humidifiers to provide moisture so that it was more comfortable for the participant to breathe the supplied gas mixture. See next page for a schematic of the gas delivery system; figure 2.1.

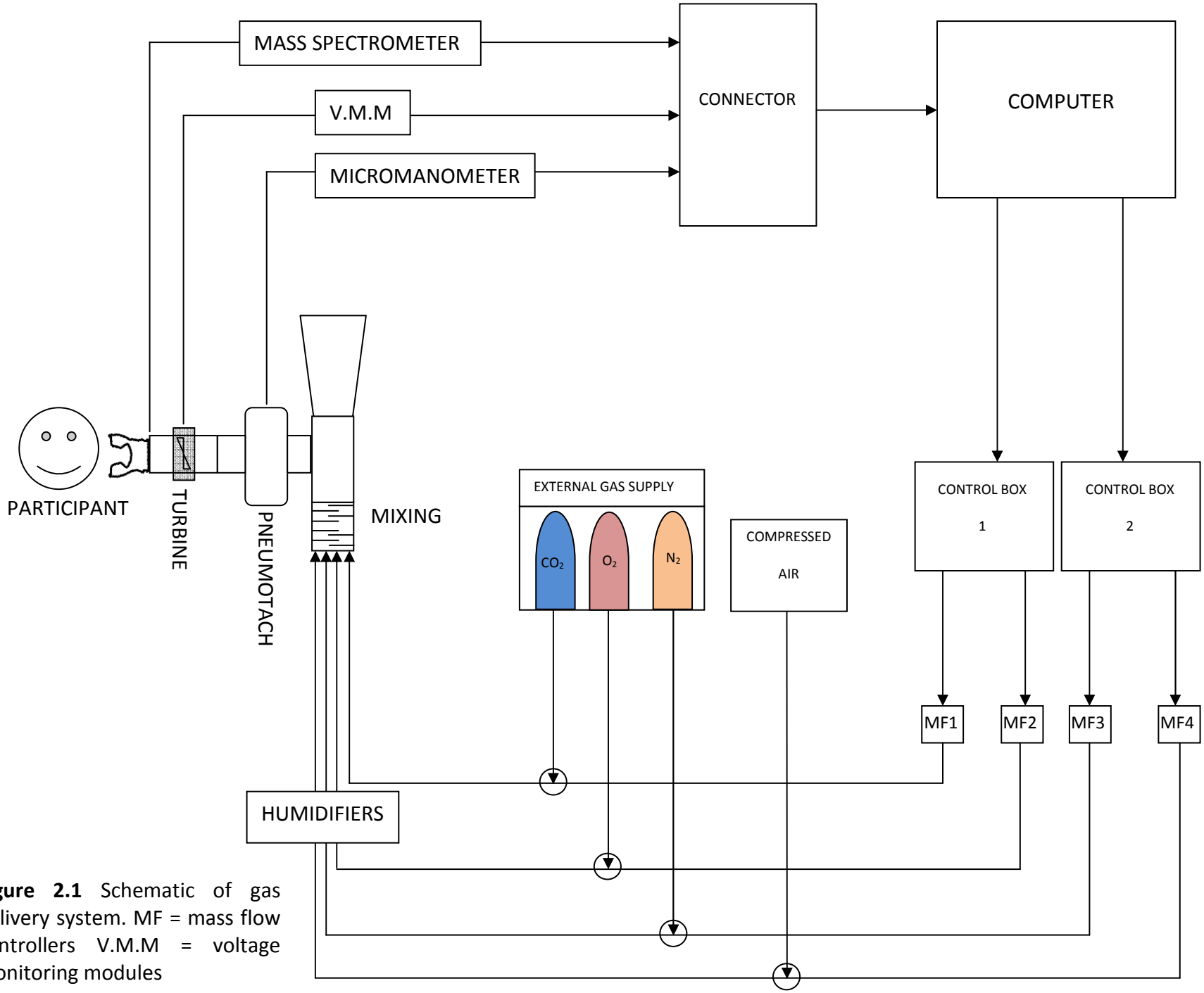

Figure 2.1 Schematic of gas delivery system. MF = mass flow controllers V.M.M = voltage monitoring modules

2.3 Respiratory Protocols

A number of respiratory protocols were used in the studies conducted in this thesis. These will now be discussed in detail.

2.3.1 Multi-frequency binary sequence (MFBS) test

A MFBS test was used to determine peripheral and central CO_2 chemosensitivity as used by Pedersen *et al.* (1999b). The test was preceded with five minutes of isocapnic euoxia ($P_{ET}O_2$ = 100 mmHg, $P_{ET}CO_2$ = 1 mmHg > normal level), following which the MFBS test was completed. The test consisted of thirteen steps of hypercapnia varying in length of exposure time. During these steps $P_{ET}CO_2$ was increased to 10 mmHg above participants' normal levels. Between hypercapnic steps $P_{ET}CO_2$ was controlled at 1 mmHg above normal. The binary sequence lasted for 1408 seconds. The MFBS covered six harmonics based on the Van den Bos Octave (Godfrey, 1993). The pulse duration was 11 seconds. During euoxic and hyperoxic MFBS tests $P_{ET}O_2$ was controlled at 100 mmHg and 220 mmHg, respectively throughout the entire protocol. See figure 2.2 below for a graphical representation of the MFBS test.

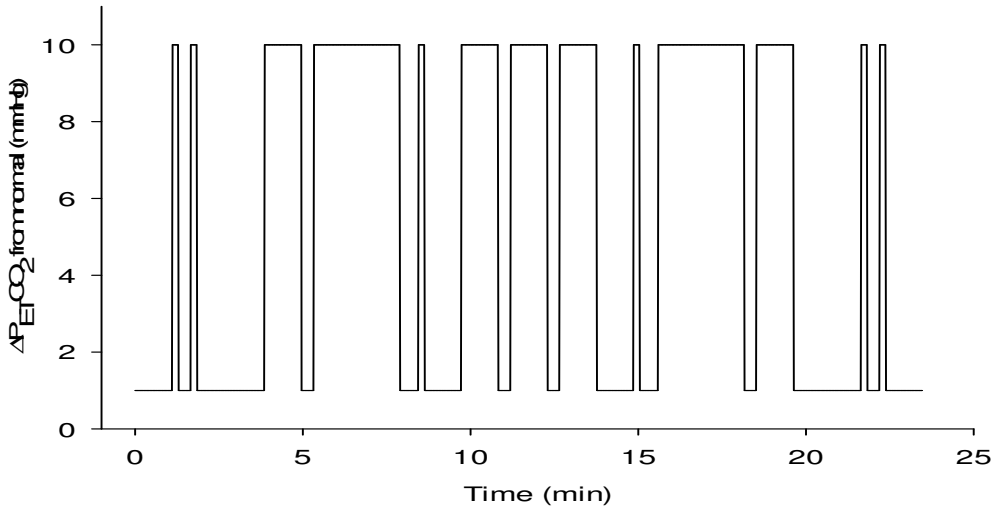

Figure.2.2 Showing the intermittent changes in $P_{ET}CO_2$ from 1mmHg above normal end-tidal to 10mmHg above during the MFBS test.

2.3.2 Hypercapnic Dose Response

A hypercapnic dose response protocol was used alongside Doppler ultrasound measurements to test the cerebrovascular reactivity of participants. The test consisted of four five-minute steps of hypercapnia. Steps were 1, 4, 7 and 10mmHg above participant's normal $P_{ET}CO_2$. The protocol was preceded with 5 minutes and was terminated with 2 minutes of isocapnic euoxia. $P_{ET}O_2$ was controlled at euoxic levels throughout. See figure 2.3 for a graphical representation of the hypercapnic dose response protocol.

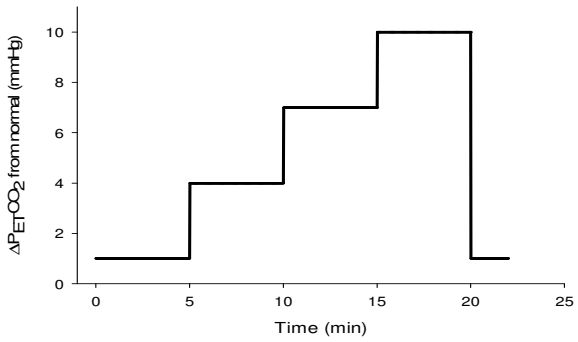

Figure 2.3 Representing the step changes in $P_{ET}CO_2$ from +1, +4, +7 and +10mmHg used during the hypercapnic dose response.

2.3.3 Acute hypoxic ventilatory response (AHVR) test

During measurement of hypoxic pulmonary vasoconstriction an AHVR test was used to expose participants to sustained hypoxia. The test consisted of five minutes of isocapnic euoxia, followed by twenty minutes of isocapnic hypoxia ($P_{ET}O_2$ = 50 mmHg, $P_{ET}CO_2$ = 1 mmHg above normal), followed by a further five minutes of isocapnic euoxia, see figure 2.4. This protocol was partnered with a control protocol of 30 minutes of isocapnic euoxia.

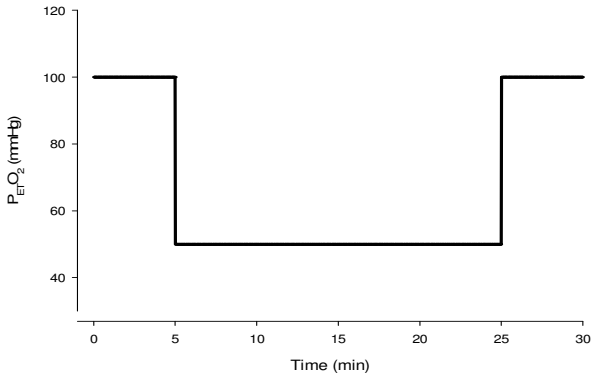

Figure 2.4 Step reduction in $P_{ET}O_2$ from 100 to 50 mmHg during acute hypoxic ventilatory response test.

During all protocols participants were sat in a comfortable chair breathing through the mouth piece. Light entertainment was provided to distract them during the protocols for the minimisation of volitional breathing that could impact on the results.

2.4 MFBS Modelling

The ventilatory responses during the MFBS test were modelled to deduce the sensitivity of both peripheral and central chemosensitivities. Total ventilation ($\dot{V}_E$) can be described as a function of central chemoreflex output ($\dot{V}_c$) and peripheral chemoreflex output ($\dot{V}_p$), where C is a trend term and *t* is time in equation 5 below.

$$\dot{V}_E = \dot{V}_c + \dot{V}_p + Ct \quad \textbf{(5)}$$

A two compartment model was used to assess both peripheral and central chemoreflexes during the MFBS test. Models of both central and peripheral gains are shown below in equation 6 and equation 7, respectively.

$$\tau_c \frac{d\dot{V}_c}{dt} + \dot{V}_c = G_c \left[P_{ET_{CO_2}} (t - d_c) - B\right] \quad \textbf{(6)}$$

$$\tau_p \frac{d\dot{V}_p}{dt} + \dot{V}_p = G_p \left[P_{ET_{CO_2}} (t - d_p) - B\right] \quad \textbf{(7)}$$

Where G_c is the central chemoreflex sensitivity, τ_c is the central chemoreflex time, d_c is the central transport delay time, G_p is the peripheral chemoreflex sensitivity, τ_p is the peripheral chemoreflex time and d_p is the peripheral transport delay time and B is a bias term equivalent to the $P_{ET_{CO_2}}$ for which $\dot{V}_c$ or $\dot{V}_p$ equal zero.

Assuming that $P_{ET}CO_2$ remains the same throughout the breath these differential equations can be solved using a set of difference equations for $\dot{V}_c$ and $\dot{V}_p$ (see *appendix 1*).

2.5 Doppler Ultrasound

A technique used to conduct a number of measurements throughout this thesis was Doppler ultrasound. Doppler ultrasound works on the principle of the Doppler Effect to produce 2D images and measure the direction and velocity of blood flow through a vessel or organ. Since its commercial availability in 1975 ultrasound has become a widely used tool in medical research, diagnosis and treatment. Being non-invasive, low-cost and giving highly reproducible data acquisition sees it preferred over other more costly and invasive techniques. It was adopted in this thesis to conduct echocardiograph measurements and for the assessment of flow-mediated dilation and cerebrovascular function.

2.5.1 Doppler Effect

In 1842 Austrian physicist Christian Doppler presented the Doppler Effect. The Doppler Effect describes how the frequency of waves emitted from an object, be they light or sound, are affected by the direction of the object's travel, relative to an observer's position.

Using the light of distant stars as an example, Christian Doppler showed that stars moving towards the earth emitted a high-wave frequency of light and therefore appearing blue in the night's sky. Stars moving away from the earth have a low frequency of light wave, appearing red; an effect termed red shift. The change in wave frequency in relation to the motion of an object is also true for the reflection of sound waves off an object. It is this principle that is used in Doppler ultrasound technology to detect the direction and velocity of blood flow and to create 2D images.

2.5.2 Doppler Ultrasound technique

Doppler Ultrasound uses a transducer containing a piezoelectric material, in this case crystals, which vibrate when an electrical current is applied to it. The vibration produces high frequency sound waves that are emitted from the transducer. The transducer also detects any sound waves reflected back towards the origin. A stationary object reflects the emitted sounds waves back to the transducer with the same frequency as the original waves. However, if the object in question, for example a red blood cell moving within a vessel, is moving away from the transducer the frequency will be lower. Conversely, it will be higher if the cell is moving towards the transducer. These changes in frequency can be depicted using the same colour scheme as that of Christian Doppler's moving stars; red showing movement away from the transducer and blue representing movement towards the transducer.

The change in frequency of the emitted sounds waves can be used to calculate the velocity of the moving object. If:

$$\Delta f = \frac{2vf\cos\theta}{C} \quad \textbf{(8)}$$

This formula can be re-arranged to calculate velocity:

$$v = \frac{\Delta f C}{2f\cos\theta} \quad \textbf{(9)}$$

Where; v is velocity, Δf is the frequency shift, C is the velocity of the sound in tissue (1540 $m.s^{-1}$), f is the transmitted frequency and θ is the angle between the direction of the flow and the beam (assumed to be 0^{o}).

2.5.3 Doppler Ultrasound Modes

The sound waves from the Doppler transducer can be emitted in two different fashions. A pulsed wave is used to measure low velocity flows at specific locations. The transducer emits

a single wave and waits for the return of the subsequent reflected wave before emitting a second. The location or depth of measurement can be changed by altering the duration of time that the transducer waits for the returning wave. However, because of the delay between pulses it does not allow for the accurate measurement of fast velocity flows. For such measurements continuous wave is used. During continuous wave Doppler the transducer is able to both emit and record returning waves continuously. This is made possible by the placing of two independent transducers aligned together within the head of the transducer, one to emit waves and one to record returning waves. However, because of the lack of pauses in emitted waves, like with a pulsed wave, the specific location of measurement cannot be made.

With both the above settings the returning frequency is converted into velocity using previously described equations 8 and 9, which is depicted in a spectral trace. The spectral trace represents flow on a positive and negative velocity y-axis, where a negative velocity represents flow away from the transducer and vice versa for a positive velocity.

2.6 Echocardiography

Certain echocardiography measurements were made in this thesis in order to calculate cardiac output and systolic pulmonary artery pressure. These parameters were continuously measured during the previously mentioned AHVR respiratory test.

2.6.1 Cardiac output

In order to determine cardiac output the following procedure was completed. First, an apical 5-chamber view of the heart was obtained, viewed through the 4^{th} & 5^{th} intercostal space,

see figure 2.5. From this image, blood flow velocity through the aortic valve, during the systolic phase of the cardiac cycle, was measured using pulsed wave Doppler. Blood flow was measured here because the aortic orifice has a fixed diameter, meaning velocity is the single changing variable. A mean of blood flow velocity for each systolic phase was then calculated from a spectral trace. The mean blood flow velocity was integrated with the duration of blood flow in order to produce a velocity time integral (VTI). VTI is the distance travelled by the blood pumped out from the heart during each beat. To measure VTI two conditions must be met, the flow through the aortic valve must be uniform and the vessel must be cylindrical.

Further to VTI measurements, the cross sectional area of the aortic orifice was calculated on a separate occasion. In order to calculate the cross sectional area of the aortic orifice the diameter (D) must first be measured via a parasternal long axis view of the heart, shown in figure 2.6.

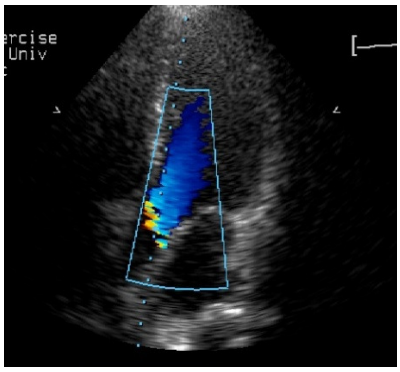

Figure 2.5 Apical 5 chamber view of the heart. Blue jet represents the blood moving away from the ultrasound beam through the aortic valve.

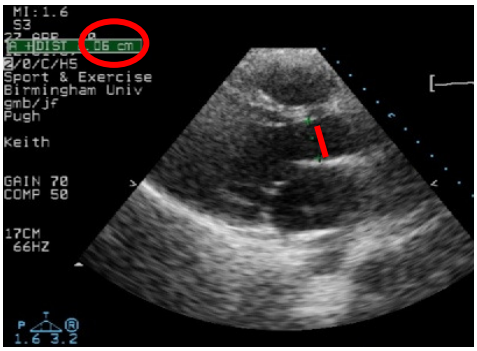

Figure 2.6 Parasternal view. Red line represents the cross-sectional area of the aortic orifice. Red circle shows the calculated value of cross-sectional area.

Stroke volume (mL) was determined using equation 10 and cardiac output ($\dot{Q}$) (L.min^{-1}) was then determined by equation 11. Where, heart rate was measured continuously using electrocardiography (ECG).

$$Stroke\ Volume = VTI\ X\ Cross\ sectional\ area \quad \textbf{(10)}$$

$$= VTI\ X\ \pi \left(\frac{D}{2}\right)^2$$

$$\dot{Q} = Heart\ Rate\ X\ Stroke\ Volume \quad \textbf{(11)}$$

2.6.2 Systolic pulmonary artery pressure (SPAP)

In order to measure SPAP using Doppler ultrasound an anatomical feature of the tricuspid valve called tricuspid regurgitation was used to our advantage. Following the closing of the tricuspid valve a small amount of blood is forced back through a small orifice in the tricuspid valve due to the pressure within the right ventricle. During this phase in the cardiac cycle, when the pulmonary valve is open, the right ventricle and pulmonary artery becomes a single chamber equal in pressure. Therefore the peak pressure difference across the tricuspid valve can be calculated using a modified version of Bernoulli's equation:

$$\Delta P = 4v^2 \quad \textbf{(12)}$$

Where ΔP is the pressure (mmHg) drop across the orifice and v is mean velocity (m.s^{-1}). For a complete explanation of the modified version of Bernoulli's equation see *appendix 2*. The peak pressure difference is assumed to change linearly with SPAP in healthy individuals because right atrial pressure is thought to be unchanged.

Tricuspid regurgitation is detectable in approximately 70% of the population. Therefore, prior to the admittance of participants into an experimental protocol measuring SPAP, screening for sufficient and clear measurement of tricuspid regurgitation was necessary.

For the measurement of tricuspid regurgitation a four chamber apical view of the heart was used, see figure 2.7. The Doppler beam was aligned at the atrial side of the tricuspid valve and a continuous spectral trace was used to determine the peak velocity of the regurgitant jet. The peak velocity was then automatically converted into the peak pressure difference by the Doppler machine. Peak velocity of the regurgantant jet was also measured using a spectral trace.

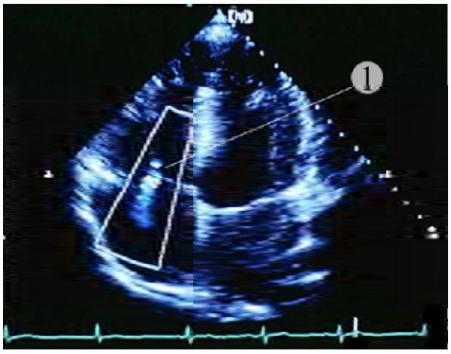

Figure 2.7 Four chamber view of the heart showing the regurgitant jet passing through the tricuspid valve into the right atrium (1).

Dorrington *et al.* (1997) showed that measurements of tricuspid regurgitation using Doppler ultrasound are representative of pulmonary vascular resistance. This was evident by a strong correlation in pulmonary vascular resistance, measured by right heart catheterisation, and tricuspid regurgitation measured using Doppler ultrasound.

2.7 Flow mediated dilation (FMD)

For an indication of vascular health and changes in vascular function, the technique of flow mediated dilation (FMD) was used in this thesis. The vascular endothelium is an important tissue lining the entire cardiovascular system. It is the link between the blood and the vessel wall and has many important functions such as thromboregulation, the control of vascular tone and the angiogenesis of new vessels (Alaiti *et al.*, 2012; van Hinsbergh, 2012). Its interaction with stimuli, both internal and external in nature, is integral to the regulation of these functions. Vascular dysfunction can lead to atherosclerosis, cardiac disease and hypertension (Gimbrone Jr & García-Cardeña, 2013). FMD is used to quantify the vascular health of individuals by measuring the vascular response to increased shear stress originating from augmented flow through the blood vessel. During an FMD, blood through the brachial artery is occluded with an inflatable arm cuff. Upon release of this arm cuff the large increase in flow through the vessel creates a shear stress stimulus on the vessel wall. In response to this shear stress the endothelium releases vasoactive substances to dilate the artery and accommodate for the increased flow through the vessel, a reaction termed reactive hyperaemia. The measurement of vasodilation, by way of change in vessel diameter with ultrasound imaging, can provide a simple and non-invasive assessment of vascular health. Artery diameter can be measured manually offline or tracked using an external software. FMD in this thesis were performed using such software. A vascular image analysis system was used to track the walls of the brachial artery continuously from black and white ultrasound images at a rate of 25 images per second.

During an FMD protocol participants lay in a supine position with their right arm stretched out to the side perpendicular to their torso. A special arm rig was used to support the

participants arm during the procedure. An inflatable cuff was placed around the forearm in a distal position from the site of measurement. A linear transducer was used to image the brachial artery. Once a clear and suitable image was found the transducer was held in place with a clamp attached to an adjustable arm. ECG was simultaneously measured throughout for determination of end-systolic and diastolic phases.

Following acquisition of brachial artery image the vascular image analysis software is calibrated to the image size and a region of interest dedicated. Following this the walls of the artery are continuously traced and the diameter of the vessel measured until the end of the procedure. Initially a two-minute baseline was completed, followed by five minutes of cuff inflation to 250 mmHg in order to obstruct blood flow through the arm. At the end of the five-minute blood flow obstruction period the cuff was deflated allowing blood to flow through the arm. A post-inflation period of three minutes was then completed or until the vessel diameter had returned to baseline levels.

Blood velocity measurements were made during baseline and cuff inflation for the certainty of occlusion. Blood velocity measurements were also made within 10 seconds after cuff release for the calculation of shear stress stimulus on the vessel wall. Shear rate was calculated using equation 13 below, where *v* is the blood velocity and D is the diameter of the vessel.

$$Shear\ rate\ (sec^{-1}) = \frac{4v}{D} \quad \textbf{(13)}$$

The percentage change from baseline to peak diameter following the vasodilatory response after cuff release is then calculated offline. This percentage change is an indication of the vascular response.

The method of FMD used during this thesis was in accordance with guidelines put forth by Thijssen *et al.* (2011). One limitation of the methodology used for FMD measurements in this thesis is the inability to measure continuous blood flow velocity measurements throughout the test. The advantage of such a procedure would be to gain insight to shear stress throughout the entire test and have instantaneous diameter and blood flow velocity values available.

2.8 Trans-cranial Doppler ultrasound (TCD)

In order to infer information about brain blood flow and cerebrovascular reactivity, TCD was used in this thesis. TCD is a non-invasive technique that uses high temporal resolution to penetrate the skull. However, anatomical acoustic windows are still manipulated for the insonation of cerebral blood vessels. Unlike the vascular and echocardiographic measurements previously described, TCD does not allow for the 2D imaging of the cerebral vessels. Therefore diameters of vessels and as a result volumetric flow cannot be determined. Only cerebral blood flow velocity (CBF_V) can be measured with the TCD system. A constant diameter of the cerebral conduit vessels is assumed to allow changes in blood flow velocity to be used as an index of changes in blood flow. This assumption has been reported to be valid (Nuttall *et al.*, 1996; Serrador *et al.*, 2000).

The measurement of CBF_V with TCD ultrasound was done according to the guidelines set out by Willie *et al.* (2011). A transtemporal approach was used to relay the Doppler beam through the anterior window to allow for near-zero angle of insonation of the middle cerebral artery. Once the middle cerebral artery was found the Doppler ultrasound probe was securely fitted in place with a head band. For the assessment of cerebrovascular

reactivity the hypercapnic dose response protocol, previously described, was used. CBF_V was recorded continuously via a spectral trace outputted to Spike 2 software, version 5.21. In addition to CBF_V, ECG and blood pressure waveform measured with finapres portapres were also outputted to Spike 2 software.

2.9 Polysomnography

During each of the overnight studies conducted in this thesis continuous polysomnography was performed. Polysomnography involves the recording of various physiological parameters to describe sleep stages, quantify arousal events and detect possible breathing events.

The physiological parameters measured during each night include brain activity via electroencephalography (EEG), eye movements via electrooculography (EOG), skeletal muscle activity via electromyographic (EMG), oxygen saturation via a pulse oximeter and heart rate via ECG. In Chapter 4 of this thesis, additional respiratory measurements of flow and chest/abdominal movements were also recorded during each night using a nasal cannula and strain straps, respectively.

In order to measure EEG, EOG and EMG gold plated cup electrodes were placed on pre-determined locations on the skin and scalp of the participant. Gold plated electrodes were used to enhance conduction of electrical activity. Prior to placement of the electrode the area was thoroughly cleaned using an alcohol wipe and conductive gel. The electrode was secured into position using conductive paste. Finally, electrodes were further secured with tape if placed on the skin or gauze with additional paste if placed on the scalp of the participant.

A simplified version of the 10-20 EEG system was used for EEG placement. First the longitudinal distance between the illion and nillion (sagittal line) was measured and the centre point marked with a red crayon. Also, the distance from one zygomatic arch to other, running through the centre point, was measured (coronal line). The final measurement made is the transverse diameter of the head. The diameter line runs through points that are 10% of the sagittal distance up from the illion and nillion (Oz and Fpz, respectively), and 10% of the coronal distance up from the zygomatic arch.

Central lobe (C3-C4) and occipital lobe (O1-O2) electrodes were then placed on either side of the sagittal line, odd numbers for the left even numbers for the right of the sagittal line, from the participant's perspective. C3-C4 electrodes are placed along the sagittal line, at 20% of the length of the sagittal line from the centre point.

O1-O2 electrodes are placed 5% of the transverse diameter either side of the sagittal line from the point Oz. Frontal lobe electrode (Fp1 and Fp2) are placed 5% of the transverse diameter either side of sagittal line from the point Fpz. Each EEG electrode is referenced to neutral electrodes placed behind the ear on the mastoid process (M1-M2). A ground electrode is placed on the point Fpz. See figure 2.8. for a representation of electrode placement.

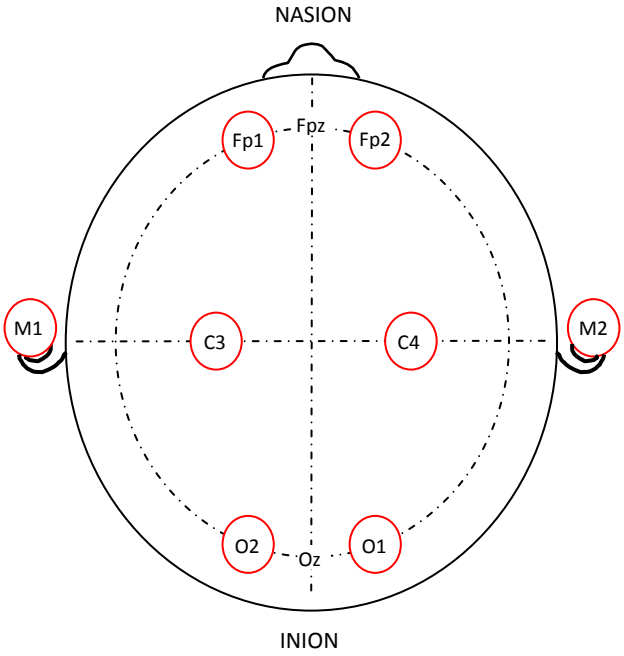

Figure 2.8. Diagram showing EEG placements (red circles) on the head using the modified 10-20 system.

An EOG electrode was placed either side of the lateral canthi for the recording of eye movements inferred from cornea-retina potential difference. The EOG electrodes were placed 1 cm out, and 1 cm above and below the lateral canthi on the right eye and left eye respectively, so that up and down as well as side to side eye movements could be recorded. An EMG electrode was placed at the base of the mandible for the recording of mylohyoid muscle activity.

All recording electrodes in addition with respiratory recording devices were connected to an Embla titanium system for amplification. Live readings from the Embla titanium amplifier were recorded using RemLogic 2.0 software on a networked computer in a room adjunct to the sleep laboratory.

2.10 Sleep stage and arousal identification and scoring

Sleep stages are dominantly defined by changes in brain activity shown by fluctuations in EEG wave forms. A number of different waveforms can be seen over the course of a night's sleep, each corresponding to a particular stage of sleep. Waveforms are shown in figure 2.9.

In addition to changes in EEG waveforms EOG and EMG alterations are also used in the scoring of sleep stages. Stages are scored along 30-second segments of recorded data or an epoch. Sleep stages were scored offline using the guidelines below, which are in accordance with those set out by Rechtschaffen and Kales (1968).

Wakefulness. A relaxed but awake state is characterised by low voltage alpha waves that make up the majority of the epoch. Eyes are closed with the possible presence of blinking and an EMG trace shows normal muscular activity.

Stage 1. Stage 1 sleep shows a cessation of an alpha waveform with the majority of the epoch being made up of theta waves. Blinking of the eyes will no longer be present but EMG still shows normal tonic activity.

Stage 2. Stage 2 is defined by the presence of K complexes and/or sleep spindles in the EEG trace against a background of mixed frequency EEG. Normal muscular tone is still present in the EMG trace.

Stage 3 (Slow wave sleep). Slow wave sleep (SWS) is defined by the occurrence of high amplitude delta waves present for the majority of the epoch. EMG activity shows a slight reduction from normal tonic activity.

REM sleep. EOG and EMG activity are more important in scoring REM sleep than EEG measurements. The appearance of saccadic eye movements across the epoch represents REM. The loss of muscular tone during REM sleep is shown by a large reduction in EMG

activity during this stage. EEG amplitude will be markedly reduced in comparison to slow wave sleep and mimic a wakefulness/stage1 state. Sawtooth waves are characteristic of REM sleep EEG.

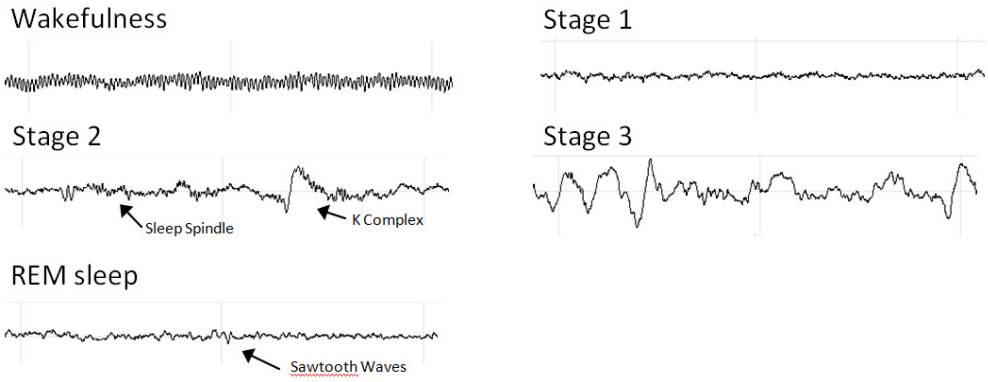

Figure 2.9 displays typical examples of EEG waveforms for sleep stages from wakefulness to REM sleep. Arrows highlight important identification features of some sleep stages.

2.10.1 Micro-arousals

In Chapter 5 in this thesis micro-arousals were elicited while the participants slept, using an external noise to cause fragmentation of sleep. The external noise was a 500ms sine wave played through speakers located 50 cm from the participants head during the night. The sound played every minute and was externally controlled by an experimenter in an adjacent room. The following guidelines were used for the detection and stimulation of micro-arousals.

For the purpose of this thesis the definition of a micro-arousal used was that according to the American Sleep Disorders Association (1992). According to this definition a micro-arousal is defined by a return to alpha waves from theta waves or a return to theta from delta waves, dependent upon the stage of sleep preceding the stimulation. During REM sleep a burst of EMG activity will been seen when a micro-arousal is occurs. A micro-arousal occurs

for between 1.5 to 3 seconds. Additional features, but not required for the scoring of a micro-arousal could be tachycardia and/or k-complex bursts.

Sleep fragmentation was initiated after three minutes of uninterrupted stage 2 sleep. The initial volume of the audio cue was 40dB. Volume was kept at the given level if a micro-arousal was stimulated. If no micro-arousal was stimulated within two attempts the computer volume was increased by pre-determined levels until such a micro-arousal was induced.

Before the stimulation of subsequent micro-arousals a return to original sleep stage for at least 30 seconds was needed. If the participant returned to wakeful stage of sleep micro-arousals were discontinued until they returned to stage 2 sleep. Typical examples of both arousals and micro arousals are depicted in figure 2.10.

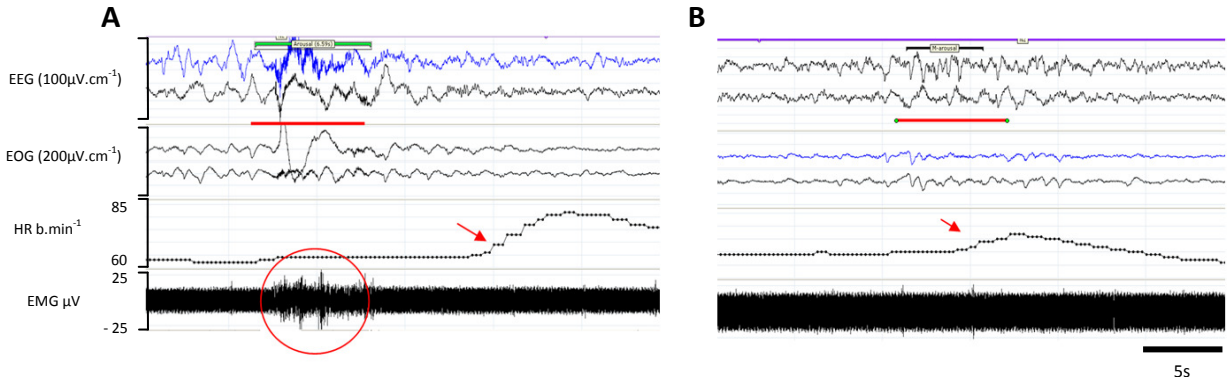

Figure 2.10 Typical examples of an arousal (A) and micro-arousal (B) during sleep. The red line highlights the typical EEG disturbance and desynchronisation during the arousal and micro-arousal, respectively. Red arrows show resultant tachycardia during each event and the red circle highlights the typical EMG burst that accompanies an arousal. The y-axis labels are the same for both traces and each represents a 30 second epoch.

CHAPTER 3.

CHANGES IN CHEMOSENSITIVITY WITH AGE

3.1 Introduction

Ageing is associated with numerous changes in respiratory function. An in depth description of the alterations in respiratory physiology with age was given in the introduction. The majority of these alterations with age are anatomical in nature. However, one additional area of respiratory function that has been reported to change with age is chemosensitivity. The particular respiratory chemoreceptive response in question is the HCVR, with the majority of research that the hypoxic chemosensitivity is unaltered with age (Ahmed *et al.*, 1991; Poulin *et al.*, 1993; Smith *et al.*, 2001; Pokorski & Marczak, 2003b). Although two earlier studies using progressive Read re-breathing techniques did find decreased hypoxic sensitivity in older individuals in comparison to younger participants (Kronenberg & Drage, 1973; Peterson *et al.*, 1981b). Also, a study in 2003 by Pokorski and Marczak (2003a) reported an enhancement in the HVR of older individuals following ascorbic acid supplementation, suggesting a decline in elders HVR. However, no age group comparison was made in this particular study.

The ageing effect on the HCVR has been researched widely with the majority of the previous research suggesting an attenuation in chemosensitivity with advancing age (Kronenberg & Drage, 1973; Peterson *et al.*, 1981b; Brischetto *et al.*, 1984; Chapman & Cherniack, 1987; Naifeh *et al.*, 1989; Poulin *et al.*, 1993). However, additional research has shown no such variation in chemosensitivity between young and older individuals (Rubin *et al.*, 1982; Garcia-Rio *et al.*, 2007). Reasons for these differences could be variations in the methodological choices used to assess chemosensitivity.

Of the research demonstrating an attenuation in hypercapnic chemosensitivity the majority adopted the method of Read's re-breathing to expose their participants to a hypercapnic

stimulus (Kronenberg & Drage, 1973; Peterson *et al.*, 1981a; Brischetto *et al.*, 1984; Chapman & Cherniack, 1987). Additional studies used the steady state method, either via a DEF system, to tightly control the exposed stimuli (Poulin *et al.*, 1993) or via an additional steady state method in the Hazinski method as used by Naifeh *et al.* (1989). All these methods assessed chemosensitivity via the calculation of ventilatory response slopes. Considering that a number of different methods of administering hypercapnia were used by these studies and that they all demonstrated a decline in hypercapnic chemosensitivity with age, this would suggest further that the method to assess chemosensitivity was to account for the different findings of Rubin *et al.* (1982) and Garcia-Rio *et al.* (2007). Rubin *et al.* (1982) and Garcia-Rio *et al.* (2007) used a method of mouth occlusion pressure to infer respiratory drive during a HCVR, the caveats of which are mentioned in section 1.2.3. No differences in mouth occlusion pressure were seen between young and old in those studies. However, although Rubin *et al.* (1982) did observe a lower mean HCVR in older individuals, it was not a statistically significant finding, possibly because of small group power.

Additional equivocal observations arise when interpreting the nature of an observed reduction in CO_2 chemosensitivity, i.e. which chemoreceptors are possibly responsible. Some studies showed a reduction in chemosensitivity during a single hyperoxic hypercapnic test, possibly inferring central chemoreceptor alteration (Kronenberg & Drage, 1973; Peterson *et al.*, 1981a; Brischetto *et al.*, 1984), while others did not (Rubin *et al.*, 1982; Chapman & Cherniack, 1987; Poulin *et al.*, 1993; Garcia-Rio *et al.*, 2007). Some of these studies reported a reduction in the ventilatory response of older participants during exposure to hypoxic hypercapnia, suggesting an alteration in the chemoreceptor interaction brought about by reduced peripheral chemoreceptor sensitivity (Chapman & Cherniack, 1987; Poulin *et al.*,

1993). These differences in findings could also explain why Garcia-Rio *et al.* (2007) and Rubin *et al.* (1982) concluded there was no change in hypercapnic chemosensitivity with age; as they only used a hyperoxic re-breathing test.

Despite the differences in findings between these studies a common theme is the use of hyperoxia for its peripheral chemoreceptor silencing ability. However, this ability is debatable. Furthermore, the use of hyperoxia could interfere with the assessment of true CO_2 chemosensitivity. The complications of hyperoxia use are explained previously in section 1.2.4. A particular confounding factor that arises with the use of hyperoxia is the effect that hyperoxia can have on the cerebrovascular bed that can directly influence the $[H^+]$ detected by the central chemoreceptors. Research has suggested that the hyperoxic vasoconstriction of the cerebrovascular bed is affected by age (Watson *et al.*, 2000), with older individuals showing less reduction in cerebral blood flow during hyperoxic exposure. An altered cerebrovascular reactivity to hyperoxia with age could create a confounding factor when comparing two different age groups with a hyperoxic hypercapnia ventilatory test.

In this initial study a pseudorandom method of chemosensitivity test was used to assess total CO_2 chemosensitivity, and to identify the sensitivities of the peripheral and central chemoreceptors without the need of hyperoxia. The total, peripheral and central chemosensitivities were assessed in old and young individuals. In order to the test the ability of hyperoxia to inhibit the peripheral chemoreceptors and assess any age differences in the inhibitory effect, participants were also asked to complete a MFBS test under hyperoxic conditions.

3.2 Methods

3.2.1 Participants

Fourteen young participants were recruited from the student body of the University of Birmingham. Sixteen older participants were recruited using flyers and newspaper articles. All participants were free of cardiovascular and respiratory disease. Participants were in a 2-hour fasted state during protocols. They were also asked to refrain from vigorous exercise and alcohol intake 24 hours prior and any caffeine intake 12 hours prior to any experimental visit. For each participant, consent was obtained and a general health questionnaire completed prior to induction into the study. Ethical approval for the study was obtained from the local ethics subcommittee within the University of Birmingham.

3.2.2 Protocol

Participants were asked to complete two MFBS tests, one under euoxic conditions and one under hyperoxic conditions. Each test was completed on a separate day. Tests were completed in a randomised order. A two-compartment model of ventilation was used to determine the central and peripheral chemosensitivities during euoxic respiratory tests. MFBS tests and the control of end-tidal partial pressures are outlined in further detail in chapter 2.

3.2.3 Data analysis

Normal $P_{ET}CO_2$ was assessed following a period of spontaneous breathing of room air through the mouthpiece. Resting minute ventilation values were obtained during the first minute of the isocapnic period.

The two-compartment model used to assess peripheral and central chemosensitivity has been previously described in chapter 2.

A two-tailed independent sample T-test was used to test for statistical significant differences in chemosensitivity during both MFBS tests between the two age groups. A paired sample T-test was used to compare with-in subject chemosensitivity differences between the euoxic and hyperoxic MFBS tests. All reported significant differences have a $P<0.05$ unless stated otherwise.

3.3 Results

3.3.1 Participants

Sixteen older participants with mean ±S.D. age and body mass index (BMI), of 70.81 ± 3.80 years and 25.61 ± 4.15 kg/m^2 respectively and fourteen young participants, 24.29 years ± 3.95 and 23.98 ± 2.85 kg/m^2, were recruited for the study. There were seven females in each age group. With the exception of one participant, an older male, all participants completed a euoxic MFBS test. Seven young participants and eight older participants completed a hyperoxic MFBS test.

Physical characteristics and normal $P_{ET}CO_2$ levels of the participants are displayed in table 3.1. There was no difference in mean BMI, resting ventilation or normal $P_{ET}CO_2$ between the two age groups.

	Age	Height (cm)	Weight (Kg)	BMI (Kg/m^2)	$P_{ET}CO_2$ (mmHg)	V_E ($L.min^{-1}$)
Old						
Mean	70.81#	167.42	71.80	25.61	37.90	9.92
±S.D	3.80	8.97	13.52	4.15	3.02	2.39
Young						
Mean	24.29#	171.86	70.59	23.98	39.61	10.84
±S.D	3.95	13.42	10.06	2.85	2.14	2.42

Table 3.1 shows mean ± S.D demographics for young (n=14) and older (n=16) groups. With the exception of age there was no difference in demographics between the two age groups. V_E = resting minute ventilation during euoxia. # = $P<0.01$

3.3.2 Ventilatory response and end-tidal gas control

Figure 3.1 shows $P_{ET}CO_2$ and $P_{ET}O_2$ gas control, and the resultant ventilatory responses during a typical MFBS test under euoxic or hyperoxic conditions. Shown in the graph of ventilation are data points representing the absolute breath–by-breath changes in minute ventilation throughout each MFBS test. A red line depicts the model ventilation derived from the MFBS two compartment model. Fluctuations in minute ventilation during both protocols follow the intermittent changes in $P_{ET}CO_2$ throughout the MFBS tests.

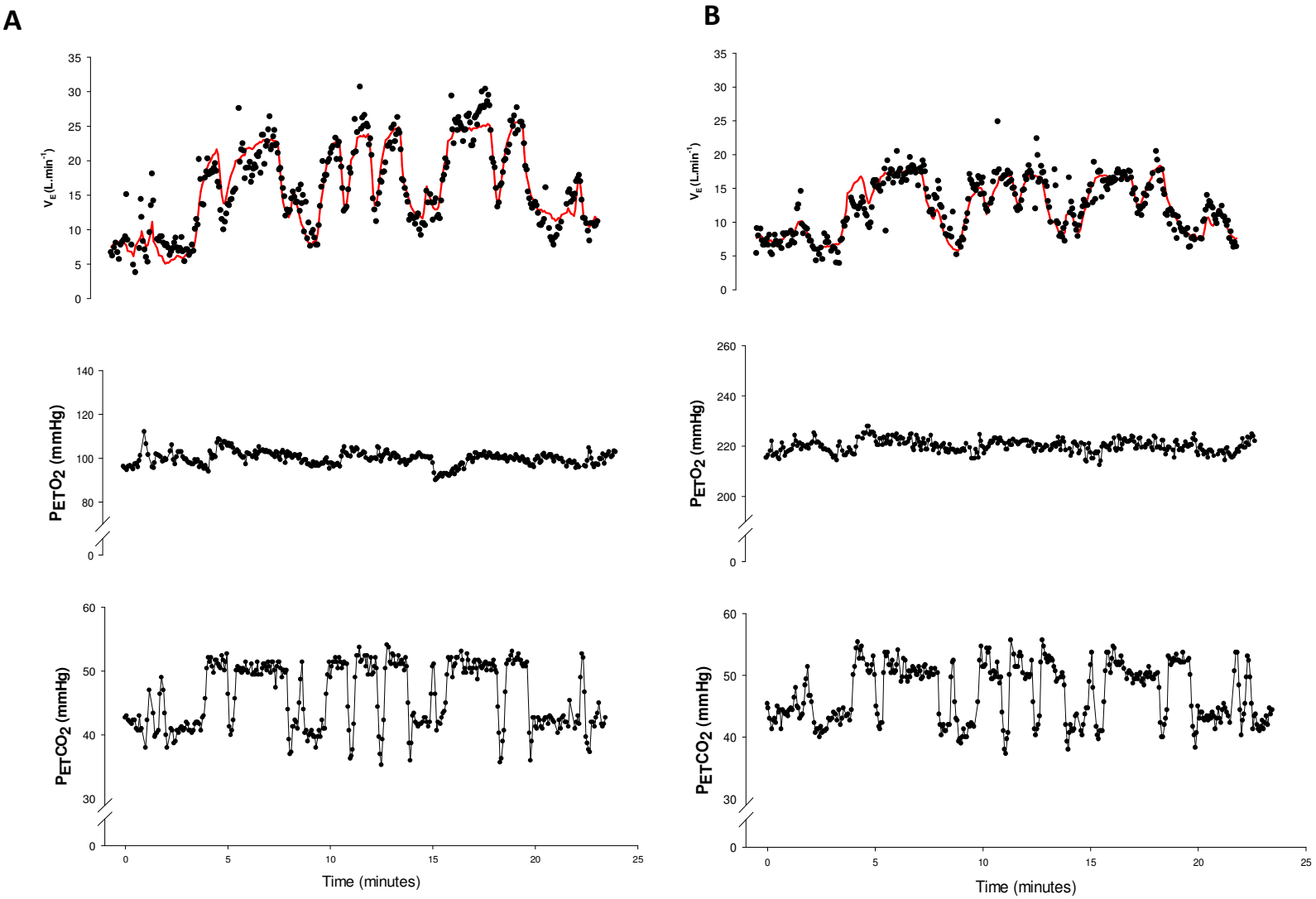

Figure 3.1. Graphs showing example ventilatory responses, $P_{ET}CO_2$ and $P_{ET}O_2$ levels during a euoxic (A) and hyperoxic (B) MFBS tests. Data is from one single participant.

3.3.3 Chemosensitivity during Euoxic MFBS test

Table 3.2 shows individual participant and mean parameters calculated from the euoxic MFBS test by the two-compartment model of ventilation. Total gain was significantly different between the two groups, with older participants showing a lower mean (±S.E.M.) CO_2 chemosensitivity than younger participants; 2.19 ± 0.22 and 3.17 ± 0.23, respectively. There was a significant mean difference in central gain between the young (2.07 ± 0.14) and older (1.21 ± 0.16) age groups. Peripheral sensitivity was indifferent between the two age groups. All gains are reported in $L.min^{-1}$ $mmHg^{-1}$. The extrapolated CO_2 apnoeic threshold (B value) was significantly different between the old and young groups, 33.16 ± 1.04 and 36.53 ± 0.75 mmHg respectively.

	Subject I.D	G_{TOT} (L.min^{-1}.mmHg^{-1})	G_P (L.min^{-1}.mmHg^{-1})	G_c (L.min^{-1}.mmHg^{-1})	B (mmHg)
	1	2.39	0.30	2.08	31.19
	3	4.40	2.55	1.85	31.97
	5	2.24	0.82	1.43	34.27
	6	1.69	1.02	2.49	37.93
	9	1.30	1.45	0.46	30.46
OLDER	10	3.51	0.92	0.88	25.85
	14	1.90	0.37	1.12	32.00
	20	1.80	0.92	1.39	39.20
	22	1.49	0.60	1.09	36.20
	23	2.31	0.63	1.30	31.63
	24	1.94	0.79	0.51	35.09
	27	2.27	1.42	0.85	38.56
	28	2.13	1.64	0.49	27.37
	29	1.98	0.65	1.33	31.09
	60	1.47	0.61	0.87	34.63
Average		2.19*	0.98	1.21*	33.16*
±S.E.M.		0.22	0.16	0.16	1.04
	2	4.45	1.37	3.08	37.55
	4	2.88	0.41	2.47	37.55
	7	2.07	0.41	1.66	37.90
	8	3.67	1.32	2.35	33.84
	12	2.53	0.59	1.94	29.85
YOUNG	16	3.71	1.40	2.31	36.94
	17	3.66	1.14	2.52	39.56
	18	2.76	0.96	1.79	38.77
	21	1.70	0.38	1.31	41.91
	25	2.78	1.39	1.39	36.62
	26	3.86	2.14	1.73	36.93
	31	4.54	1.77	2.76	36.79
	33	1.77	0.34	1.43	34.03
	34	3.91	1.91	2.00	32.91
	37	3.26	0.96	2.30	36.76
Average		3.17*	1.10	2.07*	36.53*
±S.E.M.		0.23	0.15	0.14	0.75

Table 3.2. Individual and mean ±S.E.M. model parameters for euoxic MFBS test. OLD vs. YOUNG G_{TOT} is the total CO_2 chemosensitivity, G_p is the peripheral chemoreflex sensitivity, G_c is the central chemoreflex sensitivity, B is a bias term equivalent to the $P_{ET_{CO_2}}$ for which $\dot{V}_c$ equals zero. * = significant difference; p<0.03.

3.3.4 Chemosensitivity during hyperoxic MFBS test

Table 3.3 shows the individual participant and mean parameters of the hyperoxic MFBS test calculated using the two-compartment model. The mean (±S.E.M.) difference between the young and older age groups in total gain was duplicated in the hyperoxic protocol, 2.90 ± 0.33 and 1.92 ± 0.26, respectively. The same is found for central gain 2.02 ± 0.27 and 1.25 ± 0.20 for young and older participants respectively. All values displayed in $L.min^{-1}$ $mmHg^{-1}$. There was no difference in peripheral gain and B values between the two age groups.

	Subject I.D	**G_{TOT} ($L.min^{-1}.mmHg^{-1}$)**	**G_P ($L.min^{-1}.mmHg^{-1}$)**	**G_c ($L.min^{-1}.mmHg^{-1}$)**	**B (mmHg)**
	6	1.33	0.63	0.70	35.83
	14	1.31	0.65	0.67	36.19
OLDER	22	1.36	0.42	0.94	36.82
	23	2.30	0.65	1.65	36.36
	27	2.09	0.86	1.23	39.82
	28	3.29	1.02	2.27	34.63
	60	0.88	0.27	0.61	33.87
	61	2.76	0.79	1.97	36.55
Average		1.92*	0.67	1.25*	35.97
±S.E.M.		0.26	0.07	0.20	0.62
	2	4.13	1.28	2.86	35.21
	4	2.72	0.35	2.37	35.53
	7	1.73	0.25	1.48	37.97
YOUNG	8	3.06	1.20	1.87	34.81
	17	3.85	1.02	2.83	37.24
	21	2.13	0.39	1.74	39.54
	26	2.68	1.70	0.98	37.39
Average		2.90*	0.88	2.02*	36.81
±S.E.M.		0.33	0.21	0.27	0.65

Table 3.3 Individual and mean ±S.E.M. model parameters for hyperoxic MFBS test analyzed by two-compartment model. OLD vs. YOUNG Symbols as in Table 4.2.

3.3.5 Comparison between euoxic and hyperoxic tests

When both groups are combined mean ±S.E.M. peripheral chemosensitivity levels were significantly lower during the hyperoxic MFBS test when compared to the euoxic test, 0.76 ± 0.11 and 0.94 ± 0.14 $L.min^{-1}.mmHg^{-1}$, respectively. When grouping for age the younger individuals show a significant reduction in G_p during the hyperoxia test in comparison to the euoxic MFBS test. The older participants show a trend to a reduction in G_p during hyperoxic test in comparison to euoxia, however this was not statistically significant (p=0.067). Despite the observed reductions peripheral chemoreceptor influence on ventilation was still present during the hyperoxia test. There was no within subject difference in central gain between the euoxic and hyperoxic MFBS tests within either group. There was no difference in total gain within the age groups between the two tests. Mean parameters are shown in figure 3.2.

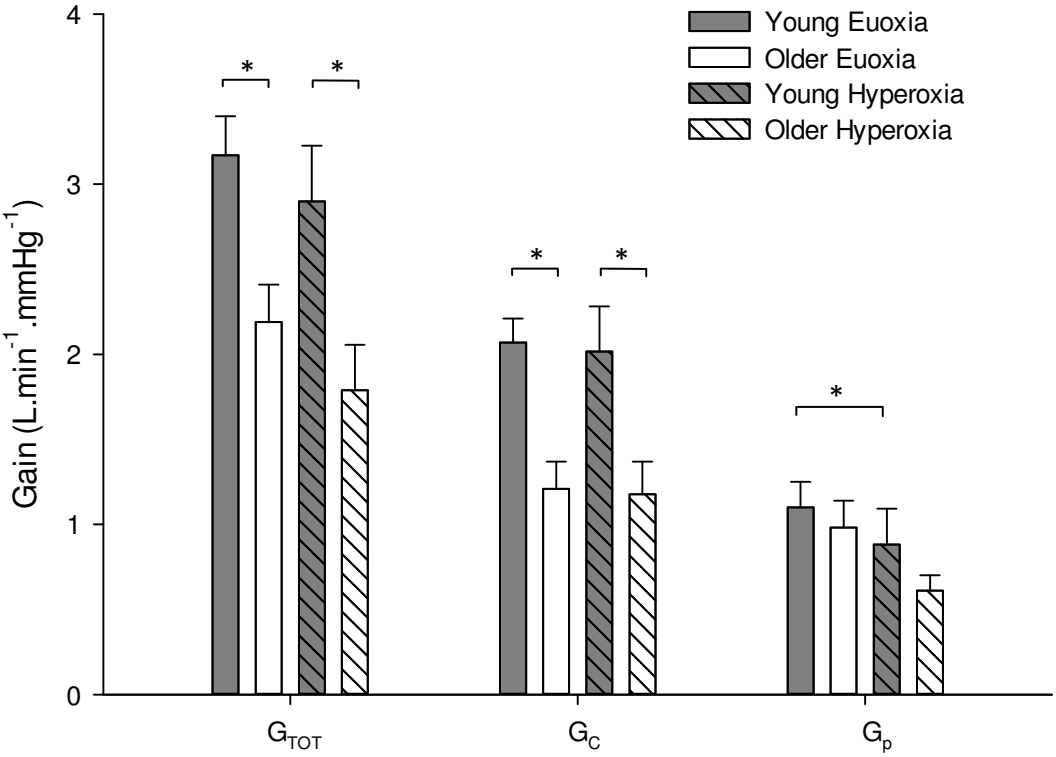

Figure 3.2 A graph showing the mean (±S.E.M.) total chemosensitivity (G_{TOT}), central chemosensitivity (G_c) and peripheral chemosensitivity (G_P) levels of both young and older individuals during both euoxic and hyperoxic MFBS tests. * = significant difference; p<0.05.

3.4 Discussion

The study presented here has confirmed that ageing is associated with a reduction in CO_2 chemosensitivity. Furthermore, it was shown that this reduction in older individuals is driven by a decline in central chemosensitivity. No differences in peripheral chemosensitivity were seen between the two age groups, indicating no peripheral chemoreceptor contribution to the reduction in total CO_2 chemosensitivity with age. The absence of change in peripheral chemosensitivity is supported by the similarity in normal $P_{ET}CO_2$ between the two groups, as normal $P_{ET}CO_2$ levels are said to be governed by the influence of the peripheral chemoreceptors on normal resting ventilation, which is also similar (Dahan *et al.*, 2007).

The results of this study support the majority of previous research showing a diminishment of total CO_2 chemosensitivity with age (Kronenberg & Drage, 1973; Peterson *et al.*, 1981b; Brischetto *et al.*, 1984; Chapman & Cherniack, 1987; Poulin *et al.*, 1993). Progressive exposure to hypercapnia using a re-breathing technique was the preferred method of gas control in most of these studies, with the exception of Poulin *et al.* (1993). Poulin and colleagues used a DEF system that allowed accurate control of $P_{ET}CO_2$, but it also allowed $P_{ET}O_2$ to be clamped at a desired level, something that the previously mentioned re-breathing studies were not able to control.

The earlier hyperoxic re-breathing studies reported a reduction in chemosensitivity (Kronenberg & Drage, 1973; Peterson *et al.*, 1981b; Brischetto *et al.*, 1984), possibly suggesting a decline in central chemosensitivity. However, this reduction during hyperoxic hypercapnia is not observed in other studies (Chapman & Cherniack, 1987; Poulin *et al.*, 1993). These studies showed no differences in response between age groups during hyperoxic hypercapnia. This observation demonstrates a peripheral chemoreceptor role in

the reduction of CO_2 chemosensitivity with age. The data presented in this study has shown a reduction in central chemosensitivity without the use of hyperoxia. Nevertheless, the difference in central chemosensitivity between the two age groups was also observed during the hyperoxic MFBS test, reinforcing the findings of the euoxic MFBS test. These observations would seem to differ with the findings of Chapman and Cherniack (1987) and Poulin *et al.* (1993).

Data presented by Poulin *et al.* (1993) and Chapman and Cherniack (1987), showing a decrease in the ventilatory response to hypoxic hypercapnia in older individuals, could suggest an alteration in the interaction of hypoxia and hypercapnia at the peripheral chemoreceptors. The changes in chemoreceptor sensitivities presented in this study could also suggest an appearance of a hypoadditive interaction between peripheral and central chemoreceptors with age, whereby the stimulation of the peripheral chemoreceptors with hypercapnia could be inhibiting the central chemoreceptor output in older individuals. However, if a hypoadditive interaction were present in our older individuals the inhibitory affect of hyperoxia on phrenic nerve discharge would augment central chemoreceptors response to hypercapnia. This appears not to be the case, as there was no difference in central gain between the euoxic and hyperoxic MFBS tests, in either age group. This observation suggests any alteration in the influence of the peripheral chemoreceptors on central chemosensitivity to be minimal.

In the older age group, no difference was found between the two MFBS tests. This observation suggests that the reduced cerebrovascular reactivity to hyperoxia in older individuals reported by Watson *et al.* (2000) may have a minimal effect on central chemosensitivity during a hyperoxic hypercapnic test. Furthermore, younger individuals

showed a similar level of total and central chemosensitivity during the hyperoxic MFBS test as they did during the euoxic test. These results suggest that the background of hyperoxia has little effect on chemosensitivity via an alterations in $[H^+]$ brought about by a reverse Haldane effect or indirectly through a cerebrovascular mechanism as was previously suggested. However, this cannot be certain because no cerebrovascular assessment was made during the present study. Also, the levels of hyperoxia used in this study, 200 mmHg, are marginal in comparison to those used by Watson and colleagues, 100% O_2.

The peripheral chemoreceptor element of CO_2 ventilatory control was reduced, but nevertheless persisted during the hyperoxic MFBS test. This observation supports that of Pedersen *et al.* (1999b), who also used a MFBS test. These results also support observations of carotid sinus nerve activity during hyperoxic exposure (Fitzgerald & Parks, 1971; Lahiri & DeLaney, 1975) and lend evidence to the suggestion that hyperoxia does not silence peripheral chemoreceptor activity and is not sufficient enough to infer solely central chemoreceptor control.

Between age groups it did appear that older individuals' peripheral chemosensitivity was unchanged between euoxic and hyperoxic MFBS tests. This suggests that ageing may impact on the peripheral chemoreceptors' response to the inhibitory effect of hyperoxia. However, it would seem more likely that the statistical power of the group of older participants that undertook a hyperoxic MFBS test, would account for the insignificant finding. This statement is supported by the observation that the reduction in the older group's mean peripheral sensitivity in the hyperoxic MFBS test in comparison to the euoxic test, tended to be greater than that of the attenuation in the younger group.

Results from this study also demonstrate a reduction in CO_2 apnoeic threshold with age. The absence of any difference in normal $P_{ET}CO_2$ between the two age groups suggests that the observed reduction in apnoeic threshold is not relative to any reduction in normal $P_{ET}CO_2$ with age. This suggests further that reduced CO_2 apnoeic threshold with age is because of a reduction in the slope of HCVR and extrapolated x-axis intercept. The reduction in apnoeic threshold in the present study supports previous findings (Rubin *et al.*, 1982; Poulin *et al.*, 1993; Garcia-Rio *et al.*, 2007). Garcia-Rio *et al.* (2007) reported a reduction in extrapolated apnoeic threshold in older individuals but reportedly showed no change in ventilatory response slope or normal $P_{ET}CO_2$, raising the question of how a reduction in extrapolated apnoeic threshold came about. A reduction in apnoeic threshold was also observed during the hyperoxic MFBS test in this present study, however the finding was deemed not to be statistically significant. This finding is most likely explained again by the low participant numbers.

3.4.1 Clinical Relevance

As previously described in section 1.5.2, the elderly population experience a greater incidence of SDB than younger individuals. It should be noted that one limitation with this study is the failure to screen participants for possible existence of SDB. This may have a possible confounding on assessed chemosensitivity levels because of the possibility of changes in chemosensitivity caused by the condition itself.

One likely cause of an increased level of apnoeas during sleep is an alteration in respiratory control. Such a mechanism is also possibly more likely in the development of SDB in older individuals for two reasons. One, because the prevalence of SDB in older generations is

independent of obesity and two, the pathophysiology of SDB in elders is characterised by a much greater occurrence of central apnoeas.

However, two elements could influence whether the alterations in chemosensitivity with age, reported in this study, contribute to the development of SDB in older individuals. One is the role of the central chemoreceptors in the pathophysiology of SDB, particularly central apnoeas and secondly, whether the changes in chemosensitivity are transferred to the sleeping state.

3.4.2 The role of the central chemoreceptors

Given the typical time constants of the two chemoreceptors it could be suggested that the fast responding peripheral chemoreceptors would be a prime candidate for both the protection against apnoeas and inducing central apnoeas. This role of the peripheral chemoreceptors mediating central apnoeas is highlighted further by Smith *et al.* (2006a) who showed the development of central apnoeas within 20 seconds of inducing an apnoeic threshold. Further evidence for the peripheral chemoreceptor role in apnoea development has been shown in carotid body resected dogs (Smith *et al.*, 2007). Data from these animals demonstrated a near absence of immediate apnoeas during induced hypocapnia, thus suggesting an integral role of the peripheral chemoreceptors in central apnoea development. The absence of a visible central apnoea in these dogs suggests the absence of a central chemoreceptor CO_2 apnoeic threshold. Further evidence for this statement is observed in animal preparations with intact and isolated carotid bodies in the same study. The carotid bodies were held in a normoxic nomocapnia environment while central hypocapnia was induced. The authors found that no central apnoeic threshold occurred

during these conditions. These results were recently supported by Fiamma *et al.* (2013) whose findings also showed no central apnoeic threshold during the isolation of carotid bodies in normoxic normocapnic conditions. However, data presented by Nakayama *et al.* (2003) does suggest otherwise, with the development of apnoeas during hypocapnia in carotid body denervated sleeping dogs. However, under these circumstances the magnitude of the hypocapnia was much greater, approximately an additional 5 mmHg below eucapnic levels. Also, the time until the induced apnoeas occurred in carotid body removed animals was on average 22 seconds longer (≈33 seconds) than those seen in the carotid body intact animals. Although these data demonstrate the possibility of a central hypocapnic threshold, it would appear that such a threshold is too slow to be responsible for the development of immediate central apnoeas, typical of SDB. For such transient apnoeas, that occur during periodic breathing, the peripheral chemoreceptors appear to be necessary and the central chemoreceptors seem to play little if any role.

These findings would therefore suggest that the reduced central chemosensitivity in older individuals observed in this study would have little or no contribution to the development of central apnoeas through a hypocapnic apnoeic threshold. Furthermore, the observed reduction in apnoeic threshold in older individuals in this study would seem to be a protective mechanism in the development of central apnoeas.

Hypocapnia is predominantly brought about by periodic breathing during in sleep. The hyperventilatory response is stimulated via the peripheral chemoreceptors and characteristic of a high chemosensitivity (Khoo *et al.*, 1982). The observation of similar levels of peripheral chemosensitivity between the two age groups would suggest that the stimulation of periodic breathing by the peripheral chemoreceptors would be minimal in

older individuals. These findings further suggest that a reduction in central chemosensitivity would not contribute to the development of central apnoeas in older individuals.

However, if the peripheral chemoreceptors are integral to a central apnoea development it would be hypothesised that when they are isolated and exposed to hypopcapnia a quick apnoea would follow. Smith *et al.* (2010) explains this is not the case. Research has shown peripheral chemoreceptor inhibition through both hypopcapnia and hyperoxia results in a marked reduction in ventilatory output but no occurrence of typical central apnoeas (Daristotle *et al.*, 1990; Smith *et al.*, 1995; Blain *et al.*, 2009). Smith and colleagues have suggested that although the peripheral chemoreceptors are integral to the development of central apnoeas a possible interactional aspect arising from the central chemoreceptor input is also present and can affect the peripheral chemoreceptor output during inhibition by hypocapnia and hyperoxia. Therefore, although research has shown peripheral chemoreceptors to play a key role in central apnoeic thresholds, alterations in central chemoreceptor sensitivity could affect the synergy between the two chemoreceptors and thus the response of the peripheral chemoreceptors to hypocapnia. Therefore, the reduced central chemosensitivity observed in this study could still play a role in hypocapnic induced apnoeas.

The ablation of the central chemoreceptors is an additional method of determining their role in respiratory control during sleep and is also a representation of the reduced central chemosensitivity observed this study. One particular study has shown that the abolishment of Pre-BötC neurons leads to breathing disturbances during sleep (McKay & Feldman, 2008). Mckay and Feldman postulated that the loss of these neurons in older individuals through neural degradation could lead to the development of SDB. One limitation of this study is that

the Pre-BötC is a site of both central chemoreception and respiratory rhythmogenesis. The dual function of the Pre-BötC means it is difficult to distinguish the cause of respiratory instability in these animals; is it the removal of a chemoreceptive region or the ablation of a key site of rhythmogenesis?

More recent research also investigating the effect of ablation of central chemoreceptive sites, created a mutant mice that lacked an RTN region (Ramanantsoa *et al.*, 2011). The RTN is a prime central chemoreceptive region governing central chemosensitivity, as was evident in the absence of HCVR response in these mice at postnatal periods; the HCVR did re-emerge in adult mice. Nevertheless, the RTN ablated mice survived, breathing stability was maintained, no apnoeas were detected and $PaCO_2$ was also maintained. This is evidence to suggest that the carotid bodies are sufficient in maintaining stable breathing and a normal $PaCO_2$. A further observation for supporting this observation is the development of periodic breathing when the carotid bodies were inhibited with hyperoxia. These observations lend weight to the suggestion that the central chemoreceptors are not essential for respiratory control. Also, it suggests that a reduction in central chemosensitivity would not contribute to the development of respiratory instability and apnoeas. Nevertheless, the RTN is one of many central chemoreceptive sites, suggesting possibly that additional redundant sites could contribute to the stability of respiratory control observed. It also remains to be seen if similar reductions in central chemosensitivity in humans will lead to such respiratory disturbances during sleep.

As described in detail in Chapter 1, reductions in chemosensitivity could have other mechanistic effects, other than reduced respiratory drive, that could promote the development of SDB. Reduced chemosensitivity could influence protective respiratory

reflexes that prevent airway collapse and promote airway reopening during obstruction (Chin *et al.*, 2012). These effects of reduced chemosensitivity could lead to a greater number of airway obstructions occurring and more prolonged apnoeic events. The prolonging of short cessations in ventilation to greater than ten seconds would deem them apnoeic events. This could be one possible mechanism for the greater occurrence of apnoeas in older individuals. However, considering the time frame of a reflex airway opening or re-initiation of ventilation that would account for this possibility, the peripheral chemoreceptors would seem to have a dominant role.

An additional role of reduced chemosensitivity in the development of apnoeic events is a reduced efferent control of upper airway tone arising from the chemoreceptors. It has been shown that chemosensitive raphe neurons have innervations to airway related vagal neurons and the possible chemosensitive region of the locus coeruleus innervates the hypoglossal nucleus, the origin of the hypoglossal nerve. Stimulation of these central chemosensitive regions can affect the airway tone and airway resistance (Kc & Martin, 2010). It is plausible that reductions in neural activity at these central chemoreceptive regions would also reduce efferent input to the upper airway and lead to a greater risk of airway collapse during sleep. The reduction in central chemosensitivity in the older individuals in this study and the observation of greater airway collapsibility in older individuals (Eikermann *et al.*, 2007), suggest this to be a possible mechanism of increased SDB in older individuals.

3.4.3 State dependent chemosensitivity

One particular limitation of this study is that chemosensitivity was measured during wakefulness. In investigating the link between chemosensitivity and SDB in elders it would have been beneficial to also assess chemosensitivity during sleep in these participants. Reasons for this statement are the alterations from wakefulness to sleep, in respiratory control and elements effecting respiratory control, i.e. cerebral blood flow. A number of studies have investigated the effect of age on the decrement of the HCVR from wakefulness to sleep. (Naifeh *et al.*, 1989; Browne *et al.*, 2003; Martinez, 2008). These studies tested the ventilatory responses to carbon dioxide exposures using a number of techniques. Martinez (2008) tested the response of the peripheral chemoreceptors during a single breath exposure to hypercapnia. Martinez observed no differences between the two age groups during NREM and REM sleep despite a reduction in peripheral chemosensitivity older individuals during wakefulness. A study using longer exposures of hypercapnia, and therefore stimulating both peripheral and central chemoreceptors, observed similar findings between the two age groups to that of Martinez, (Browne *et al.*, 2003). However, a small number of older individuals did present with a reduction in hypercapnic chemosensitivity during ten minute exposures to hypercapnic during sleep. Also, participants were told to be deprived of sleep prior to the experimentation so that daytime sleep tests could take place. Sleep deprivation could have an impact on the HCVR in both young and older participants that could act as a confounding factor in the interpretation of the authors' observations. Chapter 4 will explore the effect of sleep deprivation on CO_2 chemosensitivity. Nevertheless, from their observations the authors concluded that the increased occurrence of SDB in the older population is not linked with any alteration in hypercapnic chemosensitivity during

sleep with age. Browne *et al.* (2003) did not assess separate chemoreceptor sensitivities and neither did Naifeh *et al.* (1989).

These studies suggest a possible state dependent reduction in chemosensitivity with age, whereby reductions in chemosensitivity in older individuals only occur during wakefulness. However, one particular study used proportional assist ventilation to destabilise the respiratory system during sleep and monitor the ventilatory responses to give an indication of loop gain (Wellman *et al.*, 2007). The authors observed reductions in loop gain in older participants in comparison to younger participants during sleep. Wellman and colleagues also showed that older OSA patients had an even lower loop gain. It should be highlighted that only 3 older OSA particiaptns were tested in this study. Although these data did suggest a reduction in loop gain during sleep in older individuals, the authors did suggest that because of a more stable system in the elderly OSA patients an additional cause for the development of OSA, other than an aspect of respiratory control, was more likely. Nevertheless, the authors did speculate as to the possible mechanisms for which a low loop gain could lead to SDB and these mechanisms are similar to those put forth in this discussion and in section 1.6.4.

3.4.4 Mechanisms

One possible mechanism for the differences in central chemosensitivity observed in older individuals could be the changes in anatomical respiratory function associated with age. A reduction in muscular strength and functional vital capacity could reduce the HCVR, an observation that could be misinterpreted as an attenuated central chemosensitivity. However, previous studies have shown these age-associated changes in respiratory function

to play a minimal role in the alteration of hypercapnic chemosensitivity with age (Peterson *et al.*, 1981b; Rubin *et al.*, 1982). Furthermore, if anatomical changes in respiratory function were the cause of the reduction in total and central chemosensitivity observed in older individuals, it would be hypothesised that the HVR would also be reduced with age, this has been shown not to be the case (Ahmed *et al.*, 1991; Poulin *et al.*, 1993; Smith *et al.*, 2001; Pokorski & Marczak, 2003b). See also Chapter 6, where HVR was assessed in older and younger individuals and no change with age was found. However, it must be noted that the participant groups are different between these two studies and the mean age was slightly lower than the older group in the present study.

There are a number of possible other mechanisms for the reduction in central chemosensitivity in the older participants in this thesis. As previously stated, neural degradation with age could affect central areas of chemoreception thus affecting central chemosensitivity and total chemosensitivity (McKay & Feldman, 2008).

The state dependent differences highlighted in some of the studies referenced in section 3.4.3, could point to an additional site of neural degradation being responsible for the decline central chemosensitivity found in this study. The lower HCVR levels in older participants in comparison to younger participants were reported to occur only during wakefulness and not sleep. Also, the levels of HCVR in older individuals during wakefulness were so low that there was no reported change in HCVR from wakefulness to sleep in these elders, such a decline was seen in younger individuals. The decline in HCVR from wakefulness to sleep is thought to occur because of the reduction in chemosensitive neurons within the hypothalamus (Nattie & Li, 2010). The orexin neurons provide a wakefulness modulation of central chemoreception, which is removed at sleep onset, as

previously described in the introduction chapter. It is possible that some form of partial or complete neural degradation or an inhibition of the hypothalamus neurons with age could result in the attenuation of this modulation by the orexin neurons. Such an effect would account for the reduced in central chemosensitivity in older individuals during wakefulness reported in the present study. It would also explain why reductions in the HCVR of older individuals were reported only during wakefulness and no decline in HCVR was seen from wakefulness to sleep (Naifeh *et al.*, 1989; Browne *et al.*, 2003).

However, as stated previously neither of these studies assessed individual chemoreceptor contributions to chemosensitivity. Also, Martinez (2008) showed a state dependent difference in peripheral chemosensitivity between young and older individuals. Although not supported by the results presented here, Martinez's observations do suggest the state dependent differences she and others observed arise from changes in carotid body chemosensitivity.

Inhibition of orexin has also been shown to have little effect on resting ventilation in mice (Dias *et al.*, 2010). The results of the present study demonstrate no difference between the young and old age group in minute ventilation levels during the euoxic period. This has been observed by previous studies (Brischetto *et al.*, 1984; Poulin *et al.*, 1993; Garcia-Rio *et al.*, 2007). Although speculative because of the comparison between species, the observation lends evidence to the suggestion of some form of inhibition or neural degradation of orexin neurons in the older individuals in the present study, which may lead to the reduction in central chemosensitivity with age.

Gender differences in chemosensitivity could also explain our findings. Research has shown a reduced central but not peripheral chemosensitivity to hypercapnia in postmenopausal

women in comparison to pre-menopausal women (Preston *et al.*, 2009). Reductions in central chemosensitivity were correlated with reductions in circulating female sex hormones.

When separating the data in the current study into categories of sex in each age group, it was observed that the older females have a much lower total and central chemosensitivity than any other of the younger categories. This observation suggests that the older females are the larger contributor to the observed reduction in total and central chemosensitivity than the older males in this study. However, older males also show a significant reduction in central chemosensitivity in comparison to younger counterparts. Although a reduction in central chemosensitivity is also observed in our older females in comparison to older male participants it was not statistically significant (p=0.14), possibly due to low statistical power. These observations are shown below in figure 3.3.

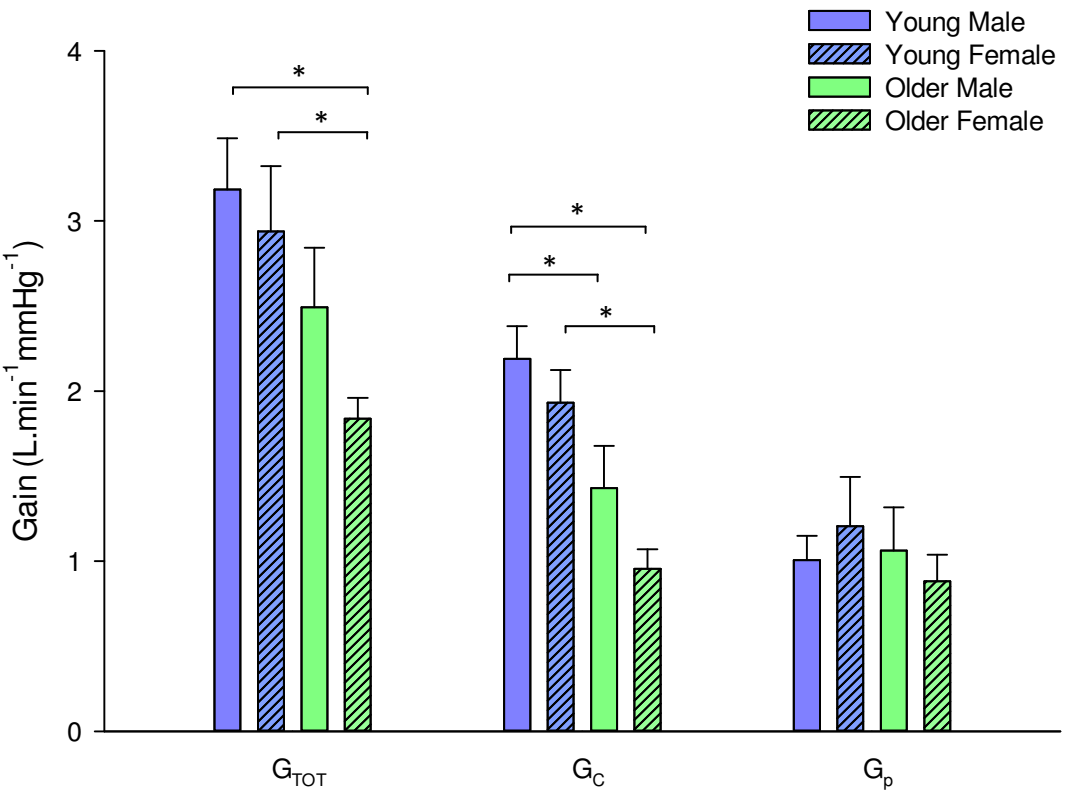

Figure 3.3 A graph showing the mean (±S.E.M.) total chemosensitivity (G_{TOT}), central chemosensitivity (G_c) and peripheral chemosensitivity (G_P) levels of both young and older individuals separated by sex. * = significant difference; $p<0.05$

One limitation of the present study is the absence of monitoring the menstrual cycle and the use of oral contraceptives in the younger age group, which could have affected the chemosensitivity of the younger females. Although no older female participants reported taking hormone replacement therapy medication, the hormone levels of these individuals were not assessed and therefore the postmenopausal status of the older female participants must be assumed.

Changes in cerebrovascular reactivity can affect HCVR and in particular central ventilatory output, as previously described. However, previous research investigating the effect of age on hypercapnic cerebrovascular reactivity suggests a decline with age (Kastrup *et al.*, 1998; Lipsitz *et al.*, 2000). A decline in hypercapnia cerebrovascular reactivity would result in an augmented central chemoreceptor ventilatory output in older individuals, an observation that is not evident in the present study.

Interestingly, a sex difference has been seen when investigating the age effect on cerebrovascular reactivity and it is similar to that suggested for the sex difference in chemosensitivity in older individuals. Kastrup *et al.* (1998) showed that older females had an attenuation in hypercapnic cerebrovascular reactivity whereas older males showed no change with age. In a number of older females who underwent hormone replacement therapy the reduction observed was restored to similar levels of their premenopausal counterparts. Despite the similarity between the hypercapnic cerebrovascular reactivity and central chemosensitivity changes seen in older females, it seems paradoxical relationship given the similar directions of the two changes with age.

In addition to the strong association between age and SDB, the Wisconsin sleep cohort study also found that being male is no longer a strong correlate with SDB in those aged over 50

years (Tishler Pv, 2003). One potential reason for this change in the divide between the sexes, is the strong increase in prevalence in the female sex, thought to be a consequence of going through menopause (Bixler *et al.*, 2001; Young *et al.*, 2003). Therefore, it is a possibility that the changes in chemosensitivity observed in older female individuals in this study could play a role in the changes in prevalence of SDB with regards to sex difference in the elderly.

3.4.5 Conclusion

To conclude, the observations in this study demonstrated that hypercapnic chemosensitivity is reduced in older individuals in comparison to younger counter parts. I was also shown, without the use of hyperoxia, that the reduction in total hypercapnic chemosensitivity in our older participants is driven by a reduction in central chemosensitivity. No age group difference in peripheral chemosensitivity was observed. Further research is needed to uncover the possible mechanism of reduced central chemosensitivity and whether these alterations in chemosensitivity contribute to the increased prevalence of SDB in the older population.

CHAPTER 4.

THE EFFECT OF SLEEP LOSS ON CHEMOSENSITIVITY AND VASCULAR FUNCTION

4.1 Introduction

Sleep loss is becoming a more common feature of our modern society, as discussed previously in the introduction chapter. Also, disruption to sleep length is a common feature of both disease states and ageing.

Epidemiological studies have highlighted the effect of chronic sleep loss on health and morbidity (Ferrie *et al.*, 2007; Cappuccio *et al.*, 2010b). Additional laboratory data have demonstrated the effect of sleep loss on more specific areas of physiological control, such as insulin resistance (Spiegel *et al.*, 1999) and gene expression (Möller-Levet *et al.*, 2013). Patients suffering from conditions where sleep disruption is a characteristic feature, will not only be at a greater risk of developing cardiorespiratory disease as a result, but will also possibly experience a worsening on the condition itself, as a result of the chronic disruption to sleep. One such condition is SDB. As previously described in the introduction, section 1.4.1, research has shown SDB patients to present with a worsening of the condition following the deprivation of sleep (Guilleminault *et al.*, 1984; Canet *et al.*, 1989; Persson & Svanborg, 1996; Thomas *et al.*, 1996). All these studies implemented a 24-hour period of sleep loss, however, a more representative form of sleep loss in our modern society, in disease states and in older individuals is a shortened sleep length on a regular basis over a prolonged period (Krachman *et al.*, 1995; Ohayon *et al.*, 2004; Redline *et al.*, 2004; Parthasarathy & Tobin, 2012).

A more recent study investigating the effect of sleep deprivation on SDB severity, did not show any effect of sleep loss on the AHI of SDB patients (Desai *et al.*, 2003). This study also investigated the effect of sleep deprivation on nocturnal respiratory features in healthy control participants. During that study authors reported no development of hypopnoeas or

apnoeas during the night in healthy controls. This study was also the only study to control participants' sleeping periods prior to the deprivation protocol. However, despite this controlling element, participants reported being slightly sleep deprived prior to the initiation of sleep deprivation, which may have influenced their findings.

A possible cause of the worsening effect of sleep deprivation on SDB could be an alteration in respiratory control, namely chemosensitivity. However, of the studies investigating the effect of sleep deprivation of SDB patients, only one has assessed respiratory control and this was in infants. Thomas *et al.* (1996) found a greater ventilatory response to short exposures of hypercapnia following sleep deprivation, thus suggesting elevated peripheral chemosensitivity in these patients. The researchers did not assess central chemosensitivity.

Investigation of the effects of sleep deprivation on respiratory control has been previously reported by a number of studies (Cooper & Phillips, 1982; Schiffman *et al.*, 1983; White *et al.*, 1983; Phillips *et al.*, 1987; Ballard *et al.*, 1990; Spengler & Shea, 2000; Stephenson *et al.*, 2000). However, there are a number of caveats to these investigations. A controlled environment and a healthy participant population unaccustomed to sleep loss are paramount for both the effectiveness of the sleep deprivation intervention and the measurement of respiratory control. Of these studies, a number have either used a participant population and/or forms of control that could be deemed to have confounding factors on the results obtained.

The participants used in earliest of the studies, Cooper and Phillips (1982), were made up of doctors and nurses. The sleep deprivation intervention was made up of each participant's night shift and a subsequent 12 hours of wakefulness. The protocol not only resulted in just an approximation of the deprivation period to which participants were exposed to (~ 27

hours), but it also lacked any clear control during this period, such as participant activity, food intake and light exposure. These elements of control are necessary as they themselves could affect circadian rhythms and respiratory control. Additionally, the use of healthcare practitioners who are accustomed to night shifts would affect the impact of the deprivation intervention on sleep and circadian disruption. Similar caveats are seen in Schiffman *et al.* (1983) were participants' night shift periods were used as part of the sleep deprivation protocol. Nevertheless, these two studies showed similar findings to an additional study, were normal healthy participants under controlled conditions were used (White *et al.*, 1983). These three studies all observed a reduction in HCVR within the range of 12 – 24%.

Conversely, further investigation by Phillips *et al.* (1987) and Ballard *et al.* (1990) found no change in respiratory responses to hypercapnia following 24 hours of sleep deprivation. However, the participants used in these two studies suffered from COPD and asthma, respectively. Although an important finding, the use of participants with respiratory illnesses in these studies makes it difficult to extrapolate results to a healthy population, as the conditions themselves may affect the respiratory chemosensitivity.

Two more recent studies that investigated the effect of a single night of total sleep deprivation in healthy individuals used a more extensively controlled protocol than previous research. Spengler and Shea (2000) asked their volunteers to complete a preliminary adaptation night of sleep in the laboratory to ensure that they were familiar with the study environment and that confounding variables were all controlled for prior to the sleep deprivation protocol. This minimized the potential confounding effect of prior sleep disruption on measured baseline values. Participants then underwent a "constant routine protocol" of controlled food intake, low light exposure and monitored activity levels. During

this period they were deprived of sleep for 24 hours. The HCVR was assessed every two hours during this period, using a Read's re-breathing test. The authors reported a 17% increase in the HCVR after 24 hours of total sleep deprivation. A second similar study also used this "constant routine" form of strict control during a 24 hour period of sleep deprivation (Stephenson *et al.*, 2000). Where Spengler and Shea used a Read's re-breathing test these authors used a modified re-breathing test, or a Duffin re-breathing test, to assess chemoresponsiveness to a stimulus of hypoxic hypercapnia. They found no change in chemosensitivity following sleep deprivation. One limitation of this study was the absence of controlled sleep periods prior to the initiation of the protocol.

As with the studies investigating the effect sleep deprivation on SDB, one common feature in all these studies is a complete deprivation of sleep for 24 hours. In the case of the more recent studies the 24-period of deprivation was primarily used to assess the 24-hour circadian rhythm of respiratory chemosensitivity (Spengler *et al.*, 2000; Spengler & Shea, 2000; Stephenson *et al.*, 2000). The shortening of sleep length across a number of consecutive days could have a more disruptive effect on respiratory chemosensitivity than the total deprivation of sleep over a single 24-hour circadian cycle.

All the studies mentioned above, with the exception of Stephenson *et al.* (2000), used a method of Read's re-breathing to exposure participants to hyperoxic hypercapnia. The possible caveats of using hyperoxic hypercapnia have been described previously. No study has attempted to separate the contributions of the peripheral and central chemoreceptors to the measured total chemosensitivity.

Additionally, as previously mentioned the condition of SDB has strong mechanistic links with the development of cardiovascular disease. The sleep deprivation characteristic of SDB could

have mechanistic properties for the increased occurrence of cardiovascular disease in sleep apnoea patients. This is supported by research reporting shorter sleep length having a greater incidence of cardiovascular disease (Cappuccio *et al.*, 2011).

One form of cardiovascular function that leads to the development of cardiovascular disease and that is also associated with SDB, is vascular dysfunction. A reduction in both brachial and cutaneous vascular reactivity has previously been reported in shift workers following a single night shift (Amir *et al.*, 2004) and following total sleep deprivation in controlled environments (Sauvet *et al.*, 2010). As with the previously mentioned research on sleep deprivation and respiratory control, these investigations all adopted a total sleep deprivation protocol. Takase *et al.* (2004a) did show a reduced vascular function in participants who reportedly slept for 80% less over a four week period. However, the uncontrolled four week period was during an exam period when participants were said to be under chronic stress.

The purpose of this study is to investigate the impact of acute sleep length restriction, across three nights, on respiratory chemosensitivity in healthy participants. Participant's peripheral and central chemosensitivity was assessed with a MFBS test. An additional aim of this study is to use nocturnal respiratory polysomnography to assess any correlation between alterations in respiratory chemosensitivity and breathing stability during reduced sleep length periods. Lastly, it is hypothesised that recurrent nights of reduced sleep length will compromise vascular function, measured using flow mediated dilation.

4.2 Methods

4.2.1 Participants

Ten participants (3 females) were recruited from the University of Birmingham student body. All participants reported having a habitual sleeping pattern of 6-9 hours between 22:00 and 08:00 hours to ensure they had a normal sleeping routine. Participants were unable to take part in the study if they had travelled to a different time zone four weeks prior to any experimental week, if they were frequent nappers or if they had been a night shift worker anytime within the year prior to taking part in the study. Participants had not been previously diagnosed with a sleeping, eating or mental condition. All participants were non-obese (BMI <30kg/m^2), non smokers and free of cardiovascular and respiratory disease.

The purpose and details of the study were provided to all participants and informed consent gained and a general health questionnaire was completed prior to the participants' implementation of the research project. Ethical approval for the study was obtained from the local ethics subcommittee within the University of Birmingham.

4.2.2 Experimental Protocol

Participants completed two experimental periods in a randomised cross over design in which each experimental week was separated by a four-week wash out period. During each experimental period participants spent five consecutive nights sleeping in a purpose built sleep laboratory. The two experimental weeks consisted of a Restriction and Control week. These experimental weeks differed in the number of hours participants were allowed to sleep each night. Each experimental week was preceded by a preliminary period of six consecutive nights of a normal sleeping period within the participants' home. The

preliminary week is conducted to ensure a stable run-in period of sleep prior to the start of each experimental week. Participants were asked to refrain from any strenuous physical exercise during experimental weeks and were told to refrain from any daytime sleeping during any preliminary or experimental weeks. A schematic of the experimental design is shown in figure 4.1.

All experimental periods began on a Sunday evening and were terminated the following Friday evening. Participants were asked to arrive at the laboratory at 19:00 hours each evening, and they were asked to not have consumed any food or caffeine for three hours prior to their arrival. Between their arrival and departure from the laboratory the following morning participants were only allowed to consume water and the control meals provided for them for evening meals (20:30) and breakfast (09:00). The purpose of the provided meals was to standardise the type of meal within and between protocols. Participants were allowed to leave the laboratory following breakfast to go about their normal daily activities.

The first night of each experimental week was used as an acclimatization period during which participants slept for eight hours between 23:00 and 07:00 hours. The following night consisted of the same sleeping hours and made up the baseline night's sleep (BL) for each experimental week. For the three consecutive nights (N3, N4 and N5) following BL the opportunity to sleep was made either shorter or longer for Restriction and Control weeks, respectively. The Restriction week consisted of an opportunity of four hours of sleep between the hours of 01:00 and 05:00. During the Control week participants had the opportunity to sleep up to ten hours between 20:00 and 08:00 hours. Each morning participants were made aware of the end the sleeping opportunity period by an alarm clock. During the hours of wakefulness participants were free to work at a computer, read, watch

movies or socialize with friends or experimenters. During the assigned sleeping periods participants were told to remain in bed, asleep or rested, with the lights off. Between the hours of 22:00 and 08:00 light levels were controlled during non-sleeping opportunity hours to a low light level of 15 Lux. The brightness of the ambient light was monitored with a Lux meter (Trotec, BF05 Lux meter).

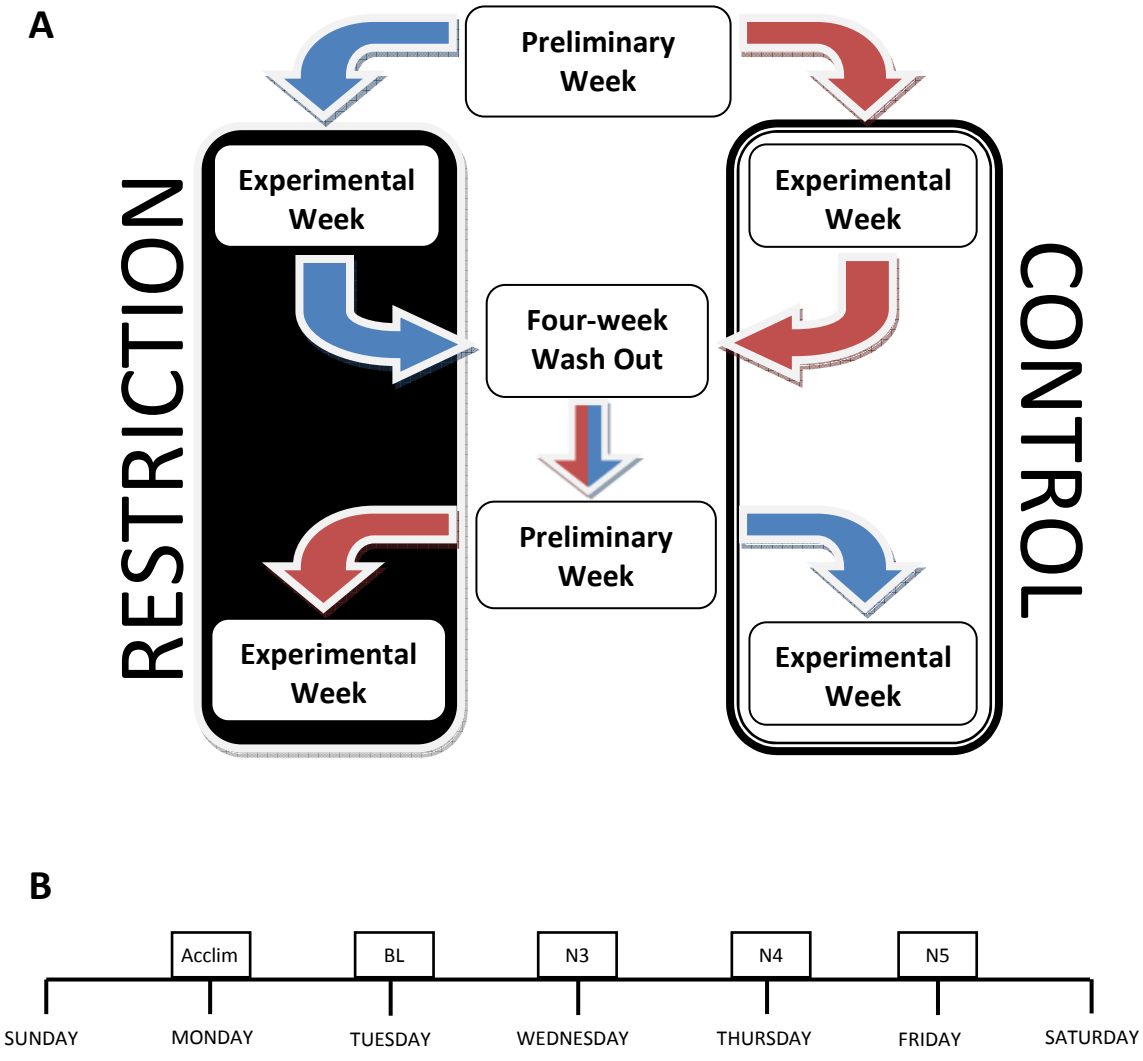

Figure 4.1 A schematic representation of the entire study. **A**. Represents the randomized cross over design of the study showing how two example participants, shown by blue and red arrows, may have completed the entire study. **B**. Displays formulation of the experimental weeks. Where, Acclim = Acclimatisation night, BL = Baseline, N3 = first night of either control or restricted sleep length and N4 and N5 represent the fourth and fifth nights, respectively.

4.2.3 Measurements

Participants were asked to wear an accelerometer watch (Actigraph GT3X) on the wrist of their non-dominant hand throughout both the preliminary and experimental weeks. The accelerometer watch was used to monitor both sleeping periods to ensure stable sleep during the preliminary week and to verify that participants refrained from sleeping during the day.

Each evening at 19:15 and each morning at 08:00 measurements of resting blood pressure were made by way of electronic sphygmomanometer (Omron 750-IT) on the participants' non dominant arm. Furthermore, participants were asked to score their level of sleepiness from 1 to 7 using the Stanford Sleepiness Scale (Hoddes *et al.*, 1972); (1 = Feeling active, vital, alert, or wide awake – 7 = No longer fighting sleep, sleep onset soon; having dream-like thoughts). The measurements of sleepiness were taken on arrival to the laboratory and on departure from the laboratory each morning.

Polysomnography was conducted each night. All other physiological measurements were initiated on the morning following each baseline night. Each morning at approximately 08:30 endothelial dependent vascular function was assessed using a flow mediated dilation test. A MFBS chemosensitivity test was performed each evening prior to the participants evening meal at 19:30. A schematic of the experimental protocols and measurement timings is shown in figure 4.2.

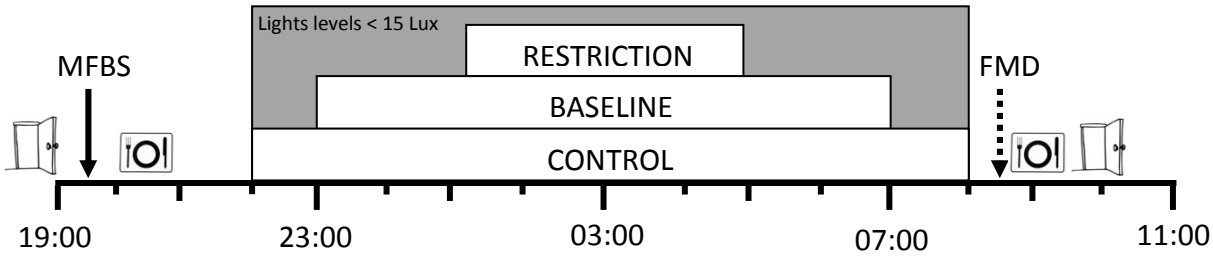

Figure 4.2 Shows the manner in which each night, with the exception of the acclimatisation night, was constructed and the times of the measurements. Clear boxes show the number of hours designated during the Control, Baseline and Restriction nights. The grey scale background shows the hours of controlled low light exposure (<15 Lux). The open doors show the times of participant arrival and departure. Knife and fork symbols show controlled evening meal and breakfast times.

4.2.4 Data analysis

A repeated measures analysis of variance (ANOVA) was used to determine significant differences within data obtained during each protocol. When a significant effect over time was determined main effects were resolved using a Fisher's least significant difference post hoc test. A paired T-test was used to assess changes from baseline to final night polysomnography measurements. Significance was determined when $P<0.05$. Values reported are mean ± S.E.M and significance was determined by $p<0.05$ unless otherwise stated.

4.3 Results

Ten healthy young participants with a mean ±S.D age and BMI of 26.00 ± 4.88 years and 22.62 ± 2.47 kg/m^2 respectively, took part in the study. An equal number of participants completed the restriction protocol first as those who began with the control protocol. All participants met the required inclusion and exclusion criteria. Results for flow-mediated

dilation (FMD) were obtained from eight participants. FMD data from two participants were removed from the data analysis because of inadequate quality of ultrasound imaging, meaning that an accurate set of data could not be obtained.

4.3.1 Sleeping time and sleepiness

Accelerometer data demonstrated similar lengths of sleep within and between the two preliminary weeks with no significant differences in the number of minutes slept each night. Furthermore, there was no difference in the time slept during the acclimatisation and baseline nights between the control and restriction weeks. The times slept during the controlled and restricted sleep length periods were significantly different for each night. Participants on average also slept longer during the first night of 10 hours of opportunity than they did on the previous baseline night. The accelerometer data demonstrated no daytime sleeping by any participants during any days of the study.

During the restriction protocol participants grew significantly sleepier throughout the experimental week; $p<0.01$. There was no change in the sleepiness scale throughout the control protocol. Results obtained from the accelerometer and the Stanford sleepiness scale are presented in figures 4.3 and 4.4 respectively.

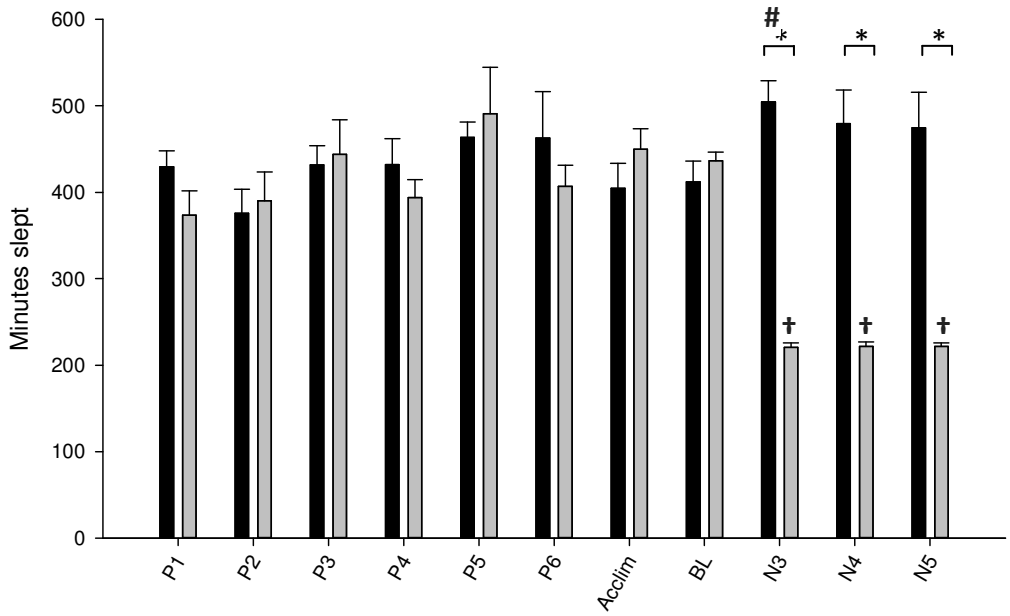

Figure 4.3 Represents the mean (±S.E.M) number of minutes slept each night during the preliminary weeks (P1-P6) and the experimental weeks (Acclim – N5). Control and restriction protocols are shown by light and dark bars respectively. * = significant difference in sleep length on equivalent nights between protocols. **† =** significantly lower than all other nights within restriction protocol with the exception of N3, N4 and N5. # = significantly different from control protocol baseline. Significance = $p<0.01$; repeated measures ANOVA.

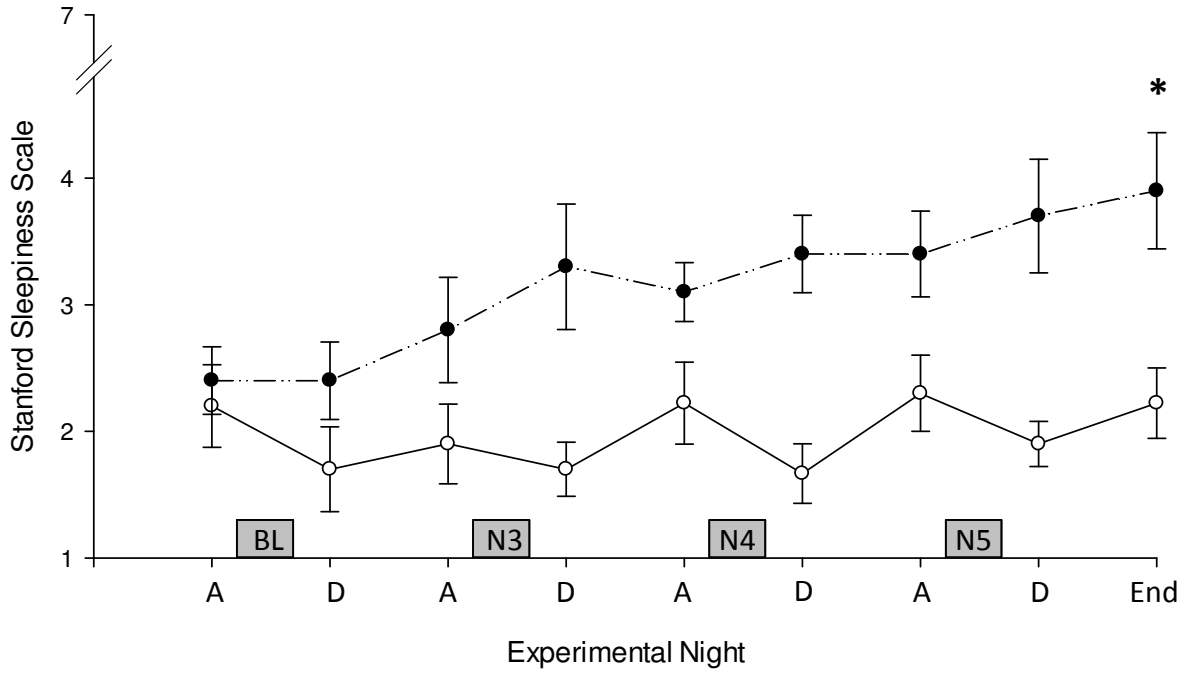

Figure 4.4 Graph represents mean (±S.E.M) Stanford Sleepiness Scale scores on arrival (A) and departure (D) throughout the experimental week, in addition to the final sleepiness score taken on the evening of the final experimental day. * = a significant increase in sleepiness across the restriction protocol week in comparison to the control week; P<0.01, Repeated measures ANOVA.

4.3.2 $P_{ET}CO_2$, Ventilation and Heart rate.

Mean $P_{ET}CO_2$ did not change throughout either protocol week. Resting minute ventilation and heart rate were obtained during euoxic eucapnia prior to the MFBS test. There was no change in resting ventilation or heart rate during the course of either protocol week. There was also no difference between the two protocols. Results are presented in table 4.1.

	Restriction				Control			
	BL	N3	N4	N5	BL	N3	N4	N5
$P_{ET}CO_2$(mmHg)	38.80	38.90	39.50	38.60	38.55	38.78	38.75	38.65
± S.E.M	0.60	0.43	0.31	0.45	0.81	0.64	0.46	0.43
V_E (L.min^{-1})	10.58	12.59	12.79	11.92	12.08	11.85	12.35	11.77
± S.E.M	1.03	1.31	1.14	0.59	0.99	0.88	1.02	1.41
HR (b.min^{-1})	60.85	63.65	65.11	66.95	65.10	62.28	67.35	68.65
± S.E.M	2.99	3.04	3.74	3.29	3.76	2.51	3.81	3.63

Table 4.1 Shows mean ± S.E.M of resting $P_{ET}CO_2$, ventilation (V_E) and heart rate (HR) prior to the MFBS test each evening during the restriction and control protocols. No differences were seen.

4.3.3 Blood pressure

There was no change in evening blood pressure measurements following either the control or restriction protocol weeks. There was no change in morning blood pressure variables during the control protocol week. There was a significant but minor decrease in mean arterial pressure of 2.18 mmHg following the restriction protocol week. Additionally, there was a significant 2.10 mmHg decrease in diastolic blood pressure following the restriction week. Blood pressure parameters are displayed in table 4.2.

		Restriction					Control				
		BL	N3	N4	N5	End	BL	N3	N4	N5	End
PM	MAP (mmHg)	90.33	89.51	90.81	90.91	88.37	91.09	92.10	90.63	90.47	89.27
	±S.E.M	3.55	2.98	3.76	2.63	3.60	2.56	2.67	2.80	2.90	2.24
	SBP (mmHg)	122.60	121.00	122.90	123.80	119.63	124.60	125.23	123.67	124.20	120.80
	±S.E.M	5.31	4.14	4.89	4.44	5.36	3.22	3.54	4.57	3.91	4.17
	DBP (mmHg)	74.20	73.77	74.77	74.47	72.73	74.33	75.53	74.11	73.60	73.50
	±S.E.M	2.38	2.62	2.77	2.15	2.60	3.66	3.81	3.60	4.04	3.23
AM	MAP (mmHg)	87.12	88.44	88.07	84.94‡		85.62	87.04	88.07	89.08	
	±S.E.M	2.80	2.64	3.83	2.70		2.20	1.68	2.17	2.46	
	SBP (mmHg)	116.90	117.67	117.53	114.57		114.07	116.53	117.63	119.10	
	±S.E.M	4.36	3.47	5.15	3.46		2.26	2.62	3.27	4.01	
	DBP (mmHg)	72.23	73.83	73.33	70.13†		71.40	72.27	73.30	74.07	
	±S.E.M	1.79	1.91	2.79	2.08		3.07	2.91	3.49	3.27	

Table 4.2 Mean (±S.E.M) systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP) for evening (PM) and morning (AM) time points are shown. † = significantly different from BL, N3 ‡ = significantly different from BL, N3, N4; $p<0.05$, repeated measures ANOVA.

4.3.4 Respiratory chemosensitivity.

There was no change in total chemosensitivity during either the restriction or control weeks. When total chemosensitivity is separated in to both peripheral and central chemosensitivity the data demonstrated small but not significant increase in peripheral chemosensitivity following both experimental weeks. Mean central chemosensitivity reduced from baseline (2.52 ± 0.61 $L.min^{-1}.mmHg^{-1}$) after the second night (1.92 ± 0.22 $L.min^{-1}.mmHg^{-1}$) and the third night of sleep loss (1.94 ± 0.21 $L.min^{-1}.mmHg^{-1}$). This translates to a relative reduction in central chemosensitivity of 23.51 ± 8.31% and 20.33 ± 8.74% following the second and third nights of sleep restriction, respectively. No changes in central chemosensitivity were seen throughout the control week. Results are shown in figure 4.6. There were no correlations with any changes in chemosensitivity and levels of sleepiness recorded using the Stanford Sleepiness Scale. There was no change in B value throughout the control week. During the restriction week there was a decrease in B value (mmHg) from the first night of sleep restriction to the final night (36.08 ± 0.46 to 33.10 ± 1.27; $P<0.05$). Also, mean B value tended to decrease from baseline (35.40 ± 0.79) to final night of sleep loss; $P = 0.054$. Changes in B value across both weeks are displayed in figure 4.5 below.

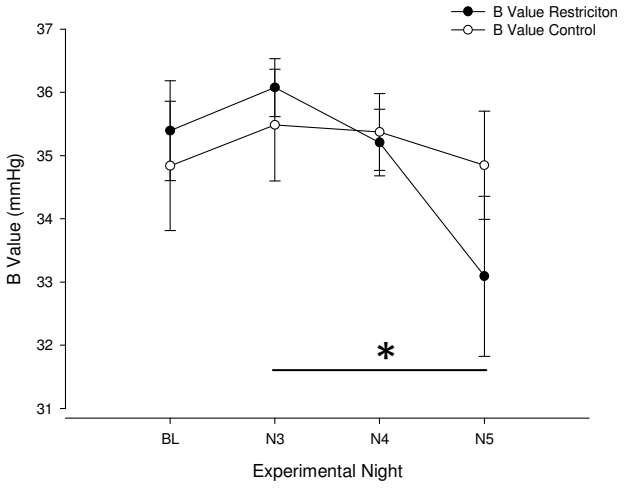

Figure 4.5. Mean ± S.E.M changes in B value across both experimental weeks. There was no change in B value across the control week. There was a significant decrease from N3 to N5 during the restriction week; * = $P<0.05$.

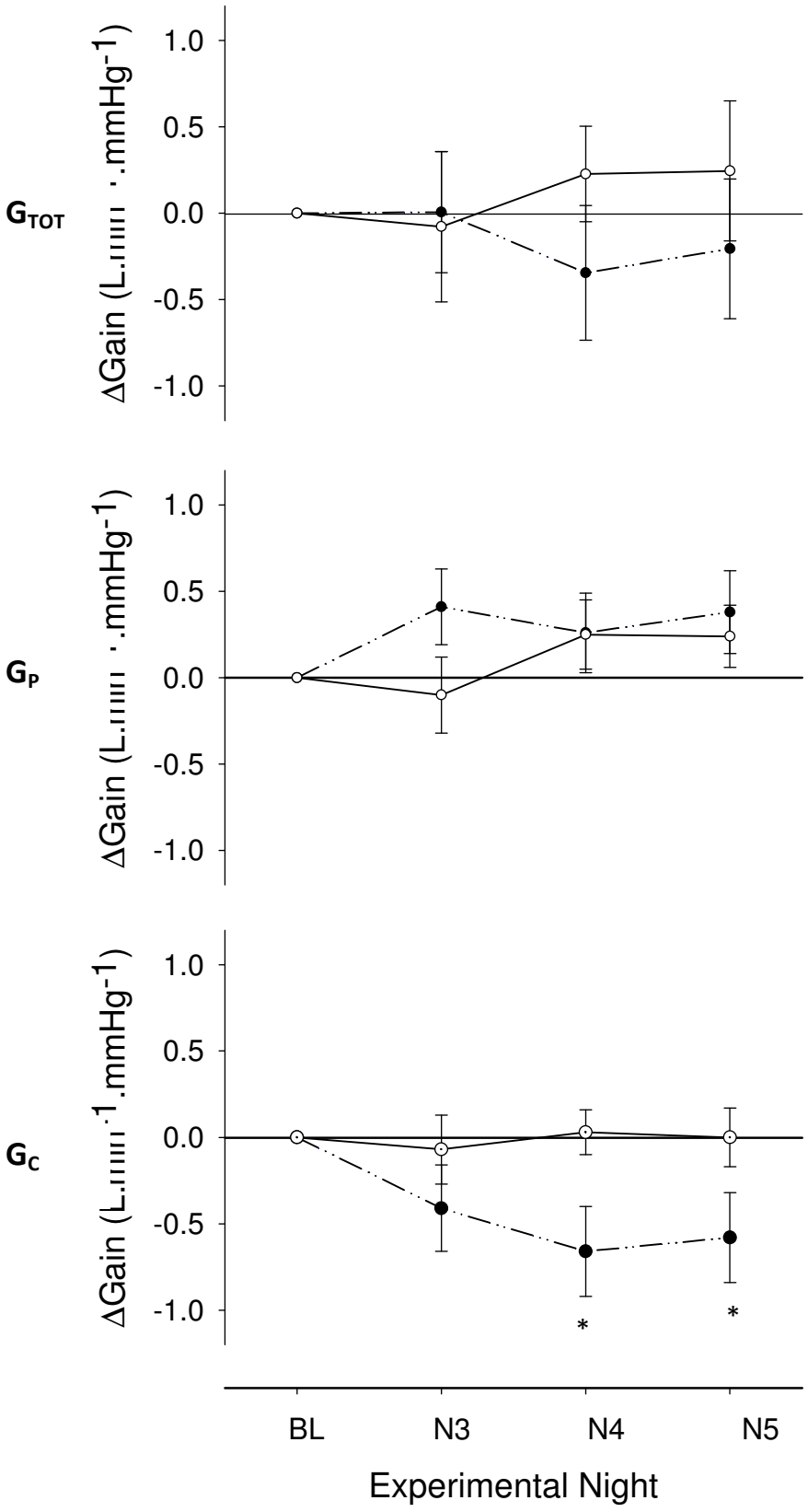

Figure 4.6 Graphs represent mean (±S.E.M) change in gain ($L.min^{-1}.mmHg^{-1}$) from baseline for total chemosensitivity (G_{TOT}), peripheral chemosensitivity (G_P) and central chemosensitivity (G_C). * = significant change from baseline ($p<0.05$), repeated measures ANOVA.

4.3.5 Vascular function

Changes in FMD response across both weeks are shown in figure 4.7. The functional hyperaemia response was unchanged throughout the control experiment week. When normalised to baseline, the mean percentage change in brachial artery diameter upon cuff release was significantly reduced by 3.36% (±0.51) following two nights of restricted sleep length in comparison to baseline. Following a third night of sleep restriction the functional hyperaemia significantly improved to return to baseline values.

There was no change in resting brachial artery diameter during either experimental week. Also a change in calculated shear rate upon cuff release was not seen throughout either experimental week. Mean resting brachial artery diameter and shear rate are displayed in figure 4.8.

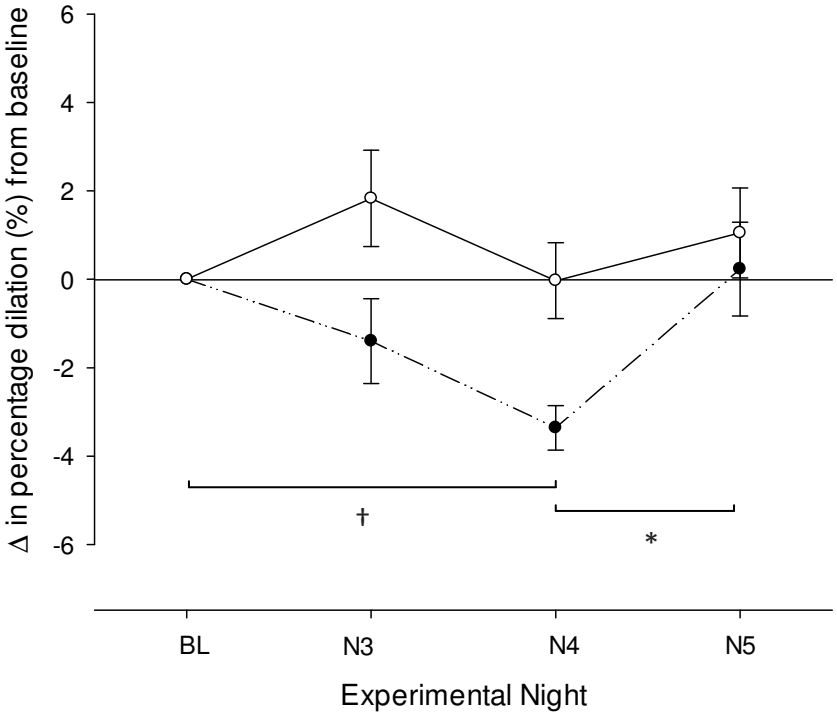

Figure 4.7 Graph displays mean (±S.E.M) changes from baseline in percentage flow mediated dilation response on cuff release for control (solid line – clear dots) and restriction (broken line – black dots) experimental weeks. Significant differences occurred during the Restriction protocol † = significant change from baseline $p < 0.03$ * = significant change from N4 to N5; $p < 0.05$. Repeated measures ANOVA.

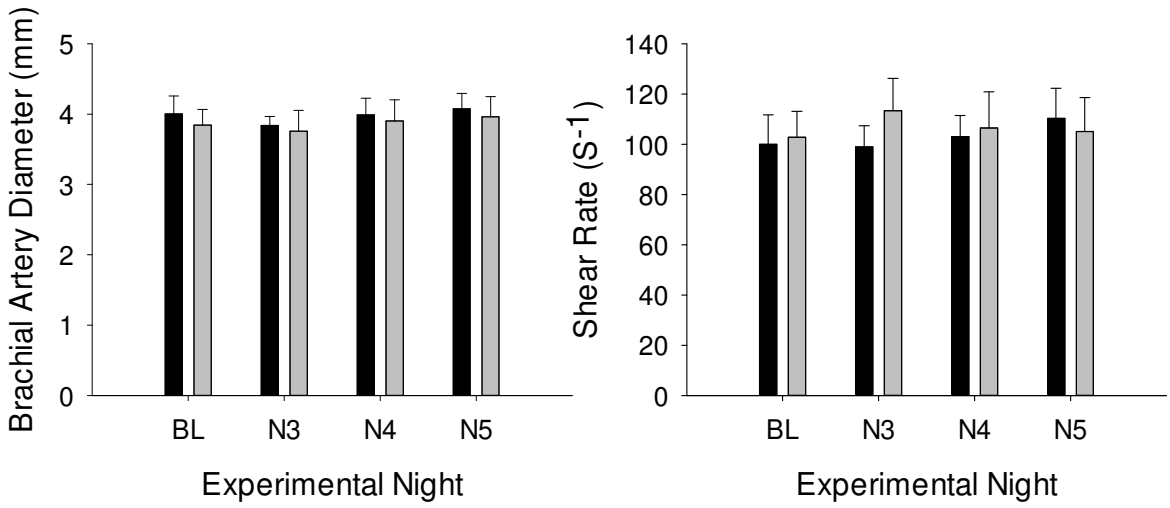

Figure 4.8 Graph displays mean (±S.E.M) of baseline brachial artery diameters (A) and calculated shear rate upon cuff release during morning FMD tests for each protocol. No significant differences were observed. Black bars represent the Control week, grey bars the Restriction week.

4.3.4 Polysomnography

Table 4.3 shows mean polysomnography results for baseline and final night for each protocol. No changes in nocturnal respiratory features, hypopnoea and/or apnoeas were observed throughout either protocol week. All apnoeas that were present were central in nature. Data did demonstrate changes in particular features of sleep architecture. Total sleep time was significantly greater between baseline and the final night of the control protocol. Unsurprisingly, the opposite change was observed between the baseline and final night of the restriction protocol. Participants showed a significant reduction in time and percentage of sleep time spent in REM between the baseline and final night of sleep restriction. During the control protocol the opposite occurred in the absolute time and percentage of time spent in REM sleep. Less time was spent in stage 1 and 2 sleep during the final night of sleep restriction in comparison to baseline. A greater arousal index during the final night of control in comparison to baseline was observed.

	Restriction		Control	
	BL	N5	BL	N5
Total Sleep Time (min)	443.22	233.55#	452.88	523.63#
± S.E.M	13.78	2.98	5.25	17.07
Sleep Efficiency (%)	97.23	99.11	97.58	93.51
± S.E.M	1.07	0.47	0.80	1.81
Sleep Latency (min)	8.22	2.85	14.44	25.69
± S.E.M	2.11	0.70	4.40	9.08
REM (min)	105.11	47.10*	103.56	134.00#
± S.E.M	7.42	5.60	8.05	10.94
Stage 1 (min)	32.06	11.20#	22.13	44.56
± S.E.M	5.74	2.02	3.71	9.57
Stage 2 (min)	220.50	88.95#	234.69	247.88
± S.E.M	10.49	3.76	8.00	16.38
Stage 3 (min)	85.56	86.29	92.50	97.19
± S.E.M	5.54	7.39	8.32	8.15
REM (%)	23.72	20.35*	22.89	25.50*
± S.E.M	1.47	2.58	1.82	1.78
Stage 1 (%)	7.32	4.77#	4.93	8.56
± S.E.M	1.32	0.84	0.84	1.92
Stage 2 (%)	49.59	38.08#	51.78	47.01#
± S.E.M	1.22	1.50	1.48	1.77
Stage 3 (%)	19.36	36.82#	20.40	18.90
± S.E.M	1.18	2.97	1.82	1.98
Arousal Index (no.hr^{-1})	4.86	3.85	4.07	4.92#
± S.E.M	0.56	0.51	0.52	0.59
Hypopnoea Index (no.hr^{-1})	0.68	0.62	0.25	0.12
± S.E.M	0.28	0.29	0.11	0.03
Apnoea Index (no.hr^{-1})	0.11	0.20	0.24	0.05
± S.E.M	0.06	0.12	0.19	0.03

Table 4.3 Shows the polysomnography data for baseline (BL) and final nights (N5) of each protocol. #= P<0.03 *=P<0.05

4.4 Discussion

The data from this study demonstrated no alteration in total respiratory hypercapnic chemosensitivity following three nights of reduced sleep length. However, the calculated contributions of the central and peripheral chemoreceptors show a reduction in central chemosensitivity in conjunction with a small but not significant increase in peripheral chemosensitivity following sleep restriction. The summation of these converse alterations in chemoreception result in the observation of an unaltered total chemosensitivity following sleep restriction. The control protocol resulted in no alterations in total, central or peripheral chemosensitivity from baseline, showing that the sleeping in the laboratory and undergoing experimental measurements during the protocol did not affect chemosensitivity in the participant population. Despite the changes in central chemosensitivity, no development of respiratory disturbances, hypopnoeas or apnoeas, were observed during sleep in the restriction protocol.

Lastly, a biphasic shift in vascular reactivity occurred during the sleep restriction protocol, as is evident in the reduction of the flow mediation dilation response following two nights of reduced sleep length. The reduction in vascular reactivity recovered to baseline levels following the third and final night of sleep restriction. No alterations in calculated shear stress stimulus or brachial artery resting diameter were seen, which may have otherwise affected the measured vascular reactivity.

4.4.1 Sleep restriction and Chemosensitivity

Where all previous research has adopted a 24-hour total sleep deprivation this present study is the first study to investigate the effect of reducing the sleeping hours over consecutive

nights on respiratory chemosensitivity. Additionally, this is the first study to assess the contributions of the central and peripheral chemoreceptors to any shown changes on total hypercapnic chemosensitivity. By assessing their individual contributions we have been able to observe alterations in chemoreception that would otherwise remain hidden when assessing central or total chemosensitivity alone.

The previous studies of the early 1980's all reported reductions in chemosensitivity following total sleep deprivation (Cooper & Phillips, 1982; Schiffman *et al.*, 1983; White *et al.*, 1983). These studies all used a Read's re-breathing test as their preferred method of gas control during HCVR tests. The gas composition of the re-breathing bags used in these studies were all made up of high CO_2 concentrations with a balance of O_2; the majority of the compositions being 7% CO_2 and 93% O_2. As shown in previous studies (Dahan *et al.*, 1990; Pedersen *et al.*, 1999b) and shown by data presented in chapter 3 of this thesis, the use of high oxygen concentrations attenuate the peripheral chemoreceptor contribution to the HCVR, but not completely. Nevertheless, it could be postulated that the reduction in chemosensitivity seen by the above studies is in its majority an effect brought about by a central chemosensitivity alteration. It is in this aspect that our results seem to support that of these previous studies. However, we did not observe an overall significant reduction in total chemosensitivity following sleep loss due to the influence of the small increases that we have seen in peripheral chemosensitivity. The use of hyperoxia in previous research would have concealed the slight enhancement of the peripheral element of chemosensitivity observed here and therefore the magnitude of the reduction in hypercapnic chemosensitivity could have been overestimated.

However, the most recent study by Spengler and Shea (2000) also uses a hyperoxic re-breathing test to establish the HCVR and reported the opposite, a minor increase in chemosensitivity following total sleep deprivation. The main differences between the protocol used by Spengler and Shea (2000) and other previous research was the use of strictly controlled behavioural and environmental conditions. The authors controlled light levels, food intake and activity levels during the deprivation period. Similar levels of control were used during the present study. In the restriction protocol, light levels were controlled at a low level of 15 Lux during the periods of prolonged awakening. Controlling light levels during these periods is integral to avoid any confounding effects that exposure to bright light, during the usual dark periods, can have on circadian rhythms (Boivin *et al.*, 1996). Any residual effect on circadian rhythm by unusual light exposure could possibly affect respiratory chemosensitivity and confound the results obtained. Also, food intake and activity levels were controlled during times under laboratory conditions. However, in our study participants were allowed to leave the laboratory during daytime and go about their daily activities. However, a restriction from exercise and a three-hour fasting period prior to their return to the laboratory in the evening were enforced. This element of freedom within the study was implemented so as to minimise the stress and boredom of laboratory conditions. Additionally, the free-living period was introduced so as to create an element of real world conditions within the study, making the study more representative of reality. The free-living aspect of the present study had no confounding effects on our measurements, as evident by the absence of any changes in chemosensitivity or vascular function during the control week. In fact, it is possible that the strict control conditions that were enforced by Spengler and Shea could have affected the overall results. It is possible that the unusual

nature of the controlling aspects, such as meals being issued every two hours, could alter circadian rhythms further and possibly effect respiratory control. Additionally, the repetitive HCVR tests conducted every two hours during the entire period of the study could have had an effect on ventilatory responses through long term facilitation. However, previous research has suggested that sequential hypercapnic challenges do not stimulate any long term facilitation in ventilation (Sahn *et al.*, 1977; Gozal *et al.*, 1995). Nevertheless, previous research has shown that repetitive hyperoxic exposure can cause carotid body plasticity that alters the HVR (Bavis *et al.*, 2007). It could be postulated that a repetitive high oxygen exposure during hyperoxic hypercapnia re-breathing tests could alter peripheral chemoreceptor chemosensitivity to CO_2 and hyperoxia, possibly interfering with the synergy between central and peripheral chemoreceptors.

An additional study incorporating the strict aspects of control during the deprivation of sleep is that of Stephenson *et al.* (2000). The authors saw no change in the ventilatory response to hypoxic hypercapnia, delivered by a modified re-breathing test. However, participants did not complete any preliminary nights of sleep, therefore it could be speculated that the baseline values of chemosensitivity may have been confounded by disruption to sleep. Furthermore, the repetitive exposure to hypoxia during the modified re-breath test could cause a long-term facilitation response in participants, thus affecting the observed chemosensitivity levels. However, such a response is unlikely, as the time between the intermittent measurements (three hours) is most likely not sufficient to result in neural plasticity and long-term facilitation.

One caveat of this present study is that no morning chemosensitivity levels were assessed. Such a measurement could have provided further information regarding the immediate changes in chemosensitivity following sleep loss. This would have also shed light on the possible changes in chemosensitivity that could have been brought about by attenuations in the daily activity levels of the participants and additional confounding factors uncontrolled during the participants'' free living day.

4.4.2 Sleep loss and SDB

As previously described in the introduction, 24-hours of sleep deprivation have been shown to result in an increased severity of AHI in SDB patients. Only one of these previous studies has recorded chemosensitivity in the patients following sleep loss (Thomas *et al.*, 1996), although they did not assess central chemosensitivity separately. Also, only a single study monitored control participants (Desai *et al.*, 2003). However, these participants reported being mildly sleep deprived prior to the initiation of the deprivation protocol. In the present study, two prior acclimatisation nights were completed by participants to ensure baseline values were recorded in non-sleep deprived individuals. In addition to chemosensitivity, nocturnal polysomnography was conducted in order to assess whether respiratory features of SDB or instabilities in breathing would occur in these healthy participants as a consequence of reduced sleep length. Despite the reduction in central chemosensitivity following sleep restriction, no individuals demonstrated a development of hypopnoea or apnoeas during sleep. These data suggest that sleep loss does not result in the development of sleep apnoea in healthy young individuals, supporting data reported by Desai *et al.* (2003). Furthermore, the data reported in the present study demonstrates that the stability of

breathing during sleep remains stable following a reduction in central chemosensitivity. This observation lends evidence to that of the role of the central chemoreceptors in the pathophysiology of apnoeic events being minimal, as described in Chapter 3; section 3.4.2.

The tended reduction in total chemosensitivity coupled with the lower of the apnoeic threshold and no change in normal $P_{ET}CO_2$, seen in the present study, would seem to represent a more stable respiratory system. The respiratory system following sleep loss provides a more stable sensitivity, a lower apnoeic threshold and a greater CO_2 reserve, all of which will reduce the likelihood of bring about central apnoeic events. The more stable system would explain why there was no development of apnoeic events or respiratory instability during the nights of reduced sleep loss. However, when separating total chemosensitivity into the differential peripheral and central chemoreceptor gains the respiratory system could be perceived to be less stable. This differential system shows a peripheral chemosensitivity tending to higher levels, possibly inferring greater instability, and where central chemosensitivity is attenuated. A higher peripheral gain would lead to greater incidence of periodic breathing (Khoo *et al.*, 1982) and a reduced central chemosensitivity could result in a decline of the hypercapnic arousal threshold (Ayas *et al.*, 2000) which may lead to longer apnoeic events and thus a greater level of hypoxemia. Despite this possibility no such events were seen in the participants during this study. This could be due to the minimal changes in peripheral chemosensitivity or that the changes in chemosensitivity seen may not have translated through into the sleeping state.

In light of these findings it could be postulated that the increased severity of SDB seen to occur in patients deprived of sleep, may not be caused by a changes in chemosensitivity,

namely a reduced central chemosensitivity. However, a number of confounding features of SDB, namely intermittent hypoxia, may result in additional alterations in chemosensitivity (Katayama *et al.*, 2005). If the stability of the respiratory system in patients is already compromised it could be vulnerable to further changes as a result of the deprivation of sleep. Therefore, the role of reduced chemosensitivity following sleep deprivation should not be ruled out as a possible cause for the increase in AHI seen in patients following sleep deprivation.

4.4.2 Mechanisms for alterations in chemosensitivity following sleep loss

Sleep deprivation directly affects cognition and arousal state (Durmer & Dinges, 2005). A decrease in arousal state is demonstrated by the increased magnitude of sleepiness in our participants during sleep restriction. A reduction in arousal state can have direct effects on breathing during wakefulness through the influence of reduced cortical arousal on ventilatory control (Shea, 1996). Such an effect during the sleep restriction protocol could explain, in some part, the effect on ventilatory chemosensitivity observed in the present study. However, if this were the case, a reduction in resting ventilation and a possible change in $P_{ET}CO_2$ would be expected, but we did not see a change in neither of these variables. Nevertheless, in three participants an attempt to increase cortical arousal at the nadir of the restriction protocol was made and chemosensitivity was assessed once more. A stimulated increase in arousal state was attempted by asking participants to yawn and stretch for 2 minutes prior to an additional MFBS test. Yawning and stretching has been suggested to have a direct effect on a number of areas within the brain to increase arousal (Walusinski, 2006). One particular area within the brain activated by the yawning response is

suggested to be the reticular formation. The reticular formation has afferent projections to major respiratory control centres in the brain stem (Martelli *et al.*, 2013). Therefore one would assume an improvement in the ventilatory control following a period of yawning and stretching caused by reticular formation activation. Results from these observations in three participants are displayed in table 4.4 below. On average, no differences between the final test of the restriction protocol and the additional MFBS test following instructed yawning and stretching were found. However, one participant did show an increase in central gain of 0.27 $L.min^{-1}.mmHg^{-1}$ from 1.62 to 1.89 $L.min^{-1}.mmHg^{-1}$. Of the three participants to do this additional test this particular individual presented with the largest decrease of central gain during the restriction protocol. Nevertheless, the mild recovery following attempted reticular formation activation is still far from the participant's original baseline gain of 2.45 $L.min^{-1}.mmHg^{-1}$.

I.D	Final Restriction Protocol Test			Additional Reticular Formation Activation Test		
	G_P	G_C	G_{TOT}	G_P	G_C	G_{TOT}
0038	1.84	1.62	3.45	2.87	1.89	4.76
0039	1.44	3.05	4.48	1.21	3.08	4.29
0040	0.84	2.27	3.11	0.73	1.77	2.51
Mean	1.37	2.31	3.68	1.60	2.25	3.85
±S.E.M	0.29	0.41	0.41	0.65	0.42	0.69

Table 4.4 Shows the individual and mean ±S.E.M chemosensitivity results for the final MFBS test of the restriction protocol and the additional MFBS test following reticular formation activation (stretching and yawning) in three participants. G_P = peripheral gain, G_C = central gain and G_{TOT} = Total gain; all measured in $L.min^{-1}.mmHg^{-1}$.

These results do not suggest that the decreased arousal effects on the reticular formation could be causing the alterations in chemosensitivity seen following sleep restriction. However, it is certainly possible that the resulting activation of respiratory centres in the

brain following stretching and yawning will not have been sustained throughout an entire MFBS test. Furthermore, the use of just three participants for this additional MFBS test does not provide sufficient data for a conclusive conclusion to be made. One limitation of the present study is the absence of a measurement of cortical arousal status during each MFBS, by way of EEG. Such measurement would have ensured the arousal status of each participant did not differ between measurements.

An additional mechanism arising from the effect of sleepiness could involve the neurochemical control of arousal and sleep. Sleep and the onset of sleepiness is governed by a number of neuromodulators working within a circadian cycle (Pace-Schott & Hobson, 2002). One particular neurotransmitter involved in sleep onset is adenosine. Adenosine is a by-product from the breakdown of adenosine-triphospahte (ATP), adenosine diphosphate (ADP) and/or cyclic adenosine monophosphate (cAMP) during metabolism. The accumulation of adenosine in basal forebrain and hypothalamic regions of the brain lead to the inhibition of the GABA neurons involved in the staving off sleep promoting regions within the brain (Pace-Schott & Hobson, 2002). Furthermore, adenosine works to inhibit orexin neurons within the lateral hypothalamus that promote arousal (Liu & Gao, 2007) thus leading to sleep onset. The increased energy expenditure of the brain during prolonged wakefulness leads to an accumulation of adenosine (Kalinchuk *et al.*, 2003). This accumulation of adenosine most likely contributes to the increased level of sleepiness during sleep deprivation.

Adenosine has direct effects on the activity of chemoreceptors. McQueen and Ribeiro (1981) observed carotid body excitation during infusion of adenosine in cats. Further work by Maxwell *et al.* (1986) showed that venous infusion of adenosine in humans resulted in an

augmented HVR, further suggesting adenosine as a peripheral chemoreceptor agonist. Maxwell and colleagues also concluded that adenosine had no impact on the central chemoreceptors. This conclusion was drawn because of the unaltered ventilatory response to hyperoxic hypercapnia during a Read re-breathing test. However, adenosine has been shown to have a short plasma half-life of approximately 1.5 seconds (Moser *et al.*, 1989). Therefore, despite the long period of infusion prior to the ventilatory response tests it could be postulated that there was no sufficient amount of adenosine needed to affect central chemoreception. Research conducted by Eldridge *et al.* (1984) showed that infusion of an adenosine agonist into carotid sinus denervated cats under isocapnic conditions resulted in a dose response attenuation in ventilatory output. Furthermore, adenosine acts as a cerebral circulation vasodilator (Morii *et al.*, 1987). Therefore, an additional cause of the reduction could be an increased cerebral blood flow and thus increased wash-out of $PaCO_2$ and a higher tissue pH. In order to rule out this possibility Eldridge and colleagues did monitor pH within the medulla in a single cat preparation. The pH was decreased, suggesting that the effects of adenosine on cerebral blood flow did not cause alkaline conditions within the medulla, which would have cause the reduction in ventilatory output. Therefore authors concluded adenosine caused an inhibitory effect on ventilation via the respiratory control neurons within the brain not the chemoreceptors. However, recent evidence has shown the importance of orexin neurons in central chemoreception of hypercapnia. Deng *et al.* (2007) showed a reduction in the HCVR in orexin knockout mice that was restored following orexin supplementation. As previously stated, adenosine is an inhibitor of orexin neurons. Therefore, it could be postulated that an augmentation in adenosine during sleep deprivation could result in the attenuation in central chemosensitivity reported in this study.

Furthermore, it is plausible that mild increases in plasma adenosine could result in the small augmentation in peripheral chemosensitivity demonstrated in this present study.

The effect of adenosine on hypercapnic chemosensitivity has been previously observed following sleep fragmentation. Liu *et al.* (2011) showed a reduction in the ventilatory response to euoxic hypercapnia (6% CO_2 in air) following 24 hours of sleep fragmentation in rats. The attenuation was reversed during the infusion of an adenosine receptor antagonist, further suggesting a role for adenosine in reduced chemoreceptor drive. Further information on the effects of sleep fragmentation is the topic of discussion in the following chapter.

Increased cerebral blood flow following sleep loss could account for the reduced central chemosensitivity shown in this study. However, limited research on the effects of sleep deprivation on the cerebral blood flow and cerebrovascular reactivity has been conducted. Using brain imaging techniques in patients suffering with depression, Volk *et al.* (1992) evaluated regional cerebral blood flow following a night of total sleep deprivation, showing increases in blood flow in the parietal lobe and left temporal lobe. Additional research by Volk and colleagues showed increases in blood flow in additional brain regions following the restriction of sleep length (Volk *et al.*, 1997). However, the aim of these two studies was to test whether assessing brain perfusion can be used as a tool to identify major depressive patients that would benefit from therapeutic sleep deprivation. Those showing higher cerebral perfusion would benefit from such an intervention. Therefore, it would be assumed that the responses of the cerebral circulation to sleep deprivation could only occur in some patients. This was seen by Volk and colleagues where 60% of their participants showed an increase in cerebral perfusion. Therefore, the effect of sleep deprivation on the cerebral

circulation could cause a reduction in the magnitude of central chemosensitivity. However, such an effect could be considered to be specific to each individuals. Further research is needed to establish if these changes occur in a healthy population and what link they have on chemosensitivity. Also, regions of the brain associated with central chemoreception, such as the brain stem, should be investigated to see if increases in cerebral perfusion, similar to those seen by Volk and colleagues, are present following sleep loss.

4.4.2 Sleep restriction and vascular function

Our results suggest that a sleep restriction over two nights leads to a deterioration in vascular function, similar to that observed following total sleep deprivation in previous research (Amir *et al.*, 2004; Sauvet *et al.*, 2010). However, the recovery of vascular function following a third night of sleep restriction is a finding not previously reported by research. There was no observed alteration in calculated shear stress stimulus or brachial artery resting diameter that may have affected the measured vascular reactivity.

Possible mechanisms for the deterioration in vascular function in our participants are increased levels of pro-inflammatory cytokines and augmentation in the stress hormone cortisol. Interleukin-6 and tumor necrosis factor-α have been both linked with reduced vascular tone and nitric oxide production and have also recently been shown to correlate with reduced vascular function following a total sleep deprivation protocol (Sauvet *et al.*, 2010). Furthermore, cortisol has been shown to be elevated following sleep loss (Leproult *et al.*, 1997) and research has also shown it to be linked with endothelial dysfunction in patients suffering with depression (Broadley *et al.*, 2006). Morning blood and saliva samples were obtained during the present study for the assessment of inflammatory markers and

cortisol levels respectively, however they have yet to be analysed and they are not available to support this argument yet. An augmentation in these cytokines and in cortisol following sleep deprivation, are very likely to account for the deterioration in vascular function observed in this study. It is also possible that attenuation in the factors that promote vascular dysfunction could account for the amelioration of reactive hyperaemia after three nights of sleep loss. A decline in these factors could stem from a desensitisation to the effects of sleep loss, such that as sleep loss progresses the increases in the release of stress hormones and proinflammatory cytokines, as a consequence of sleep loss, could be dampened. Research by Martin *et al.* (1986) reported that sleep loss has no effect on the stress hormonal response to a bout of exercise. Similar findings regarding the sympathoadrenal response to exercise were also reported following sleep deprivation (Martin & Chen, 1984). These findings would suggest that a reduction in the sensitivity of stress hormone response does not take place following sleep loss. A recent study has shown an increased sensitisation of proinflammatory cytokines in response to anaerobic exercise following 24 hours of sleep deprivation (Abedelmalek *et al.*, 2013). In agreement with research by Martin *et al.,* no change in the cortisol response to exercise was observed by Abedelmalek and colleagues. Althouht these studies would seem to suggest that the possibility of any desensitization to sleep loss and the subsequent release of factors inducing vascular dysfunction to be unlikely, such response were reported during exercise bouts. Nevertheless, additional evidence against a desensitisation argument is a reported sensitisation to stress related disorders in individuals who report sleeping less (Meerlo *et al.*, 2008).

An additional mechanism for the recovery in endothelial function could be an increase in blood pressure that would increase the shear stress stimulus and thus nitric oxide release. However, our results indicate no augmentation in blood pressure during the sleep restriction protocol. The majority of research suggests an increase in blood pressure following total sleep deprivation via either systolic or diastolic elevations (Lusardi *et al.*, 1999; Kato *et al.*, 2000a; Ogawa *et al.*, 2003; Sauvet *et al.*, 2010; Yang *et al.*, 2012; Sunbul *et al.*, 2013), however, we only saw a small decrease in diastolic blood pressure. Reasons for this finding could be the difference in the nature of sleep deprivation in our study with these studies all conducting a total sleep deprivation protocols. However, Lusardi *et al.* (1996) reported elevated morning blood pressure following just a single night of restricted sleep length. In that study participants slept between 02:00 and 07:00 and their blood pressure was monitored continuously for 24-hours. This highlights two possible limitations in the timings of blood pressure assessment in our study. In our study we assessed blood pressure at absolute timings during each protocol; 19:15 and 08:15. By doing so we assessed morning blood pressure while participants were in a different state in each protocol. During the control protocol participants would have just woken up and during the restriction protocol participants had already been awake for three hours. If a prolonged period or numerous timings of blood pressure assessment had been made in our study, similar to Lusardi *et al.* (1996), then both absolute and relative assessments to sleep and awake timings could have been made. Nevertheless, an augmentation in blood pressure or shear stress was not seen at the time of the flow mediated dilation test, therefore it could not account for the increase in dilation seen on the final morning of sleep loss.

Although a biphasic response in endothelial function was observed in the present study, it does not rule out the possibility of vascular dysfunction being a causal factor in the relationship between shortened sleep length and the increased incidence of cardiovascular disease. It remains to be seen what effect further consecutive nights of sleep loss and/or more intermittent occasions of acute sleep loss will have on endothelial function and more long term effects such as vascular remodelling and atherosclerosis.

In summary, data from this study demonstrates that an acute reduction in sleep length across three consecutive nights can result in alterations in chemosensitivity by way of reduced central chemosensitivity. However, this reduction in the central chemosensitivity is not large enough to affect total chemosensitivity significantly. Also, the study showed that a reduction in central chemosensitivity during wakefulness does not affect respiratory stability or lead to a development of apnoeas and hypopnoeas during sleep, in healthy young individuals. However, it still may contribute to sleep apnoea development in individuals at greater risk because of additional abnormalities. Lastly, the study has demonstrated that sleep restriction over consecutive nights affects reactive hyperaemia in a biphasic manner. This effect could contribute to the increased occurrence of cardiovascular disease in those reporting to sleep less than 7-8 hours a night.

CHAPTER 5.

EFFECT OF A SINGLE NIGHT OF FRAGMENTED SLEEP ON CO_2 CHEMOSENSITIVITY AND CEREBROVASCULAR REACTIVITY

5.1 Introduction

In the previous chapter it was shown that the restriction of sleep length resulted in alterations in hypercapnic chemosensitivity, driven by changes in central chemosensitivity. Such a debilitative effect on chemosensitivity may result in the worsening of respiratory disorders that are characterised by sleep disruption. However, the model of sleep restriction that we used was not a truly accurate representation of the form of sleep disruption that happens in such conditions, for example, the sleep fragmentation that is experienced by sleep apnoea patients and COPD patients. Therefore, the primary aim of the present chapter is to investigate the effects of sleep fragmentation on respiratory chemosensitivity. SDB has been shown to progressively worsen throughout the night with apnoeas becoming longer and more frequent (Charbonneau *et al.*, 1994). Also, SDB is a chronic condition that progressively worsens over time (Young *et al.*, 1993). Sleep fragmentation has been suggested as one possible reason for the progression of sleep apnoea both throughout a night's sleep and over longer periods of time. Research has demonstrated a reduction in the arousal response to chemical stimuli (Bowes *et al.*, 1980; Li *et al.*, 2014), an attenuated reflex opening of the airway during artificially induced apnoeas (Brooks *et al.*, 1997) and an increased vulnerability to airway collapse (Sériès *et al.*, 1994) following sleep fragmentation. One suggested mechanism for the observed changes in apnoea severity following sleep fragmentation is an alteration in respiratory control that is caused by the disruption to sleep. However, research is divided on the effect sleep fragmentation could have on the HCVR. Three previous studies have investigated the effect of induced sleep fragmentation on hypercapnic respiratory chemosensitivity. Espinoza *et al.* (1991a) induced sleep fragmentation in humans, and by using a Read's re-breathing test they observed no effect on

the sensitivity of the ventilatory response. Similar results to progressive hyperoxic hypercapnia have been seen in sleep fragmented dogs (Bowes *et al.*, 1980). Nevertheless, Bowes and colleagues did observe a reduction in the arousal response to both hypercapnia during sleep, with a 3.4 mmHg increase in the arterial CO_2 needed to trigger an arousal.

In contrast, a recent study by Liu *et al.* (2011) observed a 50% reduction in the HCVR in sleep fragmented rats. HCVR returned to baseline levels just four hours after waking. Based on this finding, Liu and colleagues suggested that previous research made assessments of chemosensitivity too long after awakening and therefore assessed ventilatory responses after changes in chemosensitivity had returned to baseline values. Furthermore, Liu and colleagues tested a nomoxic hypercapnia ventilatory response in their rats whereas previous research had adopted hyperoxic hypercapnic re-breathing tests (Bowes *et al.*, 1980; Espinoza *et al.*, 1991b). It is plausible that peripheral chemosensitivity could be driving the observed alterations in Liu's study and that alterations in peripheral chemosensitivity were partially concealed by the use of hyperoxia in previous research.

Liu *et al.* (2011) also demonstrated that the inhibition of adenosine A1 receptors following sleep fragmentation restored the HCVR to baseline levels. Research has shown adenosine plays an important role in the sleep-wake cycle (Porkka-Heiskanen *et al.*, 2002) and following sleep fragmentation there is an accumulation of adenosine in the brain (McKenna *et al.*, 2007). Given the central nature of cortical arousal and the resultant central adenosine accumulation it is postulated that the central chemoreceptors were responsible for the observed attenuation in HCVR by Liu and colleagues. This makes it unlikely that the peripheral chemoreceptor contributes to the observed reductions. However, no previous

research has assessed the effect of sleep fragmentation on separate chemoreceptor sensitivities.

Adenosine also acts on the cerebral blood vessels to stimulate vasodilatation and increase cerebral blood flow (CBF) (Morii *et al.*, 1987; Kusano *et al.*, 2010). The increase in adenosine levels following sleep fragmentation could cause vasodilation that would contribute to a reduction in ventilatory drive through reduced tissue CO_2/pH at the central chemoreceptors. Conversely, sleep fragmentation has also been suggested to have a negative effect on cerebrovascular reactivity. Qureshi *et al.* (1999) showed that sleep fragmentation was a strong predictor of a greater overnight reduction in cerebrovascular reactivity in SDB patients. This observation by Qureshi and colleagues in parallel with the alterations in chemosensitivity observed by Liu *et al.* (2011) would seem paradoxical, because a reduction in cerebrovascular reactivity to hypercapnia would result in a greater central ventilatory output, that could be interpreted as an increased chemosensitivity (Fan *et al.*, 2010). No previous research has measured cerebrovascular reactivity in addition to chemosensitivity following simulated sleep fragmentation to see if such a paradox occurs.

In the present study participants were subjected to a control night and a night of sleep fragmentation in which micro-arousals were induced. Morning chemosensitivity was assessed with a MFBS test in order to quantify the individual chemoreceptor sensitivities following sleep fragmentation with the hypothesis of reduced central chemosensitivity. The present study also examined the hypothesis that cerebrovascular reactivity to CO_2 will be reduced from the evening before to the morning following sleep fragmentation, as shown in Qureshi *et al.* (1999). Such an observation would highlight the occurrence of a paradox in

reduced cerebrovascular reactivity and central chemosensitivity following sleep fragmentation.

5.2 Methods

Nine participants were recruited from the student body of the University of Birmingham. All participants were free of cardiovascular and respiratory disease. All participants reported having a habitual sleeping pattern of 6 to 9 hours between 22:00 and 08:00 to ensure that they had a normal sleeping routine. Participants were excluded from the study if they had irregular sleeping routines or if they were frequent nappers. Participants were also excluded from the study if they had been previously diagnosed with a sleeping, eating or mental disorder. All participants were non-obese (BMI<30kg/m^2) and non-smokers. Participants gave informed consent and they completed a general health questionnaire prior to attending the laboratory for the experimental sessions. Participants were asked to refrain from consuming food or caffeine for three hours prior to visiting the laboratory on each visit. They were also asked to refrain from vigorous exercise and daytime sleeping while taking part in the study. Ethical approval for the study was obtained from the local ethics subcommittee within the University of Birmingham.

5.2.1 Experimental Protocol

Each participant slept for two consecutive nights in a purpose built sleep laboratory at the School of Sport and Exercise Sciences. The lights in the laboratory were turned off at 23:00 hours and participants were awakened by an alarm clock at 07:00 each morning. The initial night served as the control night and the second as the fragmentation night. During the

control night participants had the opportunity to sleep normally and uninterrupted for eight hours. Conversely, during the fragmentation night micro-arousals were induced by an audible sound during all sleep stages. Guidelines for micro-arousal stimulation are described in section 2.10.1. Sleep stages and arousals were assessed each night using polysomnography. No respiratory parameters were measured during sleep to avoid any additional arousals during the control night caused by the discomfort of wearing a nasal cannula.

Each evening prior to sleep (21:00) and each morning following awakening (07:30) cerebrovascular reactivity was assessed via continuous measurement of unilateral middle cerebral artery blood flow velocity (CBF_V) with TCD (described in section 2.8). A hypercapnic dose response test was used to assess hypercapnic cerebrovascular reactivity. During the test, four steps of increasing $P_{ET}CO_2$ were administered (1, 4, 7 and 10 mmHg above each participant's normal $P_{ET}CO_2$). Each step was five minutes long and $P_{ET}O_2$ was maintained at euoxic levels of 100 mmHg. The test is described in greater detail in section 2.2.2. Blood pressure and heart rate were monitored continuously throughout by a finger photoplethysmography and electrocardiography, respectively. Following the assessment of cerebrovascular reactivity each morning participants were given a 20-minute break following which respiratory chemosensitivity was assessed using a MFBS test. Figure 5.1 is a schematic representation of the experimental protocol, where PM1 and AM1 represent the evening and morning measurements that flank the control night, respectively. PM2 and AM2 are the measurement periods on either side of the fragmentation night.

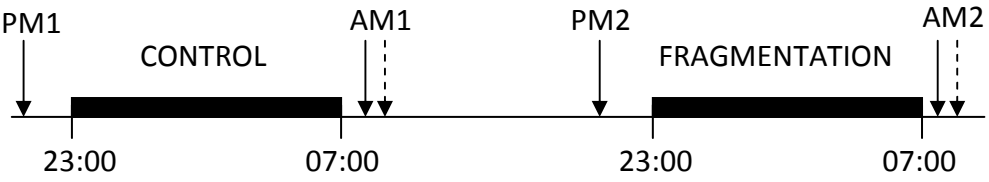

Figure 5.1 Schematic of the experimental protocol. Black boxes represent nights of sleep in the laboratory. Solid arrows show the timing of cerebrovascular reactivity test and broken arrows show the timing of the MFBS test.

5.2.2 Data analysis

Resting ventilation, heart rate, CBF_V and mean arterial pressure values were obtained immediately prior to the initiation of the first five minutes of eucapnic euoxia. Normal $P_{ET}CO_2$ values were recorded prior to initiation of the eucapnic euoxic period.

Central and peripheral chemosensitivity during the MFBS test was assessed by a two-compartment model, as described in section 2.4. A paired sample T-test was used to test for statistical significance between chemosensitivity values.

Averages of CBF_V and mean arterial pressure during for each step of the hypercapnic dose response test were taken during the final minute of each step. Cerebrovascular resistance (CVR) was calculated by a division of mean arterial pressure and CBF_V. Cerebrovascular reactivity to CO_2 ($\Delta CBF_V/\Delta CO_2$) was assessed using linear regression analysis of each response and differences in the slope of the linear regression lines were assessed statistically. The same procedure was used to analyze cerebrovascular resistance reactivity to CO_2 (CVR/CO_2).

Slopes of the linear regression lines for the ventilatory responses step increases in $P_{ET}CO_2$ during the cerebrovascular reactivity test were also calculated. Values for ventilation and $P_{ET}CO_2$ were obtained from averages of the final minute of each step, when a steady state had been achieved.

Paired sample t-test was used to test for significant differences between morning and evening measurements and between morning chemosensitivity tests. All data is presented as mean (±S.E.M.) unless stated otherwise and significance was assumed when $P<0.05$.

5.3 Results

Nine participants (3 female) with a mean age and BMI of 21.8±1.1 years and 24.6±1.4 kg/m^2, respectively, took part in the study. All participants completed both nights and all measurements in the study. Polysomnography data from one participant was incomplete and therefore removed from the presented results because of an inability to analyze fully.

5.3.1 Resting values

There was no significant difference in resting minute ventilation, CBF_V, mean arterial pressure or normal $P_{ET}CO_2$ across any of the time points. Heart rate was significantly lower in AM2 compared to PM2 and tended to decrease from AM1 to AM2 ($P=0.07$). All resting values are displayed in table 5.1.

	PM1	AM1	PM2	AM2
V_E (L.min^{-1})	11.04	9.66	11.09	9.61
±S.E.M.	0.71	0.90	0.43	0.81
$P_{ET}CO_2$ (mmHg)	39.14	39.36	38.78	38.23
±S.E.M.	0.96	0.66	0.69	1.15
HR (b.min^{-1})	65.78	59.00	68.67	58.78*
±S.E.M.	3.22	3.28	2.70	2.74
MAP (mmHg)	84.58	83.91	80.53	77.35
±S.E.M.	3.41	2.51	3.35	3.31
CBF_V (cm.s^{-1})	61.04	60.82	61.52	57.21
±S.E.M.	6.19	3.79	5.72	4.25

Table 5.1 Mean ±S.E.M. resting minute ventilation (V_E), $P_{ET}CO_2$, heart rate (HR), mean arterial pressure (MAP) and cerebral blood velocity (CBF_V) for all evening and morning periods. *=significant difference from prior evening ($p<0.05$)

5.3.2 Chemosensitivity MFBS test

There was no change in mean peripheral chemosensitivity between AM1 and AM2; 1.6 ± 0.2 and 1.7 ± 0.3 L.min^{-1} mmHg^{-1}, respectively. Central chemosensitivity was reduced from AM1 to AM2; 1.9 ± 0.4 to 1.2 ± 0.2 L.min^{-1} mmHg^{-1}, respectively. The addition of these two reveal no significant alterations, despite an overall reduction, in total chemosensitivity from AM1 to AM2; 3.5±0.5 to 2.9±0.3 L.min^{-1} mmHg^{-1}, respectively. Individual and mean changes in peripheral, central and total chemosensitivities following fragmentation are shown in figure 5.2. A reduction in calculated apnoeic threshold (B value) was also observed following fragmentation; 36.9 ± 0.7 to 34.1 ± 0.7 mmHg, $P<0.03$. There was no correlation between the changes in chemosensitivity and the number of micro-arousals and arousals that occurred during the fragmentation night.

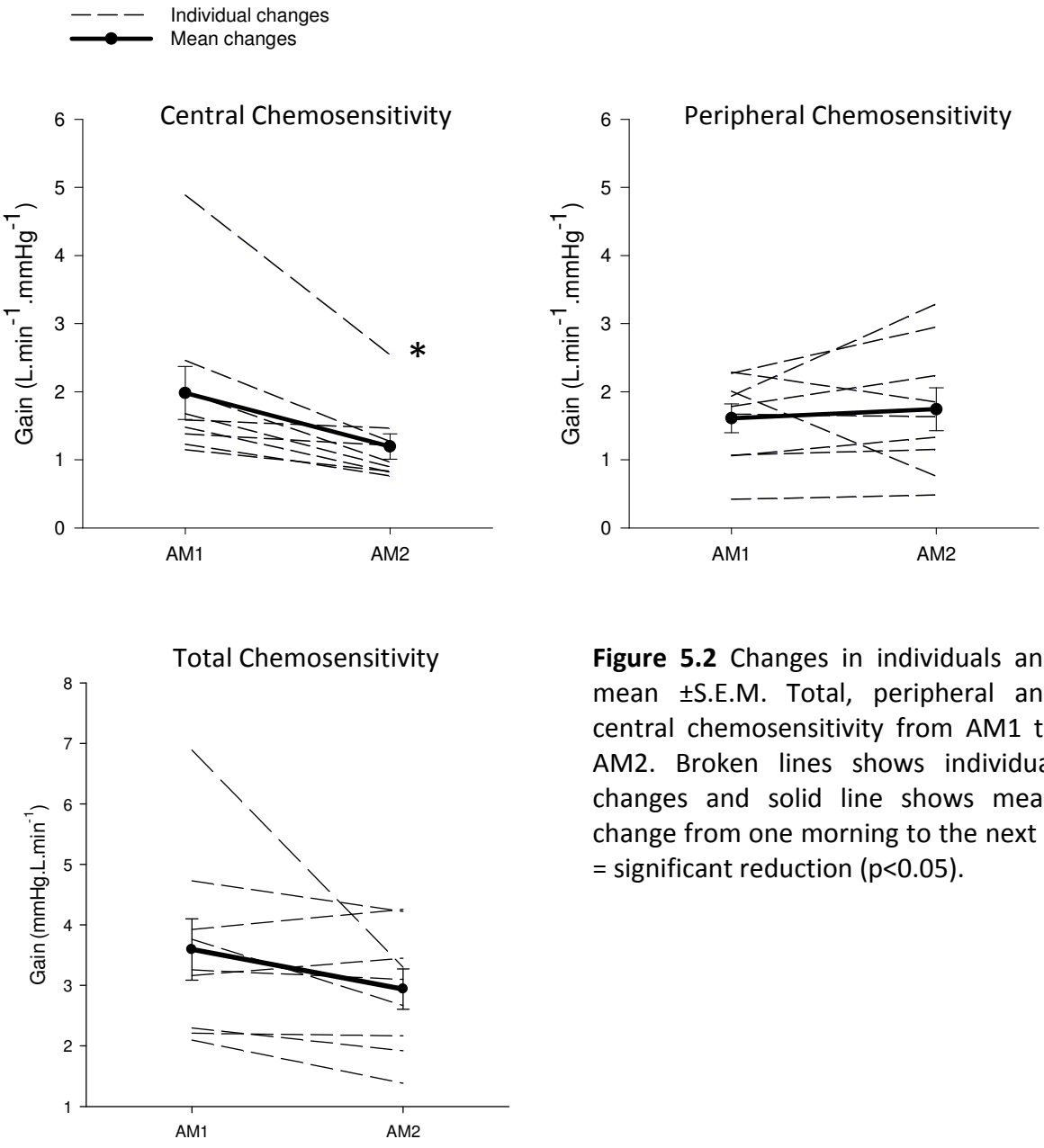

Figure 5.2 Changes in individuals and mean ±S.E.M. Total, peripheral and central chemosensitivity from AM1 to AM2. Broken lines shows individual changes and solid line shows mean change from one morning to the next * = significant reduction ($p<0.05$).

5.3.3 Cerebrovascular Reactivity Test

$P_{ET}CO_2$, $P_{ET}O_2$, minute ventilation and heart rate values during the hypercapnic exposure are displayed in Figure 5.3. There were progressive increases in heart rate and minute ventilation throughout the hypercapnic dose response test. There was no difference between any of the evening and morning periods in these progressive increases.

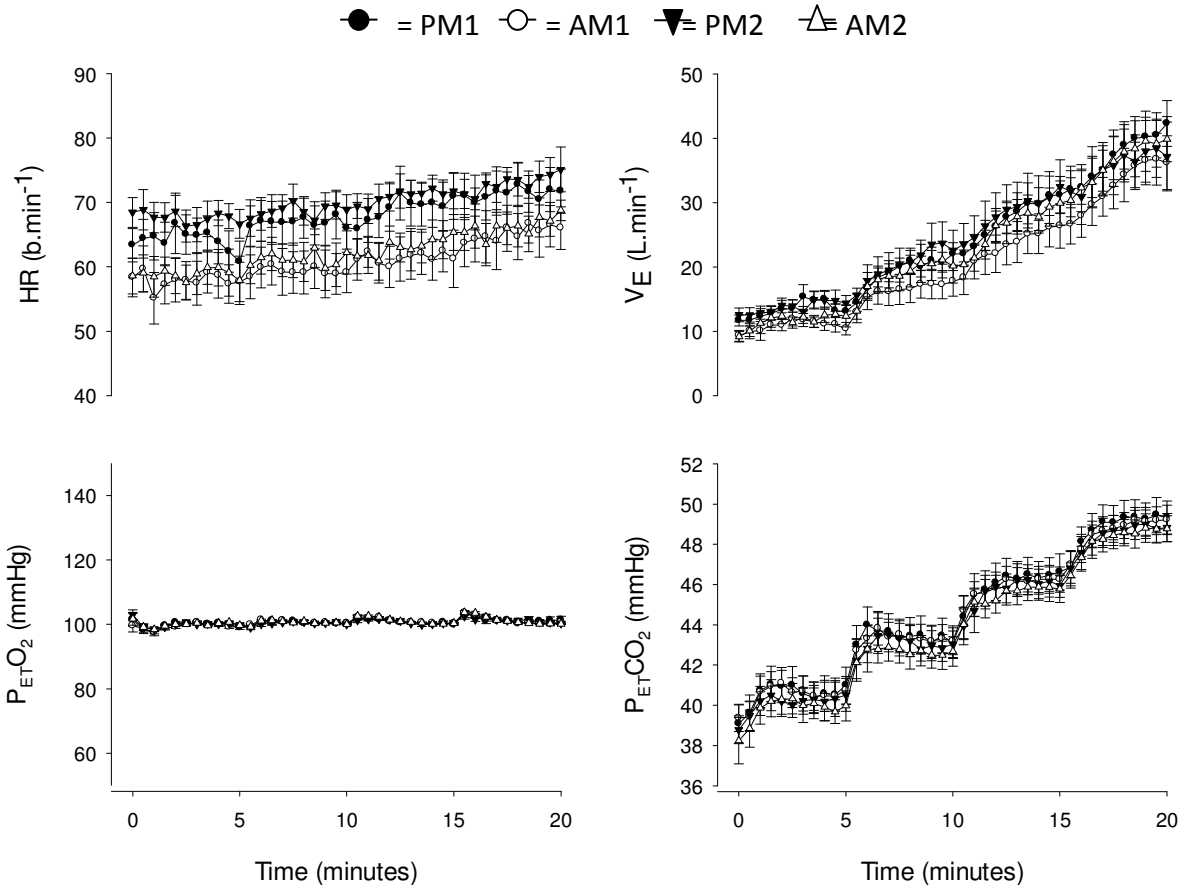

Figure 5.3 Heart rate, minute ventilation, $P_{ET}O_2$ and $P_{ET}CO_2$ during the hypercapnic dose response test for each period (mean ±S.E.M.).

A progressive increase in CBF_V and a progressive decrease in CVR were observed throughout all hypercapnic dose response tests. There was no difference in cerebrovascular responses between PM1 and AM1 or PM2 and AM2. The absolute and relative cerebrovascular responses to the hypercapnic exposure are displayed in figure 5.4. Cerebrovascular reactivity and cerebrovascular resistance reactivity to CO_2, presented as the slope of the linear regression line, are shown in table 5.2. No differences in either reactivity values were observed across any evening or morning time points.

The mean slopes of the ventilatory responses to step increases in CO_2 are also shown in table 5.2. There was no difference found between any of the tests. These values give a representation of the total chemosensitivity of the participant during the cerebrovascular reactivity test. There was no difference between these mean total chemosensitivity values and the values obtained during the MFBS test at AM1 (3.10 ± 0.35 vs 3.59 ± 0.51 $L.min^{-1}$ $mmHg^{-1}$) and AM2 (2.96 ± 0.32 vs 2.94 ± 0.33 $L.min^{-1}$ $mmHg^{-1}$).

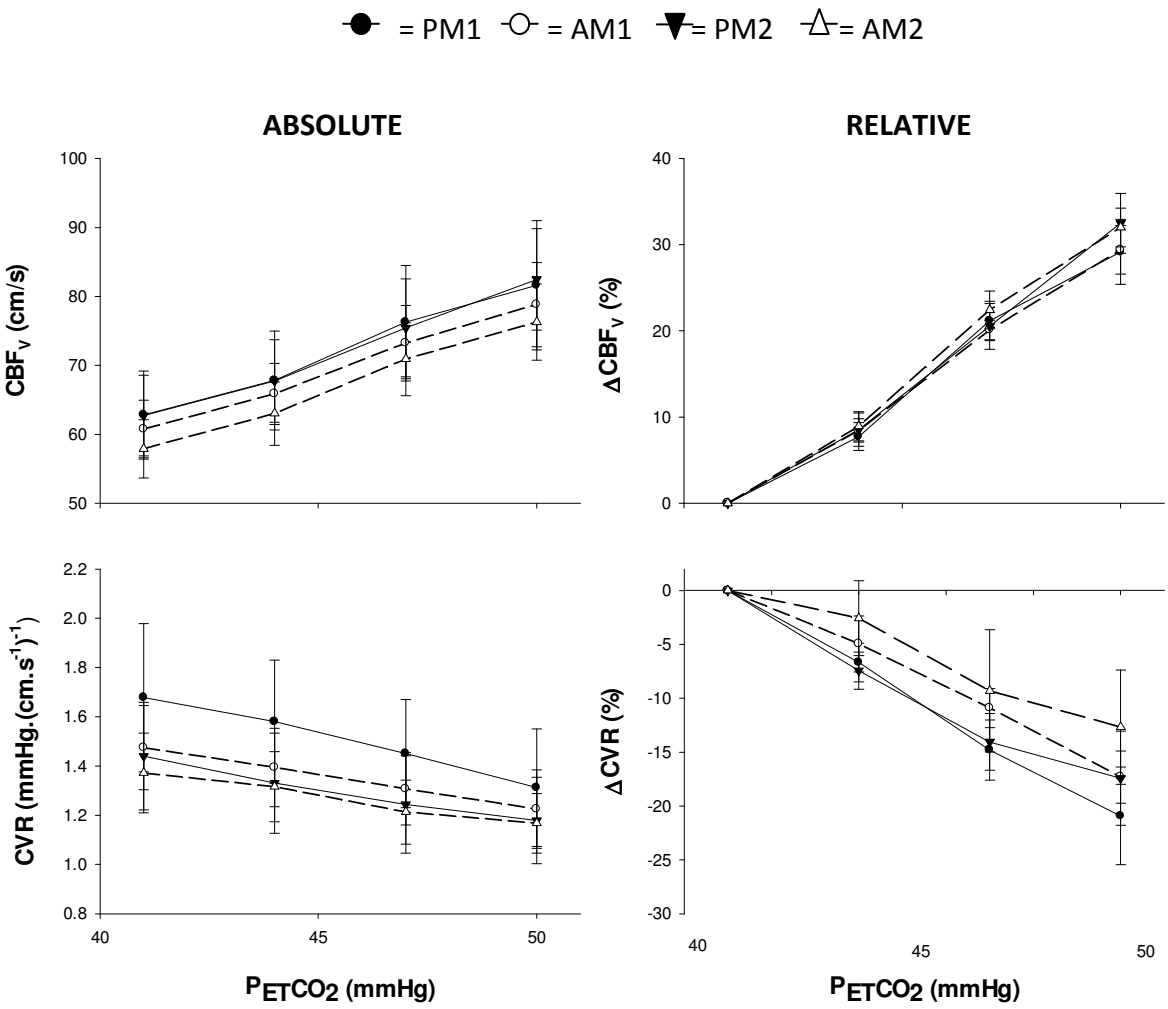

Figure 5.4 Mean ±S.E.M. of absolute and relative cerebral blood velocity (CBF_V) and cerebrovascular resistance (CVR) responses to changes to step increases in $P_{ET}CO_2$. No significant differences between measurement periods were observed.

	PM1	AM1	PM2	AM2
CBF_V/CO_2 ($cm.s^{-1}$)	2.22	2.13	2.36	2.08
±S.E.M.	0.39	0.28	0.26	0.18
CVR/CO_2 ($mmHg^{-1}.cm.s^{-1}$)	-0.04	-0.03	-0.03	-0.02
±S.E.M.	0.01	0.00	0.01	0.01
$\Delta\%CBF_V/CO_2$	3.45	3.50	4.19	3.69
±S.E.M.	0.48	0.60	0.58	0.26
$\Delta\%CVR/CO_2$	-2.30	-2.11	-1.79	-1.60
±S.E.M.	0.04	0.08	0.10	0.13
$\Delta V_E/CO_2$ ($L.min^{-1}\,mmHg^{-1}$)	3.11	3.08	2.96	2.96
±S.E.M.	0.38	0.35	0.42	0.32

Table 5.2 Cerebrovascular reactivity (CBF_V/CO_2), cerebrovascular resistance reactivity (CVR/CO_2) and ventilatory chemosensitivity ($\Delta V_E/\Delta CO_2$) during the cerebrovascular reactivity test at each period measurement; presented as mean ±S.E.M.

5.3.4 Polysomnography

The mean time spent in each sleep stage and number of arousals and micro-arousals for each night are displayed in table 5.3. There was no difference in the total time spent asleep. There was a reduction in both the relative and absolute time spent in SWS from control to fragmentation. Absolute and relative time spent in stage 2 sleep was increased from the first night to the next. There was no difference in the time spent in REM sleep between the two nights. The fragmentation night was successful in increasing the number of arousals and micro-arousals compared to the control night. There was an increase in the mean number of micro-arousals per hour of sleep from control night to fragmentation night (micro-arousal index). There was no significant increase in arousal index (P=0.08). Despite the lack of reduction in percentage time spent in REM sleep, more arousals occurred throughout REM sleep during fragmentation than in the control night; 13.50 ± 2.95 and 6.88 ± 1.27, respectively.

	Control	Fragmentation
Total Sleep Time (min)	464.81	474.88
± S.E.M.	38.54	3.67
Sleep Efficiency (%)	96.36	95.49
± S.E.M.	0.72	1.79
Sleep Latency (min)	13.00	9.96
± S.E.M.	2.03	2.60
REM (min)	94.31	92.88
± S.E.M.	8.81	7.04
Stage 1 (min)	22.81	37.00*
± S.E.M.	3.65	6.29
Stage 2 (min)	236.19	260.25
± S.E.M.	11.94	10.90
SWS (min)	80.46	53.69#
± S.E.M.	8.25	5.73
REM (%)	21.51	20.84
± S.E.M.	1.61	1.37
Stage 1 (%)	5.21	8.37*
± S.E.M.	0.84	1.44
Stage 2 (%)	54.43	58.54
± S.E.M.	2.14	2.05
SWS (%)	18.89	12.15#
± S.E.M.	2.33	1.35
Arousals	38.88	49.63#
± S.E.M.	3.74	5.69
Micro-arousals	40.50	186.38#
± S.E.M.	6.77	10.99
Arousal Index (no.hr^{-1})	5.34	6.78
± S.E.M.	0.48	0.90
Micro-arousal Index (no.hr^{-1})	5.70	25.18#
± S.E.M.	1.01	1.37

Table 5.3 Sleep architecture and arousal parameters during each night. Significant differences between each night are displayed by a hastag or star; # = $p<0.03$ * = $P<0.05$

5.4 Discussion

It was hypothesized that sleep fragmentation would result in the reduction of respiratory chemosensitivity driven characterised by a reduction in central chemosensitivity. Following a single night of sleep fragmentation a significant reduction in central chemosensitivity was observed. No change in peripheral chemosensitivity was observed from the control morning to the fragmentation morning measurements. Although an overall reduction in total chemosensitivity of 14.5% following a night of fragmented sleep was seen, this finding was not deemed statistically significant. It could be suggested that the slight augmentation in peripheral chemosensitivity maintained total chemosensitivity at an unchanged level. The finding of unchanged total chemosensitivity does match with the ventilatory sensitivity data obtained during the cerebrovascular reactivity tests. The similarity of results obtained for total chemosensitivity across the two tests highlight the importance of assessing the differential gains of each chemoreceptor, since assessing just total chemosensitivity will mask selective effects at each chemoreceptor.

A second hypothesis of a paradoxical reduction in cerebrovascular reactivity following fragmented sleep was not observed, as no differences in cerebrovascular reactivity were found between any measurements across the entire study. The absence of an alteration in cerebrovascular reactivity suggests a more central molecular mechanism for a reduction in central chemosensitivity following the fragmentation of sleep and not an indirect effect of CO_2 delivery to the central chemoreceptors.

5.4.1 Method of sleep fragmentation

Previous studies have used a strong form of simulated sleep fragmentation by either forcibly waking participants and awaiting hand gestures of recognition (Espinoza *et al.*, 1991a) or placing animals in a rotating drum to bring about interruptions in sleeping rats (Liu *et al.*, 2011). These methods resulted in a complete or near abolishment of SWS and REM sleep. This form of sleep fragmentation is not an accurate model of the fragmentation experienced in SDB. Despite a reduction in the amount of time spent in each stage these stages are still present in sleeping patients (Charbonneau *et al.*, 1994). Furthermore, there are significantly more micro-arousals than awakenings in SDB patients (Martin *et al.*, 1997). In the present study we attempted to increase the number of micro-arousals and arousals, while reducing the length but maintaining the presence of SWS and REM sleep stages. An audible tone was used successfully to increase the number of arousals and micro-arousals during sleep in our participants. Although there was a clear increase in micro-arousal index there was no significant increase in arousal index (P=0.08) from the control to the fragmentation night. A small increase in mean total sleep time during the fragmentation night is the most likely reason for this insignificant increase in arousal index.

Although there was an increase in the number of arousals and micro-arousals during the fragmentation, this did not translate into changes in all aspects of sleep architecture. During induced sleep fragmentation with micro-arousals in humans, Stamatakis and Punjabi (2010) observed reductions in percentage time spent in both SWS and REM sleep and an increase in the time spent in stage 1 sleep. In the present study, a reduction in SWS and an increase in stage 1 sleep were seen. However, no change in the time spent in REM sleep was observed. The stimulation of micro-arousals and arousals during REM proved to be difficult in the

present study with the use of an audible tone just 0.5ms in duration. In addition to an audible tone Stamatakis and Punjabi also used mechanical vibrators placed under the bed to induce arousals during sleep, something that was not used for the present study. Nevertheless, we achieved a greater number of arousals in REM sleep during the fragmentation night than in the control night.

5.4.2 Chemosensitivity and sleep fragmentation

This is the first study in humans to show a reduction in central chemosensitivity following sleep fragmentation. Previous studies have shown no change in the ventilatory responses to hypercapnia in humans (Espinoza *et al.*, 1991b) and dogs (Bowes *et al.*, 1980). However, more recently an attenuation in the ventilatory response to normoxic hypercapnia was observed in awake rats (Liu *et al.*, 2011). Possible methodological limitations in the assessment of ventilatory responses in the previous studies could account for their findings. A Read's re-breathing test was used in the earlier studies to assess ventilatory chemosensitivity, where PO_2 was not strictly controlled. A background of hyperoxia was also administered during the test that could confound the results of the HCVR test. Finally, as mentioned previously the assessment of ventilatory chemosensitivity during wakefulness may have been performed following the recovery to baseline values.

SDB is characterised by sleep fragmentation and leads to chronic excessive daytime somnolence in patients. Alterations in respiratory chemosensitivity as a consequence of chronic sleep fragmentation could place patients at greater risk of developing further apnoeic events and more prolonged events that could account to the observed worsening of the condition over time (Young *et al.*, 1993; Brooks *et al.*, 1997). Brooks *et al.* (1997) used a

model of airway occlusion in sleeping dogs to investigate the progression of sleep apnoea over time. Apnoeas were induced by a balloon surgically placed within the animal's airway. When the animal fell asleep the balloon was inflated to cause an occlusion of the airway and when an arousal occurred the balloon was deflated. Animals were subjected to obstructive apnoeas during sleep for a period of three months. In a separate protocol, the authors also exposed the animals to chronic sleep fragmentation over a similar period of time. Authors induced apnoeas in the animals prior to and following the chronic sleep fragmentation protocol, to assess the time until arousal occurred. Following both protocols the animals showed that it took longer for an arousal to occur during induced obstructive apnoeas, resulting in prolonged apnoeic events. The similarity in observations following both the chronic sleep fragmentation and apnoea protocols, suggests that sleep fragmentation was responsible for the deterioration of the arousal response. These findings by Brooks and colleagues suggest a role of sleep fragmentation in the reported progressive worsening of SDB over time and throughout the night. An earlier study by the same group (Bowes *et al.*, 1980) reported a reduced arousal response in sleeping dogs following an acute period of sleep fragmentation. The authors showed an impairment of the arousal response to exposures to both hypoxia and hypercapnia, suggesting that the deterioration in arousal response in these animals is caused by a reduction in the chemoreception following sleep fragmentation.

Previous research has shown the arousal response to hypercapnia, in the absence of mechanoreceptor input, to be centrally mediated (Ayas *et al.*, 2000). Furthermore, given the suggested peripheral chemoreceptor "silencing" ability of hyperoxia it could be postulated that a reduction in arousal response to a hyperoxic hypercapnia, as observed by Bowes and

colleagues, would demonstrate a reduction in central chemosensitivity. However, the ability of hyperoxia to inhibit the influence of CO_2 on the carotid bodies is disputed (Pedersen *et al.*, 1999a). A more recent study has observed a reduction in the centrally mediated arousal response to hypercapnia following 4 weeks of sleep fragmentation in mice (Li *et al.*, 2014). Data from the present study would seem to support these findings, showing a reduction in central chemosensitivity in humans following a single night of sleep fragmentation, without the use of hyperoxia. However, it should be noted that hypercapnic arousal thresholds may not be mediated by ventilatory chemoreceptors.

A reduction in centrally mediated hypercapnic arousal response and chemosensitivity following sleep fragmentation could account for the prolonged apnoeic events and greater O_2 desaturation observed throughout the nights in sleep apnoea patients. The chronic occurrence of fragmented sleep in sleep apnoea patients could also contribute to the observed progressive worsening of the condition over time.

Greater airway collapsibility has also been shown to occur in humans, following a single night of sleep fragmentation (Sériès *et al.*, 1994). Authors observed that sleep fragmentation resulted in an increase in P_{crit} value, thus resulting in an airway more vulnerable to airway collapse. A reduction in upper airway dilator tone could account for a greater risk of airway collapsibility following sleep fragmentation. This increased likelihood of airway occlusion would result in a greater number of apnoeic events throughout the night and over time, and thus, a greater AHI in SDB patients. Central chemoreceptive areas of the brainstem have been shown to have projections to hypoglossal nerve and can affect airway tone (Jordan & White, 2008; Kc & Martin, 2010). Given this, it is possible that a reduction in central

chemosensitivity could lead to a greater risk of airway collapsibility following fragmented sleep and also lead to a greater risk of obstructive apnoeas in sleep apnoea patients.

As with the previous chapters a reduction in central chemosensitivity coupled with a reduction in the apnoeic threshold and subsequent greater CO_2 reserve following fragmented sleep, would most likely reduce the likelihood of central apnoeas occurring during sleep.

5.4.3 Mechanism

The recent observations by Liu *et al.* (2011) have demonstrated that the increased accumulation of adenosine following sleep fragmentation (McKenna *et al.*, 2007) acts on inhibitory adenosine A1 receptors to attenuate the HCVR. The infusion of an A1 receptor antagonist ameliorated the decrease in hypercapnic chemosensitivity. Adenosine, a by-product of metabolism, plays a key role in the promotion of sleep via inhibition of arousal centres within the brain (Porkka-Heiskanen *et al.*, 2002; Bjorness & Greene, 2009). The accumulation of adenosine following a period of prolonged wakefulness inhibits arousal promoting neurons within the mesopontine tegmentum region and the basal forebrain to bring about sleep (Rainnie *et al.*, 1994; Alam *et al.*, 1999; Strecker *et al.*, 2000; Thakkar *et al.*, 2003). Adenosine also inhibits orexin neurons within the hypothalamus to promote sleep onset (Liu & Gao, 2007; Thakkar *et al.*, 2008).

With adenosine seeming to be the responsible inhibitory neurotransmitter causing reduced central chemosensitivity following sleep fragmentation, the site of inhibition remains unanswered. It is possible that residual adenosine could stimulate vasodilatation in the cerebrovascular bed (Morii *et al.*, 1987; Kusano *et al.*, 2010) resulting in an increased blood

flow and reduced tissue hydrogen ion accumulation. However, no effect of sleep fragmentation on CBF_V was observed in this present study. As described in chapter 4, an additional possibility is the inhibitory effect that adenosine has on orexin neurons within the hypothalamus. Therefore, it is postulated that if sleep fragmentation results in an accumulation of adenosine within the hypothalamic region, as it does following deprivation of sleep for 6 hours (Thakkar *et al.*, 2008), it would inhibit orexin neurons leading to the attenuated central chemosensitivity observed here, and the reduction in HCVR observed by Liu and colleagues.

Inhibition of orexin neuron activity either through antagonist administration or through orexin deficiency in knockout mice appears to have little effect on resting minute ventilation (Deng *et al.*, 2007; Dias *et al.*, 2009; Dias *et al.*, 2010; Li & Nattie, 2010). No difference in morning resting ventilation was observed in this present study. Given that orexin neurons seem to play a small role in resting ventilation, this null effect on resting ventilation can be seen to lend weight to the possible contribution of hypothalamic orexin neurons in the reduction in central chemosensitivity following sleep fragmentation.

A recent study by Li *et al.* (2014) provides further evidence for the possible role of orexin neurons in the reduction in central chemosensitivity associated with sleep fragmentation. In that study the authors exposed mice to sleep fragmentation over a period of 4 weeks. In addition to having a debilitative effect on the arousal response to hypercapnia, the chronic fragmentation of sleep resulted in the dysfunction of arousal neurons. The arousal-associated orexin neurons were reported to exhibit a reduced response to CO_2 exposure, characterised by a reduction in the marker of neural activity c-Fos. This response was still evident following 2 weeks of recovery sleep in the mice, suggesting a role for neural

degradation and not a temporary inhibition of neurons by adenosine. The authors prescribed a model of acute and chronic sleep fragmentation. In this model, acute sleep fragmentation results in an increase in sleep drive or inhibition of arousal neurons, which reduces the arousal responses to hypercapnia. A similar effect would account for the reduction in central respiratory chemosensitivity observed in the present study. In the model put forth by Li *et al.* (2014), a chronic period of sleep fragmentation causes a prolonged degradation of the arousal neurons. The degradation of neurons leads to persistence of the reduced arousal responses in these mice. It could be suggested that this would also translate into a persistent reduction in central chemosensitivity.

Li *et al.* (2014) also observed a reduction in locus coeruleus activity following chronic simulation of sleep fragmentation. In conjunction with being important for the maintenance of arousal, the locus coreuleus is chemosensitive, and so it could also play a role in the reduction of central chemosensitivity in this study. Also, orexin neurons have a governance over additional chemoreceptive regions within the brain stem, namely the RTN and medullary raphe (Li & Nattie, 2014). These regions should also not be ruled out as possible locations of reduction central chemosensitivity. Additionally, the possibility of the changes in chemosensitivity illustrated in the present study, being a result of day-to-day variations in chemosensitivity should not be ruled out.

5.4.2 Cerebrovascular reactivity

An additional hypothesis of this study was the paradoxical reduction in cerebrovascular reactivity to hypercapnia in conjunction with attenuated central chemosensitivity following fragmented sleep. Qureshi *et al.* (1999) observed a greater evening to morning reduction in

cerebrovascular reactivity in patients and showed the magnitude of fragmentation during sleep was a strong predictor of the reduced vascular reactivity. No alterations in the sensitivity of CBF_V or CVR response to hypercapnia, between any time points during this study, were observed. These data suggest that sleep fragmentation did not affect cerebrovascular reactivity and that it had no contribution to the reduction of respiratory chemosensitivity observed here and in research conducted by Liu *et al.* (2011). However, one possible caveat of this study is that only middle cerebral artery blood flow velocity was measured. It could be argued that measurement of posterior cerebral artery would give a more accurate measurement of the perfusion of the chemosensitive regions within the brainstem. Also regional differences in cerebrovascular reactivity between the middle and posterior cerebral arteries have recently been shown (Skow *et al.*, 2013). Therefore, a change in the posterior cerebral artery reactivity to CO_2 following sleep fragmentation, which would influence central chemosensitivity, cannot be ruled out.

The data also demonstrated no overnight changes in cerebrovascular reactivity across either control or fragmented night's sleep. This observation was not expected, as it is an occurrence that has been reported previously by a number of investigations (Ameriso *et al.*, 1994; Meadows *et al.*, 2005). However, one recent study has also observed no overnight change in cerebrovascular reactivity or conductance in healthy controls (Ryan *et al.*, 2014), and a recent study from the same research group has reported an increase in cerebrovascular reactivity from evening to morning (Strohm *et al.*, 2014). Ryan *et al.* (2014) suggested that methodological limitations of previous research, in particular the inadequate control of hypercapnic administration and the absence of maintain isoxia throughout the stimulus, as potential reasons for the differences in the observed findings. In this present

study a dynamic end tidal forcing system was used to accurately control participant's $P_{ET}CO_2$ and $P_{ET}O_2$ throughout. Also, the reported reductions in cerebrovascular reactivity were small, with a 0.7 cm/sec/mmHg reduction from evening to morning as observed by (Meadows *et al.*, 2005). Therefore, a possible reason for not seeing an evening to morning reduction in this study could be due to power, with previous research using greater numbers of participants than were used in this present study.

Additionally, Qureshi and colleagues showed that an additional predictor, although not as strong as fragmentation, was the magnitude of the hypercapnia experienced during the night. Repetitive exposure to hypercapnia could lead to a desensitisation of the vascular bed to hypercapnia and thus a reduction in cerebrovascular reactivity to hypercapnia. Human research has shown an increase in hypoxic cerebrovascular reactivity following exposure to intermittent hypoxia during wakefulness (Kolb *et al.*, 2004; Foster *et al.*, 2005). However, no such research has been conducted on the effects of exposure to intermittent hypercapnia on cerebrovascular reactivity, during wakefulness or sleep.

The observations of this study do not rule out the effect of chronic sleep fragmentation on cerebrovascular reactivity. Also, chronic sleep fragmentation could play a role in the proposed reduction in cerebrovascular reactivity to hypercapnia in sleep apnoea patients (Qureshi *et al.*, 1999; Durgan & Bryan, 2012). However, the suggestion of reduced cerebrovascular reactivity in SDB patients has been countered by additional studies reporting no such alteration (Foster *et al.*, 2009; Ryan *et al.*, 2014).

5.4.3 Limitations

There are a number of caveats to this present study that should be noted. Only a single night of fragmented sleep was induced here. Additional fragmentation nights could have possibly yielded further decrements in chemosensitivity and possibly cerebrovascular function. Also, additional fragmentation nights would have taken into account the possible occurrence of day-to-day variability of chemosensitivity that could have been a potential reason for the reported findings.

The lack of an acclimatisation night prior to the control night means that baseline values could be considered to be affected by the unfamiliar sleeping environment during the first night, which may have caused arousals during sleep. However, considering the increases in arousals from control night to fragmentation in this study, such a limitation could be considered to have a marginal effect on the findings.

It would have been beneficial to obtain data referring to the somnolence levels of the participants. Such data would have shown whether sleepiness could have played a role in the reduced central chemosensitivity, as possibly occurs in Chapter 4, or whether it was caused primarily by the fragmentation of the sleep cycle.

In summary, acute sleep fragmentation results in reduced morning central chemosensitivity to hypercapnia. CO_2 cerebrovascular reactivity appears to play no role in the observed reduction in chemosensitivity in this study as no alterations were demonstrated. Alterations in chemosensitivity caused by sleep fragmentation could directly impact the development and progression of apnoeic episodes in sleep apnoea patients. Further research is needed to investigate longer more chronic periods of sleep fragmentation, combined with intermittent exposures to hypercapnia and/or hypoxia during the night. Furthermore, a more selective

paradigm for the simulation of fragmentation during particular sleep stages could reveal if NREM or REM sleep has more influence on the changes in central chemosensitivity following fragmented sleep.

CHAPTER 6.

HYPOXIC PULMONARY VASOCONSTRICTION RESPONSE IN OLDER INDIVIDUALS

6.1 Introduction

As described previously, the condition of sleep disordered breathing (SDB) has strong mechanistic links with hypertension, metabolic disorders, and stroke (Somers *et al.*, 2008). One particular form of hypertension associated with SDB is pulmonary hypertension (Atwood *et al.*, 2004; Sajkov & McEvoy, 2009). It is thought that 20-40% of all SDB patients suffer from some degree of pulmonary hypertension (Sajkov & McEvoy, 2009).

Intermittent hypoxia (Zieliński, 2005) and the intrathoratic pressure swings that SDB patients experience (Marrone *et al.*, 1994) have been shown to be mediators of pulmonary hypertension in this population. The progressive hypoxia during an apnoea results in the vasoconstriction of the pulmonary circulation, thus gradually increasing pulmonary artery pressure during an apnoeic event (Marrone & Bonsignore, 2002). Also, the magnitude of pulmonary artery pressure experienced during the apnoeic events increase throughout the night (Marrone *et al.*, 1994). This is possibly due to the progressive lengthening of apnoeic events during the night (Charbonneau *et al.*, 1994) and thus the greater level of resulting hypoxemia. The repetitive nature of hypoxic challenges that SDB patients experience lead to pulmonary vascular remodelling and the development of daytime pulmonary hypertension (Stenmark *et al.*, 2006). If left untreated, pulmonary hypertension in time affect the right side of the heart causing right ventricular hypertrophy leading to possible heart failure (Zieliński, 2005).

The prevalence of SDB is largely age dependent. In the middle-aged population the prevalence of SDB is considered to be 2% of females and 4% of males (Young *et al.*, 1993), whereas in the older generations the prevalence is much greater affecting approximately 45-62% of those older than 60 years (Ancoli-Israel & Ayalon, 2006; Wolkove *et al.*, 2007).

Furthermore, a systematic review (Atwood *et al.*, 2004) found that age was positively related to the number of SDB patients presenting with pulmonary hypertension. A greater occurrence of pulmonary hypertension in older sufferers of SDB suggests that not only are older individuals more likely to develop SDB, but they are also at greater risk from suffering the co-morbidities arising as a consequence of SDB. Despite the presence of sleep apnoea, ageing alone is associated with mild increases in daytime pulmonary artery pressure (Reeves *et al.*, 2005; Lam *et al.*, 2009). As with the systemic circulation, stiffening and reduction in overall compliance of the pulmonary circulation with age could explain the mild hypertension observed in older individuals (Reeves *et al.*, 2005). However, in older people who suffer from SDB, the stiffening of the pulmonary circulation in addition to the effect of hypoxia on the pulmonary vasculature would result in greater increase in pulmonary artery pressure during each apnoea. This could explain the greater prevalence of pulmonary hypertension in older individuals with SDB seen by (Atwood *et al.*, 2004).

Ageing is associated with a gradual fall in PaO_2 (Cerveri *et al.*, 1995) more than likely because of an increasing mismatch in regional ventilation and regional perfusion in the ageing lung (Sorbini *et al.*, 1968). Given the gradual decline in PaO_2 associated with advancing age it could be suggested that ageing results in a sensitisation to hypoxia. A sensitisation to hypoxia in the pulmonary circulation would result in a greater vasoconstrictive response to acute hypoxia (Balanos *et al.*, 2002). Therefore, it could be suggested that older individuals could present with a greater HPV response than younger individuals.

An augmented HPV response in older individuals could result in even greater pulmonary artery pressure swings during apnoeas. Such a response places older SDB sufferers at greater

risk of developing pulmonary hypertension as a consequence. The effect of ageing on the HPV response has yet to be investigated.

In this study the pulmonary hemodynamics, assessed using echocardiograph, of young and older individuals were compared during normoxia and acute hypoxic exposure. It was hypothesised that older individuals would present with higher pulmonary artery pressures during normoxic breathing in addition to a greater pulmonary artery pressure response during exposure to hypoxia.

A secondary hypothesis of this study was to compare to hypoxic sensitivity of the older and younger individuals to examine if any age effects on HVR exist. This investigation has been conducted by a number of previous studies, with the majority of these reporting no change in the hypoxic sensitivity with age (Ahmed *et al.*, 1991; Poulin *et al.*, 1993; Smith *et al.*, 2001; Pokorski & Marczak, 2003b), with exception of Kronenberg and Drage (1973) and (Peterson *et al.*, 1981a). These studies represented hypoxic sensitivity as Δventilation/ΔSaO_2, where SaO_2 represents oxygen saturation. There is some disagreement in the portrayal of hypoxic sensitivity as a change in O_2 saturation because of a number of reasons. First, because the oxygen saturation is not the actual stimulus for the peripheral chemoreceptors, secondly the ventilation-SaO_2 relationship is not always linear and lastly a change in the position of the oxygen dissociation curve could influence the interpretation of the HVR, if measured as a change in SaO_2 (Teppema & Dahan, 2010). Thus, possible changes in the position of the oxygen dissociation curve with age (Ivanov & Chebotarev, 1984) could mean that the comparison of hypoxic sensitivity across two age groups is confounded if measured by Δventilation/ΔSaO_2. A recent suggestion put forth by Teppema and Dahan (2010) to overcome the potential caveats of using Δventilation/ΔSaO_2 to represent the hypoxic

sensitivity, is to instead portray the sensitivity as Δventilation/ΔlogP_aO_2, as ventilation is a linear function of log$PaCO_2$, within the hypoxic range. Therefore hypoxic sensitivity was assessed in the two age groups and represented as both the Δventilation/ΔSaO_2 and Δventilation/ΔlogP_aO_2, to see if any differences occurred.

6.2 Methods

Young male participants were recruited from the student body of the University of Birmingham. Older male participants were recruited from the local community. All participants were free of cardiovascular and respiratory disease. During each visit participants were in a three hour fasted state and were asked to refrain from vigorous exercise and alcohol intake 24 hours prior to any trial, in addition to refraining from caffeine intake 12 hours before. After receiving detailed information about the study and its risks informed consent was obtained and a general health questionnaire completed by each participant. Ethical approval for the study was obtained from the local ethics subcommittee at the University of Birmingham.

6.2.1 Experimental procedure

Eligible participants visited the laboratory on two separate occasions for two protocols, a control and hypoxia protocol as described in section 2.3.3. In brief, the hypoxic protocol consisted of an initial five-minute period of eucapnic euoxia (1 mmHg above normal $P_{ET}CO_2$ and $P_{ET}O_2$ = 100 mmHg). This initial period was then followed by a 20 minute period of eucapnic hypoxia ($P_{ET}O_2$ = 50 mmHg). The protocol was concluded with a further five minutes of eucapnic euoxia. The control protocol consisted of 30 minutes of eucapnic

euoxia. These protocols were conducted at similar times of day, with no longer than two weeks between them. The order of the protocols was randomised and during each visit participants were blinded to the protocol they were undertaking.

6.2.2 Measures

During each protocol spectral traces of tricuspid regurgitation and aortic valve blood flow were recorded non-invasively using Doppler echocardiography. The methods used to gain the required measurements are described in further detail in the section 2.6. At a later time averages of three or more spectral envelopes from each spectral trace were obtained for the calculation of SPAP and cardiac output ($\dot{Q}$). A baseline value of SPAP and $\dot{Q}$ was calculated prior to the initiation of each protocol. SPAP was then assessed for each minute and $\dot{Q}$ for every two minutes of the protocols. Participants were asked to lie on a comfortable bed in a semi-supine position, rotated to their left side, to allow for echocardiography measurements to be taken. Heart rate and respiratory waveforms were recorded continuously throughout each protocol.

6.2.3 Data analysis

To assess the SPAP steady state responses to hypoxia and make comparison between the two age groups a model was fitted to the data. The model fitted an asymptote curve to the SPAP data during euoxic baseline and hypoxic stimulus period. Three parameters were derived from the model curve fitting. M1 represents the baseline values for SPAP and M2 represents the asymptote for SPAP values during the hypoxic stimulus period. τ is a time constant of the hypoxic asymptote. For the model equation see appendix 3. The model was

fitted to individual data, using a Matlab program, from which differences in groups means were assessed statistically using an independent sample T-test.

Differences in hypoxic chemosensitivity were assessed between two age groups. The sensitivity of the HVR was calculated both via $\Delta ventilation/\Delta SaO_2$ and $\Delta ventilation/\Delta log P_aO_2$.

A repeated measures ANOVA was used to assess the interaction between time and each protocol in addition to any differences between the two age groups. Independent sample T-tests were used to test differences in demographics between the two groups. Values reported are mean ± S.E.M and significance was determined by $p<0.05$ unless otherwise stated.

6.3 Results

Twelve young participants with mean (± S.D) age of 20.33 ± 0.98 years old and BMI of 24.14 ± 2.08 kg/m^2, and nine older participants (mean age of 55.89 ± 5.23 years old and BMI of 27.22 ± 1.91 kg/m^2) took part in this study.

6.3.1 Gas Control and Ventilation

Figure 6.1 shows the controlled end-tidal partial pressures and corresponding ventilatory responses during both the hypoxia and control protocols. During the hypoxia protocol $P_{ET}O_2$ was quickly reduced from 100 to 50 mmHg and was accurately controlled at this hypoxic level for twenty minutes until quickly returning to a euoxic level. During the control protocol $P_{ET}O_2$ and $P_{ET}CO_2$ controlled at eucapnic euoxic levels. Mean normal $P_{ET}CO_2$ was similar between the young and older groups, 40.6 ± 0.4 and 40.2 ± 1.0 mmHg, respectively. So too was mean $P_{ET}O_2$ (older group = 102.53 ± 1.06, younger group = 97.73 ± 1.79).

There was no difference in minute ventilation between the young and older age groups throughout the control protocol; 13.30 ± 0.07 and 11.87 ± 0.05 L.min^{-1}, respectively. During the hypoxic period minute ventilation significantly increased upon exposure to hypoxia.

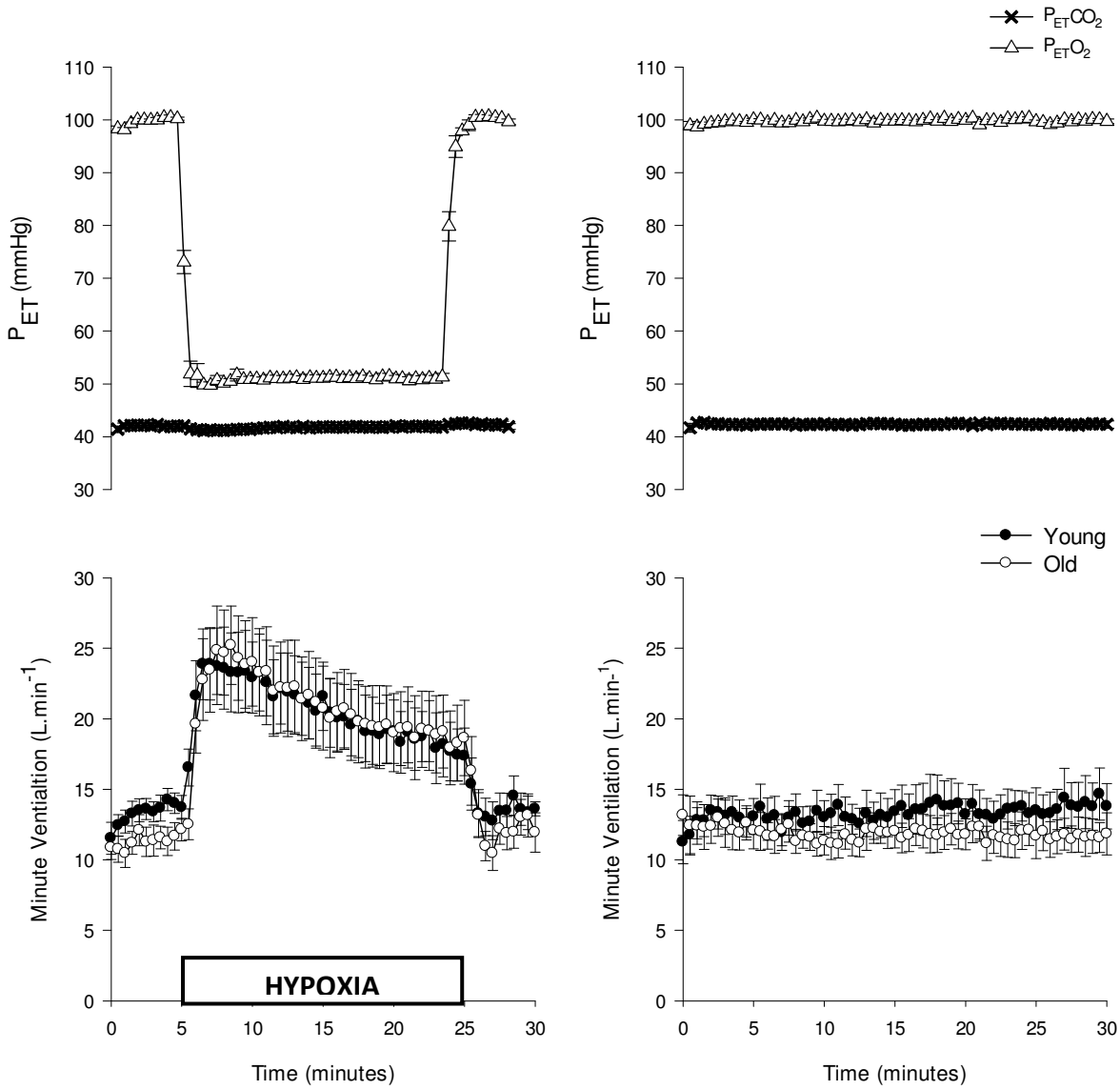

Figure 6.1 Graph showing mean (±S.E.M) values of end-tidal gases during the each protocol (top panels) and the ventilatory responses (lower panels). The right panel correspond to the data obtained during the control protocol and the left panel is the hypoxic protocol.

There were no differences in calculated hypoxic sensitivity between the two age groups. The results, presented as both $\Delta Ventilation/\Delta SaO_2$ and $\Delta Ventialtion/\Delta logP_aO_2$ are shown in table 6.1.

		$\Delta Ventilation/\Delta SaO_2$ ($L.min^{-1}.\%^{-1}$)	**$\Delta Ventilation/\Delta LogP_aO_2$ ($L.min^{-1}$)**
Older	Mean	-1.36	-47.53
	±S.E.M	0.31	7.52
Younger	Mean	-1.61	-39.18
	±S.E.M	0.69	7.50

Table 6.1 Shows the mean ±S.E.M calculated hypoxic sensitivities for both age groups. No difference was seen between the groups; $p>0.05$

6.3.3 Systolic Pulmonary Artery Pressure

The mean SPAP for both protocols are shown in figure 6.2. SPAP, was higher in the older group throughout the control protocol when compared to the young participants; 22.01 ± 0.03 and 18.91 ± 0.04 mmHg respectively. The augmented SPAP in the older group was also observed during the initial euoxic phase of the hypoxia protocol. Upon initiation of the hypoxic phase, SPAP rose to a peak pressure of 28.34 ± 1.15 mmHg in young participants and 38.23 ± 2.04 mmHg in the older group. On the return to euoxic levels SPAP fell in both groups. During the five minute euoxic recovery phase SPAP not return to baseline values in either group.

6.3.2 Cardiovascular responses

Mean $\dot{Q}$ values for both hypoxia and control protocols are displayed in figure 6.2. During the control protocol $\dot{Q}$ was similar between the two age groups; young = 4803.06 ± 27.07 $mL.min^{-1}$, older = 4704.52 ± 17.70 $mL.min^{-1}$. $\dot{Q}$ during the hypoxia protocol increased in both

groups during the hypoxic phase. The magnitude of augmentation was similar between the two groups. Q̇ return to baseline levels in the post hypoxic phase.

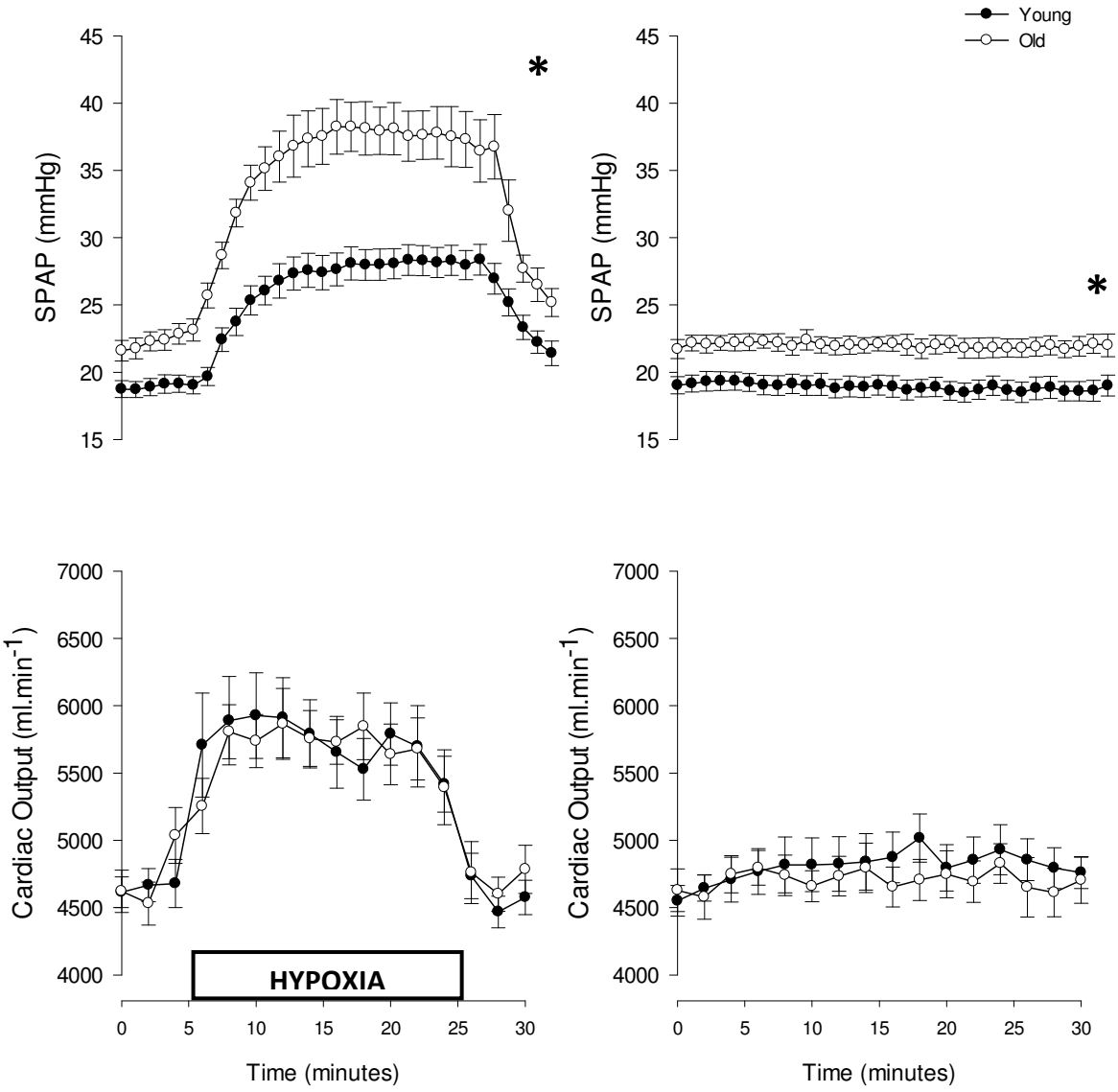

Figure 6.2 Graphs showing mean (±S.E.M) systolic pulmonary artery pressure (SPAP) and Cardiac output, top and bottom graphs respectively. The left panel shows data obtained during the hypoxic protocol and the control protocol data is shown in the left panel. * = significant difference between the two age groups; $p<0.05$.

Heart rate and stroke volume results are displayed in figure 6.3. In both age groups heart rate increased at the onset of hypoxia. Between the two age groups, there was no difference in the heart rate response during the hypoxic protocol. Mean heart rate during the euoxic phases and control protocol were also similar between the two age groups. In both age groups stroke volume was unchanged throughout both control and hypoxic protocols. During the hypoxic protocol mean stroke volume was slightly lower in the older individuals when compared to the younger age group. Mean stroke volume for the older and younger age group during the hypoxic protocol was 74.42 ± 0.28 and 81.33 ± 0.31 mL, respectively. For each age group similar Mean of stroke volume to those seen in the hypoxic protocol were observed during the control protocol; old = 74.68 ± 0.15mL and young = 80.61 ± 0.33 mL. However, these levels were not statistically significant between the two age groups ($p=0.09$).

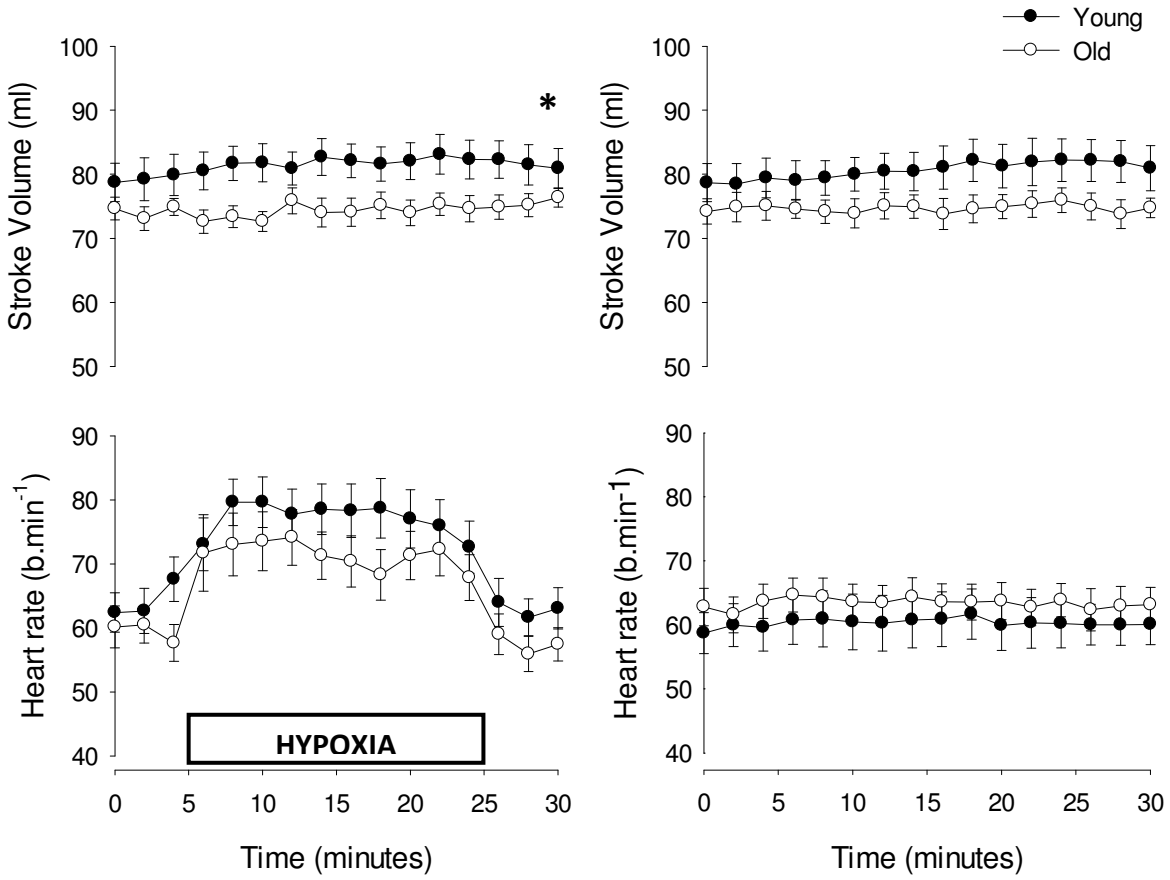

Figure 6.3 Graphs showing mean (±S.E.M) stroke volume and heart rate, top and bottom graphs respectively. Graphs in the left panel shows data measured during the hypoxic protocol and the data from the control protocol is shown in the right panels. * = significant difference between the two age groups, $p<0.05$.

6.3.4 Model Parameters

Table 6.2 shows the model parameters of the two age groups. The asymptote for SPAP during euoxic was greater in older individuals when compared to younger participants, M_1 = 21.96 ± 0.75 and 18.73 ± 0.66 respectively. The same was observed during hypoxic exposure, with the older age group showing a mean M_2 of 38.59 ± 2.20 and the younger participants mean M_2 was 28.73 ± 1.19. The time constant for the hypoxic response were not different between the older and younger age groups, τ = 1.96 ± 0.37 and 2.13 ± 0.16 minutes,

respectively. The sensitivity of the response ($M_2 - M_1$) was greater in the older age group when compared to younger participants, 16.63 ± 2.42 and 10.00 ± 1.01, respectively.

		M_1 (mmHg)	M_2 (mmHg)	τ (minutes)	M2-M1 (mmHg)	Sum of squares
Young	Mean	18.73*	28.73*	2.13	10.00*	15.71
	±S.E.M	0.66	1.19	0.16	1.01	4.14
Older	Mean	21.96*	38.59*	1.96	16.63*	89.4
	±S.E.M	0.75	2.20	0.37	2.42	30.0

Table 6.2 Showing the mean ±S.E.M parameters and residual sum of squares for SPAP during the hypoxic protocol derived from the modelling analysis. *=P<0.03.

6.4 Discussion

The primary hypothesis of the study predicted correctly that older individuals would have a greater SPAP during exposure to acute hypoxia when compared to younger participants. We observed a greater augmentation in SPAP from baseline values in older participants than younger participants during hypoxia, shown by significantly greater response sensitivity (M1-M2) in older individuals.

No difference in hypoxic sensitivity was seen between the two age groups. This finding is in accordance with previous research (Ahmed *et al.*, 1991; Poulin *et al.*, 1993; Smith *et al.*, 2001; Pokorski & Marczak, 2003b). It was also shown in this present study that the representation of the hypoxic sensitivity, either as a ratio of $\Delta ventilation/\Delta SaO_2$ or $\Delta ventilation/\Delta \log P_aO_2$, made no difference to the interpretation of the hypoxic sensitivity between the age groups. This finding suggests that any change in the oxygen dissociation curve because of ageing has minimal if any effect on the haemoglobin saturation. However, it must be mentioned that the $P_{ET}O_2$ levels used in this study to represent the PaO_2 of the

participants, is an indirect measure of true PaO_2. Alterations in the ventilation-perfusion ratio, which occur in older individuals (Lalley, 2013), could confound the assumption that $P_{ET}O_2$ is a representation of PaO_2. Therefore, changes in the hypoxic sensitivity when represented as $\Delta ventilation/\Delta log P_aO_2$ could still occur with age.

Also, it was hypothesized that older individuals would present with a higher normal SPAP than younger participants. This hypothesis was proven correct by the finding of a greater SPAP, of ≈3 mmHg, in older individuals, during the euoxic control protocol and euoxic baseline phases of the hypoxic protocol. This observation also supports previous findings (Reeves *et al.*, 2005; Kovacs *et al.*, 2009; Lam *et al.*, 2009). Similar levels of resting $\dot{Q}$ between the two age groups during both protocols indicate that a reduced compliance to blood flow within the pulmonary circulation could be the cause of augmented SPAP during normoxia. A stiffening of the pulmonary vessels with age would account for such attenuated compliance. A reduction in compliance has been suggested in previous research by Reeves *et al.* (2005). Reeves and colleagues suggest that stiffening in the vascular walls of pulmonary arteries leads to a reduction in compliance of the pulmonary circulation and limits its ability to accommodate increased blood flow without substantial increases in pulmonary pressure. Lam *et al.* (2009) measured pulmonary artery pressure using similar techniques to those used in the present study and assessed systemic vascular stiffness, calculated as the difference between diastolic and systolic blood pressures. Authors showed a significant positive correlation between the two, thus lending further evidence that the pulmonary circulation experiences stiffening similar to that observed within the systemic circulation with age. The stiffening of the pulmonary vessels with age has also been demonstrated by data collected in isolated human pulmonary vessels (Harris *et al.*, 1965). The authors showed

a reduction of pulmonary trunk distensibility in individuals who had died above the age of 70 years, in comparison to those who had died before 30 years of age.

During exercise $\dot{Q}$ is greatly augmented and as a result pulmonary artery pressure in older individuals is markedly increased when compared to younger participants (Kovacs *et al.*, 2009; Mahjoub *et al.*, 2009). Mahjoub *et al.* (2009) observed alarmingly high levels of pulmonary artery pressure (60 mmHg) during exercise in 36% of individuals aged 60-70 years and 50% of those aged 70-80 years. Authors suggest that a cause of augmented pulmonary pressure in these individuals is an increased left atrium pressure as a result of left ventricle stiffening with age (Abhayaratna *et al.*, 2006; Ha *et al.*, 2009). The diastolic dysfunction in these participants results in the reduced clearance of blood from the pulmonary veins, therefore increasing blood volume within the pulmonary circulation. A reduction in compliance of the pulmonary vessels to this back up of blood within the pulmonary vessels would additionally explain the increase in pulmonary pressure seen during exercise in older individuals.

It could be postulated that the increases in $\dot{Q}$ during exposure to hypoxia could result in a greater pulmonary artery pressure response in older and less compliant vessels, as seen during exercise. However, Balanos *et al.* (2005) quantified the indirect influence of $\dot{Q}$ on the pulmonary artery pressure response to hypoxia, suggesting that $\dot{Q}$ contributed to as little as 5% in healthy volunteers during exposure to hypoxia. A more robust linear model was recently established to reinforce the findings of Balanos *et al.* (2005), showing a 0.66 ± 0.32 $mmHg.L.min^{-1}$ contribution of $\dot{Q}$ to pulmonary artery pressure (Croft *et al.*, 2013).

However, the results of Croft *et al.* (2013) and Balanos *et al.* (2005) were obtained in healthy young individuals and so their calculated contribution of $\dot{Q}$ may not apply for data obtained

from older participants. Nevertheless in the present study, older participants were seen to have a greater mean sensitivity (M2-M1) of the SPAP response to hypoxia than younger participants. This suggests that the augmented $\dot{Q}$ during hypoxia had a minimal effect on the response of SPAP in older individuals. Therefore, it can be concluded that older individuals have a more sensitive HPV response than younger individuals. However, an assumption made here is that the contribution of the reduced pulmonary vessel compliance to pulmonary artery pressure is constant during the increased $\dot{Q}$ experienced during the hypoxia protocol.

This is the first time an age associated augmentation in the HPV response in humans has been demonstrated. Previous research conducted in animal isolated lung preparations has observed the opposite effects of ageing on vasculature responses to hypoxia (Tseng *et al.*, 1992). Similar reductions were also seen between young and middle aged rats (Tucker *et al.*, 1982) suggesting a reduced sensitivity of the pulmonary vascular bed to hypoxia. Species differences and preparation of pulmonary vessels during experimentation could explain the different observations to the ones in the present study.

6.4.1 Implications

Previous research has shown that the older population have a higher prevalence of SDB (Ancoli-Israel & Ayalon, 2006; Wolkove *et al.*, 2007) and as previously mentioned, pulmonary hypertension is one of a number of cardiovascular diseases linked with SDB. The main mediator of its development in sleep apnoea patients is intermittent hypoxia. It is thought, that the mechanisms with which intermittent hypoxia causes pulmonary hypertension are similar in nature to those that cause pulmonary hypertension following chronic hypoxia;

pulmonary vasoconstriction and vascular remodelling (Bosc *et al.*, 2010). Given that data in this present study has demonstrated a greater HPV response in older individuals, it can be suggested that older sufferers of SDB are at a greater risk of developing pulmonary hypertension, as a result of frequent apnoeic events. Also, these results could explain why the literature review by Atwood *et al.* (2004) demonstrated that sleep apnoea patients presenting with a degree of pulmonary hypertension tend to be older.

Furthermore, hypercapnia has also been shown to cause vasoconstriction in the pulmonary circulation (Balanos *et al.*, 2003). Given this evidence by Balanos and colleagues, it can be postulated that hypercapnia experienced during apnoeic events, in tangent with hypoxia, could further contribute to the development of pulmonary hypertension in sleep apnoea patients. This is highlighted by research suggesting the existence of additional mechanisms, other than hypoxia, for the development of pulmonary hypertension in SDB patients. Such research has shown that the treatment of SDB with CPAP can result in a greater reduction in pulmonary artery pressure than O_2 supplementation, a treatment designed to remove the element of hypoxia in SDB patients (Sajkov *et al.*, 2002). These findings were recently interpreted in an extensive review by Sylvester *et al.* (2012), where the authors suggested that as much as 40% of pulmonary hypertension seen in sleep apnoea patients is caused by mechanisms arising from SDB that are different from hypoxia. Hypercapnic pulmonary vasoconstriction is one possible mechanism. However, it remains to be evaluated whether the hypercapnic pulmonary vasoconstriction response in older individuals is also increased in comparison with younger individuals.

Nevertheless, evidence for the importance of CO_2 in the vasoconstriction of the pulmonary circulation in older individuals, can be shown by the reported age differences in response of

the pulmonary circulation to poikilocapnic hypoxia. Poikilocapnic hypoxia conditions occur at high altitude because of the hyperventilation caused by hypoxia. An excessive HPV response at altitude can lead to the development of high altitude pulmonary oedema. Research has shown that older individuals are less likely to experience high altitude pulmonary oedema than younger individuals (Hultgren *et al.*, 1996). This suggests that under poikilocapnic hypoxia, older individuals experience a lower HPV response than younger individuals. This is the opposite of that shown during eucapnic hypoxia in the present study. The contrast in the HPV response of older individuals during poikilocapnic and eucapnic hypoxia highlights CO_2 as an important mediator in the HPV response of older individuals. Hypocapnia plays a protective role at high altitude, causing a vasodilation within the pulmonary circulation (Balanos *et al.*, 2003). Therefore, a lower HPV response in older participants during poikilocapnic hypoxia would suggest a greater vasodilatation of the pulmonary circulation in response to hypocapnia. An alteration of this kind in older people could occur in parallel with the possible augmentation of vasoconstrictive response to hypercapnia mentioned above.

An additional reason suggested by Hultgren *et al.* (1996) for their findings, was that older individuals ascending to high altitude have a more cautious accent profile than that of younger individuals. A slower ascent profile can lower in the incidence of high altitude pulmonary oedema (Bärtsch *et al.*, 2005).

As described previously, a stiffening of the pulmonary circulation could have implications in the ability of the pulmonary vessels to accommodate large increases in $\dot{Q}$. A decrease in the pulmonary circulation compliance to increases in blood flow limits the flow through them and will have subsequent upstream implications. An augmentation in right ventricular afterload is one such implication. Considering that the pulmonary circulation receives the

entire $\dot{Q}$, an increase in the right ventricle afterload brought about by a reduction in the compliance of the pulmonary arteries, may restrict blood flow to the left side of the heart, and thus blood supply to the systemic circulation. Such influence on left ventricular function may, as a result, led in a reduction in exercise capacity. Therefore, it is suggested that the stiffening of the pulmonary circulation could limit the exercise capacity of older individuals.

An increase in the HPV response with age would be unlikely to impact on the exercise capacity of the individual. This is because the hypoxemia experienced during exercise within the pulmonary circulation only occurs at the pulmonary arteries. The pulmonary arteries have been shown to play a lesser role in the HPV response than the pulmonary veins (Marshall & Marshall, 1983; Marshall & Marshall, 1988). Therefore, it is unlikely that mixed venous hypoxemia during exercise will stimulate a HPV response that could influence exercise capacity. However, during conditions of systemic hypoxia HPV could act as a limiting factor to exercise capacity, not just because PaO_2 is reduced but also because mixed venous PO_2 will be in even lower than during normoxia. Recent studies support this statement, suggesting that pulmonary artery pressure and HPV are limiting factors of exercise capacity at high altitude (Naeije *et al.*, 2010; Naeije, 2011; Pavelescu *et al.*, 2013). Therefore, the greater vasoconstriction of the ageing pulmonary circulation in response to hypoxia could limit the exercise capacity at high altitude via greater right ventricular afterload.

A greater than normal sensitivity to hypoxia could lead to a much greater restoration of ventilation following apnoeic events, caused by the apnoea related hypoxemia. Such a breathing response could lead to periodic breathing and cyclic central apnoeic events. This is one possibility for the greater number of central apnoeas in older individuals (Bixler *et al.*, 1998; Bixler *et al.*, 2001). However, previous studies and data in this present study show that

age is not associated with a change in hypoxic sensitivity, therefore making hypoxic sensitivity an unlikely cause for the greater apnoeic events in older individuals.

6.4.2 Possible mechanisms

Age associated structural changes in the pulmonary circulation (Mackay *et al.*, 1978) could cause the stiffening of vessels and lead to the increase in pulmonary pressures during normoxic conditions. One particular structural change suggested is an increase in collagen content within the pulmonary vessel walls. However, research has reported both an increase (Farrar *et al.*, 1965) and decrease (Mackay *et al.*, 1978) in collagen content in vessels obtained at autopsy from elderly individuals. Despite the contrasting findings in these studies regarding collagen content, both studies reported that vessels from the eldest individuals had the greatest degrees of stiffness, suggesting collagen may not be the cause for the increase in pulmonary vessel stiffness. An additional connective protein that could be causing increased pulmonary artery stiffness in older individuals is elastin. Hosoda *et al.* (1984) reported an increase in elastin content within the pulmonary arteries of older individuals. This finding suggests that the increased elastin content is the cause of the greater stiffness and reduced compliance of the pulmonary vessels of older individuals.

There are numerous complex mediators of HPV that include cellular interactions within the pulmonary artery smooth muscle cell, endothelium modulators and neuronal influences (Sylvester *et al.*, 2012; Swenson, 2013). Therefore, it is reasonable to suggest that not one single mechanism is responsible for the observed sensitization of HPV in older participants. Furthermore, ageing is associated with alterations in a number physiological functions that are also mediators of pulmonary vasoconstriction, thus increasing the likelihood of more

than one mechanism being responsible for an increased HPV response. Such alterations with age could result in a greater pulmonary artery pressure during both normoxic and hypoxic conditions.

Ageing is thought to be associated with a reduction in PaO_2 (Cerveri *et al.*, 1995). Such a reduction could lead to a sensitisation of transcription factors called hypoxic inducible factors (HIF-1). HIF-1 is an important transcription factor in oxygen homeostasis in a number of systemic functions (Lee *et al.*, 2004). A heterodimer with α and β subunits, HIF-1 is constantly degraded and almost undetectable in the blood under normoxic conditions. However, during hypoxia the degradation is followed by the forming of a stable heterodimer with HIF-1β. Previous research has shown a mechanistic involvement of HIF-1 in the HPV response to both acute (Smith *et al.*, 2006b) and sustained hypoxia (Balanos *et al.*, 2002). A sensitization of HIF-1 with age could play a role in the augmented HPV response shown in this study. However, no difference in $P_{ET}O_2$ was seen between the two age groups, therefore such a mechanism is unlikely.

An important co-factor in HIF-1 regulation is iron (Ivan 2001; Jaakkola 2001). Iron is important for the degradation of stable HIF-1 and a reduction of iron levels would result in a greater level of stable HIF-1. If, during acute hypoxia, iron is chelated using desferrioxamine the HPV response is augmented (Smith *et al.*, 2008). The reverse effect of iron status is also true, with iron supplementation showing a reduced level hypoxic pulmonary hypertension in Peruvian high altitude dwellers (Smith *et al.,* 2009). Given this evidence, it could be suggested that the common occurrence of anaemia in older individuals (Salive *et al.*, 1992) would not only increase in the HPV response to acute hypoxia, but also increase the

normoxic pulmonary artery pressure levels, similar to that shown in the results of the present study.

An additional mechanism for a greater HPV response could lie within the pathway that mediates the constriction of smooth muscle cells. During hypoxic exposure there is a release of intracellular Ca^{2+} from the sarcoplasmic reticulum leading to increased actin-myosin binding and therefore vasoconstriction of pulmonary smooth muscle cells. One intracellular mediator of the signalling pathways resulting in Ca^{2+} release is the redox state of the cell. The redox state is defined by the ratio of oxidation and reduction within a cell and is affected by the production and degradation of ROS. Hypoxia results in a greater generation of ROS (Duranteau *et al.*, 1998). It is thought that this greater production of ROS during hypoxia signals the vasoconstriction of the smooth muscle within the pulmonary circulation via Ca^{2+} release. Superoxide has been suggested to play a key role in such signalling pathways (Schumacker, 2011; Swenson, 2013). Ageing is associated with a greater generation of ROS (Hensley & Floyd, 2002). Additionally, previous research demonstrates a greater generation of superoxide in ageing rats, and this has been linked with a reduction in endothelial function in these animals (Hamilton *et al.*, 2001). The greater presence of ROS with advancing age could lead to elevated extracellular $Ca2^{+}$ and greater constriction of pulmonary vessels, not only during hypoxic stimulation but also during normoxic levels.

Lastly, factors released from the endothelium can act in autocrine and paracrine fashions to bring about the vasoconstriction of the vasculature. One particular endothelium-dependent vasoconstrictor that mediators HPV, and pulmonary hypertension is endothelin 1 (Chen *et al.*, 1995). There is a greater presence of endothelin 1 with advancing age and is associated with the endothelial dysfunction observed in older individuals (Donato *et al.*, 2009; Westby

et al., 2011). The greater presence of endothelin 1 could also be impacting on pulmonary vessels causing vasoconstriction during normoxia and a greater HPV response.

Changes in structural

In summary, we observed a greater HPV response in healthy older individuals in comparison to younger counterparts. The greater vasoconstriction during hypoxic exposure could place the older generation at greater risk of developing pulmonary hypertension as a consequence of SDB.

Chapter 7.

GENERAL DISCUSSION

The work presented in this thesis has revealed a number of original findings in two topics of physiology, chemosensitivity and vascular physiology. Below is a general discussion of the findings in this thesis and the impact they have in their respective areas. Also, suggestions for the direction of future research are given where necessary.

7.1 Chemosensitivity

In the initial study of this thesis the effect of ageing on chemosensitivity was investigated. Caveats of previous research, investigating this effect, arise when regarding the use of hyperoxia and re-breathing methods to assess the effect of age on chemosensitivity. Also, there is equivocal evidence for the chemoreceptor responsible for the age-associated reduction in hypercapnic chemosensitivity. In using a novel approach to assessing chemosensitivity in older individuals, the MFBS test, the research in the initial study showed that ageing is associated with a reduction in chemosensitivity driven by a decrement in central chemosensitivity. The data presented in this thesis were the first to conclude, without the use of hyperoxia, that a reduction central chemosensitivity is responsible for the reduction in total chemosensitivity seen in older individuals.

Chapter 4 built on previous research investigating the effect of 24 hours of total sleep deprivation on respiratory control by incorporating a model of sleep loss not used previously. This model saw participants complete three successive nights of restricted sleep length, in conjunction with a separate control protocol of three normal nights of sleep. The loss of sleep over consecutive nights allowed for the investigation of the effect of sleep deprivation across a number of circadian cycles instead of a single 24-hour rhythm, as used by previous studies. Also, the use of a MFBS test in this study allows the assessment of

central and peripheral chemosensitivity, something not done previously. Data from chapter 4 demonstrated a reduction in central chemosensitivity following consecutive nights of restricted sleep length. Similar findings were shown following a single night of fragmented sleep in chapter 5.

The lack of any age effect on hypoxic sensitivity was also reinforced in Chapter 6, whereby no differences in hypoxic sensitivity slopes were found between the two age groups. Data was represented as both $\Delta Ventilation/\Delta SaO_2$ and $\Delta Ventilation/\Delta \log P_aO_2$, to avoid any possible confounding influences potential changes in the oxygen saturation curve with age could have on the interpretation of hypoxic sensitivity when represented as a change in SaO_2. The null finding here would suggest that no such influences occur with age. However, the age range of this particular older group could still be considered young in comparison to a population aged 70+ years, in which such changes in the oxygen dissociation curve and hypoxic sensitivity could occur.

The unchanged hypoxic sensitivity across the age groups reported in this thesis would appear to rule out the possibility of the increased prevalence of SDB with age being caused by hypoxic ventilatory sensitivity. Therefore, if respiratory control does have a role in the increased prevalence it is most likely to be brought about through the changes in central chemosensitivity to CO_2.

7.1.1 Central chemosensitivity and sleep apnoea

The changes in central chemosensitivity observed in this thesis could contribute to the development of SDB. Reductions in chemosensitivity with age could lead to the eventual development of SDB in older individuals, thus accounting for the increased prevalence of

SDB in this population. Also, the reduction in central chemosensitivity caused by sleep loss could play a role in the observed increase in severity of SDB in patients who have been deprived of sleep. Lastly, a decrement in central chemosensitivity following sleep fragmentation could account for the progressive worsening of SDB throughout the night and over time.

Evidence originating from a number of studies and summarised in Smith *et al.* (2010), would suggest that central chemosensitivity plays little role in the development of central apnoeic events. Central apnoeas are a prominent feature of the increased occurrence of SDB with age. Therefore, it would seem unlikely that a reduction in central chemosensitivity with age contributes to the greater prevalence of SDB in older individuals. However, a reduction in the central chemosensitivity could result in the demise of the interactional nature between the two chemoreceptors. This could cause instability in nocturnal ventilation and central apnoeas to occur.

Although central chemosensitivity may not have a causal role in central apnoea development, it may still contribute to the development of obstructive events. A decline in central chemosensitivity with age would affect the central chemoreceptor modulation of upper airway muscle tone. Such an affect would account for the increased vulnerability of airway collapse in older individuals previously reported by Eikermann *et al.* (2007). This effect would cause more frequent obstructive apnoeic events to occur in older individuals. Also, a decrease in chemosensitivity could reduce the protective airway reflexes arising from the chemoreceptors, rendering the airway more vulnerable to collapse. The possible effects of attenuated chemosensitivity on upper airway muscle activity and reflex responses, are

supported by the recent research conducted by Chin *et al.* (2012). Chin and colleagues suggested a reduced chemosensitivity leads to a greater risk of airway collapse and thus greater severity of SDB.

In chapter 4 it was shown that despite the decline in central chemosensitivity in sleep deprived individuals, no apnoeas or hypopnoeas occurred. Despite these observations, it does not exclude the possibility that in those who suffer from SDB, a decrease in central chemosensitivity contributes to the progression of the condition. This data also lends weight to the suggestion that the effects of age on chemosensitivity may have little contribute to the increased prevalence of SDB. However, as with the condition of SDB, additional residual effects of ageing such as, reduced airway muscle strength and impairment of pharyngeal sensory activity could, along with impaired central chemosensitivity, lead to the development of SDB.

An additional effect of reduced central chemosensitivity, which may lead to a greater occurrence of apnoeas and would affect the severity of the apnoeas themselves, is a reduction in the arousal response to hypercapnia. An attenuated arousal response to hypercapnia with age would augment the length of the apnoeic events, leading to more severe hypoxemia. However, data presented by Eikermann *et al.* (2007) suggests no difference in arousal threshold between younger and older volunteers, but arousal threshold was measured in that study via the magnitude of the negative pressure within the epiglottis at the time of arousal. Such an arousal is brought about by the lung and airway mechanoreceptors, which are stimulated by the persistence of inspiratory drive during an obstructive apnoea. Therefore, it is still possible that an alteration in hypercapnic arousal

threshold with age could still occur. Such an occurrence would prolong central apnoeas, where inspiratory drive and therefore mechanoreceptor stimulation is absent.

Also, apnoeic events, characterised by short pauses in ventilation, are common occurrences even in healthy sleeping individuals. These events may not be classed as apnoeas relating to SDB, because they are short in length, less than the clinical threshold of 10 seconds for an apnoeic event, and because they do not cause a clinical desaturation (a decrease in oxygen saturation of ≥4%). However, a decline in the arousal response to hypercapnia could evolve these normally short pauses in ventilation into clinical apnoeic events by augmenting their length to greater than 10 seconds long and thus causing a ≥4% decrease in oxygen saturation. This evolution of pauses in ventilation into clinical apnoeic events by a reduction in arousal threshold could be what is causing an increased level of central apnoeas in older individuals.

Observations in chapters 4 and 5 would suggest the reduction in central chemosensitivity caused by the disturbance to sleep could be one possible mechanism for the worsening of SDB throughout the night and over time. A decrement in the ability to respond to a hypercapnic stimulus during an apnoeic event could lead to the lengthening of apnoeic events in sleep apnoea patients leading to more severe hypoxemia. This suggestion has been recently highlighted by Li *et al.* (2014) and explain in greater detail in chapter 5.

7.1.2 Accelerated ageing

Results from this thesis suggest that disruption to sleep and advancing age result in similar alterations in chemosensitivity, with a reduction in central chemosensitivity being demonstrated in each study. This similarity in the nature of altered chemosensitivity

following the disruption of sleep raises the possibility that sleep disruption, in young individuals, could be used as a model of accelerated ageing. Previous investigations have also suggested sleep loss as a model of accelerated ageing. An epidemiological study conducted by Ferrie *et al.* (2011) showed a decline in cognitive function in individuals reporting shorter sleeping hours. The cognitive dysfunction in these individuals was comparative with 4 – 7 years of increased age. It could be argued that this observation of accelerated ageing was caused by a reduction of sleep length over a chronic period of years and not as an effect of acute sleep loss, as used in this thesis. However, an earlier study by Spiegel *et al.* (1999) reported a similar model of accelerated ageing following just six nights of restricted sleep length. The authors reported impaired insulin sensitivity in young healthy sleep restricted individuals. The decrement of insulin sensitivity in these young participants was comparable to older healthy individuals. More recent and striking evidence for the accelerated ageing model, is the greater appearance of cellular ageing in individuals that reportedly sleep less (Jackowska *et al.*, 2012). Telomere length is the length of the protective strand of nucleotides located at the end of each chromosome. Following each replication of a DNA strand the length of the telomere becomes shorter. Therefore, the measured length of the telomere can give an indication of cellular age. Jackowska *et al.* (2012) has shown that individuals reporting sleeping periods of five hours or less have a shorter telomere length than those sleeping a normal period of seven hours, regardless of age. The data in this thesis would also support this concept of accelerated ageing with sleep loss, given the similar reductions in central chemosensitivity with both age and sleep loss. Data presented in chapter 5 suggests that sleep fragmentation can also be deemed a model of accelerated ageing.

7.1.3 Aged associated sleep disturbance and chemosensitivity

Ageing itself is characterised by a greater occurrence of sleep disruption, with both shorter sleep and more fragmented sleep being reported. It is reasonable to suggest that the sleep loss and fragmentation experienced could contribute to the alterations in chemosensitivity with age shown in this thesis.

However, the sleep disruption simulated in this thesis is acute, either three nights of reduced sleep or one night of fragmented sleep. Sleep disruption in older individuals is a chronic occurrence over many years. Furthermore, the disruption of sleep with age could be considered to be a natural occurrence and not a voluntary deprivation of sleep, as is the case in this thesis. One feature of the sleep disruption and sleep loss is a greater level of sleepiness, as is demonstrated in this thesis and by others (Bonnet, 1986; Magee *et al.*, 1987). However, daytime sleepiness is seen to decrease with age when assessed subjectively with questionnaires (Bixler *et al.*, 2005). This decrement in daytime sleepiness level would suggest that the form of sleep disruption experienced with advancing age is different to that experienced in this thesis. However, an alteration in the perception of sleepiness, as an adaptive consequence of the greater occurrence of sleep disruption in older individuals, could explain the reduction in daytime sleepiness with age. A more objective measure of sleepiness is a multiple sleep latency test, whereby the latency of sleep onset is measured across a number of daytime napping periods. Previous research, using this test, has reported that age is not associated with a greater occurrence of daytime sleepiness (Hoch *et al.*, 1992), therefore neglecting the suggestion of an alteration in perception as a cause. Also, this further suggests that ageing is associated with a different form of sleep disruption than that used in this thesis. One other mechanism for the similar levels of sleepiness over the

age groups could be that older individuals experience a greater level of daytime napping (Buysse *et al.*, 1992). An increased level of daytime napping could result in a lower level of sleepiness in older individuals, as a greater recovery from the accumulated sleep loss is made. If age-related sleep disturbance were to have a causal role in the alterations in respiratory chemosensitivity seen with age, the mechanism for these alterations and the changes in chemosensitivity shown following sleep disruption should be mutual. One possible mechanism that could be responsible for the decrement of central chemoreception in both circumstances is the effect CBF can have on $[H^+]$ within the cerebrospinal fluid. An augmentation in cerebrovascular reactivity to hypercapnia reduces cerebrospinal fluid $[H^+]$, thus reducing the magnitude of central chemoreceptor drive. However, as previously described in chapter 3, ageing is associated with a reduction in the cerebrovascular reactivity to hypercapnia. Also, no alterations in the responsiveness to hypercapnic of the cerebral vasculature were seen following sleep fragmentation in chapter 5. Therefore, it seems unlikely that an indirect effect of cerebral blood flow or *mixer gain*, was responsible for the reduction in central chemosensitivity. Therefore, it seems a more central, *controller gain,* mechanism could be at play in the attenuated central chemosensitivity.

A common mechanism suggested in all the chapters regarding chemosensitivity, is an inhibition or degradation of chemosensitive orexin neurons. In chapters 4 and 5 it was postulated that the reported accumulation of adenosine within the brain following prolonged wakefulness and fragmented sleep (Porkka-Heiskanen *et al.*, 1997), acted on adenosine A1 receptors expressed on orexin neurons (Thakkar *et al.*, 2002) and in turn reduced central chemosensitivity. This hypothesis is supported by recent evidence by Liu *et al.* (2011) reporting a revival of the reduced HCVR caused by sleep fragmentation following

the inhibition of adenosine A1 receptors. It was also hypothesised, given the role of adenosine in SWS (Bjorness & Greene, 2009), that the reduction in SWS activity in chapter 5 would result in the accumulation of adenosine, which would act upon orexin neurons, reducing central chemoreception.

Given this suggested role of orexin in the decline of central chemosensitivity following disrupted sleep in this thesis, it can also be considered that the greater occurrence of sleep disruption and also decline in SWS activity with age would result in similar effects.

7.1.4 Orexin and Ageing

In chapter 3 it was described that the decrement in chemosensitivity with age appeared to be state dependent, with previous research reporting that the reduced chemosensitivity in older individuals is seen during wakefulness but during sleep (Naifeh *et al.*, 1989; Browne *et al.*, 2003). Given that the work in this thesis has shown that the reduction in chemosensitivity with age is centrally driven, it was postulated that the state dependent observations of previous research would suggest an alteration in the state dependent orexin chemoreceptive neurons in older individuals.

Further evidence for a decrease in orexin neuron activity with age has been reported in animal models. A decline in the measured orexin receptor messenger RNA has been reported to occur in older mice (Terao *et al.*, 2002). This observation is supported by additional research showing a decline in orexin peptide gene expression within the lateral hypothalamus of older rats (Porkka-Heiskanen *et al.*, 2004). Later research has shown a 40% reduction in the orexin neurons within the lateral hypothalamus region, in rats (Kessler *et al.*, 2011). In addition to a loss of neurons within the lateral hypothalamus, more recent

research has reported a decline in the modulation activity of orexin neurons in older rats (Stanley & Fadel, 2012). Despite these observations, human research is limited because of the clear obstructions to such measurements in human participants. Nevertheless, the measurement of orexin levels in the cerebrospinal fluid, via lumbar puncture, can give an insight into the levels of orexin within the brain. Kanbayashi *et al.* (2002) assessed cerebrospinal fluid orexin levels across the age span, from 0-79 years but found no differences driven by age. This result demonstrates that the occurrences of reductions in orexin levels and function seen in older rats may not occur in humans. However, it must be highlighted that the measurements made by Kanbayashi and colleagues are just an index of the levels within the brain and do not portray specific brain regions, as can be done in animal models. Furthermore, the assessment of orexin levels does not provide information of functional changes of the neurons themselves, with age.

Further indirect evidence of reduced orexin function with age can be extrapolated from reductions in other functions associated with orexin neurons. The orexinergic system has been researched widely over the last 10 years and has been shown to play a role in numerous physiological functions (Kukkonen, 2013; Leonard & Kukkonen, 2014). Such functions include sleep and wakefulness control, sympathetic activation in response to stress, and appetite control. An alteration in these functions with age would indirectly suggest a decline in orexin activity. However, it must be voiced that additional and redundant controllers could also work to alter these functions.

Ageing is associated with changes in food intake (de Boer *et al.*, 2013), characterised by a reduction in calorie intake (Briefel *et al.*, 1995) and a lower appetite (Clarkston *et al.*, 1997)

in humans. Injection of orexin into the hypothalumus increases food intake in rats (Sweet *et al.*, 1999). Further research has shown this feeding response to orexin injection, to be reduced in older animals (Kotz *et al.*, 2005). These observations lend evidence to the reduction of orexin neuron function with age.

Orexinergic neurons are also integral in the regulation of wakefulness and sleep (Sakurai, 2007). Such control is evident in its most extreme in the condition of narcolepsy, where orexin is absent (Nishino *et al.*, 2000). As has been described previously, ageing is associated with debilitative changes in sleep regulation. However, it would be assumed that a reduction in orexin neurons with age would yield symptoms in older individuals that are representative of narcolepsy. Such a symptom would include excessive daytime sleepiness (Overeem *et al.*, 2001), a symptom that, as previously described, is not apparent in older individuals. Nevertheless, additional characteristics of narcolepsy do have parallels with healthy ageing, such as an increased frequency of nocturnal arousals from sleep, greater sleep disturbance and lower SWS activity (Overeem *et al.*, 2001; Roth *et al.*, 2013). These similarities in sleep architecture between narcoleptics and older individuals lend further weight to the possibility of orexin neuron degradation with healthy ageing, which could affect central chemosensitivity.

Given that orexin seems to decrease with age and orexin deficiency also appears to cause sleep disruption, does the prior hypothesis that increased sleep disruption with age is a cause of reduced chemosensitivity still stand? Or is the reduction in orexin with age resulting in both an increased level of sleep disruption and a reduced chemosensitivity in older individuals? Recent data by Li *et al.* (2014) would suggest that chronic sleep fragmentation

can have a long term debilitative effect on orexin neurons. Given this observation, it could be suggested that a vicious cycle of cause and effect occurs, whereby inhibition or neural degradation of orexin neurons causes increase sleep fragmentation, which in turn would lead to a further inhibition or degradation of orexin neurons. Therefore, the attenuation of orexin neuron activity and the increased disturbance of sleep with age could have equally important contributions to the reduction of hypercapnic chemosensitivity in older individuals reported in this thesis.

7.1.5 Orexin and Sleep Apnoea

If the reductions in chemosensitivity shown in this thesis are mediated by a decline in orexin function it would seem unlikely that such an alteration following sleep disruption or with age would contribute to the development of SDB. This is because orexin neurons appear to be state dependent, active during wakefulness and inhibited during sleep, and therefore would have little effect on respiratory control during sleep. However, there is evidence to suggest that orexin neurons are still active during sleep in a sleep stage dependent manner and can influence the cycling of sleep stages during the night (Kiyashchenko *et al.*, 2002). Furthermore, data presented by Dias *et al.* (2009) did show that orexin inhibition during sleep had a small attenuating effect on HCVR of 9%, suggesting that orexin neurons, are still active during sleep. If orexin neurons do influence respiratory control during sleep and have an effect on the development of SDB, one would hypothesise that orexin deficiency may affect respiratory control during sleep. Research conducted by Han *et al.* (2010) compared the appearance of SDB in 130 narcoleptic patients with 117 healthy controls and found a greater AHI level in the narcoleptic patients than in controls. However, the mean AHI within

the patient population was still below clinical levels, 2.8 $hour^{-1}$. Nevertheless, there was a much greater number of mild and moderate SDB sufferers in the narcoleptic patients than in the control group. Previous research by Nakamura *et al.* (2007) supports these findings, as the authors noted a development of sleep apnoea in orexin defiant mice. These observations do suggest a possible link between reduced orexin neuron activity and SDB, which may be linked with an alteration in respiratory control.

However, contradicting data from the study conducted by Han *et al.* (2010) suggests that orexin deficiency in these patients did not affect the HCVR during wakefulness. A reduced HVR in narcoleptics was also reported in that study. Such a finding goes against all previous findings in animals demonstrating chemoreceptive characteristics of orexin neurons that affect the HCVR during inhibition. It is suggested by Han (2012) that a possible species difference occurs between humans, and mice and rats, whereby orexin neurons are not sensitive to CO_2. Also, the finding of unaltered HCVR in orexin deficient patients by Han and colleagues would seem to go against the suggestion that orexin inhibition/degradation is a possible mechanism for the reduced central chemosensitivity in older individuals presented in this thesis. If orexin neurons inhibition does occur with age and plays a role in the reduced hypercapnic sensitivity seen in this thesis it could be suggested that the neurons play little role in the hypoxic sensitivity, as this has been shown in this thesis and by others to be unaffected by age. However, Han *et al.* (2010) reported a reduction in HVR in their narcoleptic patients, suggesting that orexin neurons do in fact influence hypoxic sensitivity. This finding would seem to further hinder the possibility of orexin neurons being inhibited or degraded with age. However, it should be highlighted that in the study conducted by Han and colleagues a Read's re-breathing test was used to assess hyperoxic hypercapnia. In

addition all the caveats highlighted previously in this thesis, in comparison to other techniques the re-breathing test has been shown to continuously overestimate the steady state CO_2 sensitivity of the participants tested (Berkenbosch *et al.*, 1989; Mohan *et al.*, 1999). These caveats and the different chemosensitivity assessment could account for the finding of similar HCVR levels between narcoleptic patients and healthy controls by Han *et al.* (2010). Furthermore, differences in cerebral perfusion in narcoleptic patients when compared to healthy controls (Yeon Joo *et al.*, 2005), could account for similarities in measured chemosensitivity levels observed by Tan and colleagues.

In summary, this thesis has shown, without the use of possible confounding hyperoxia, that ageing is associated with a reduction in central chemosensitivity. Similar alterations in chemosensitivity were observed following two forms of sleep disruption, fragmented sleep and reduced sleep length. These similarities in changes in respiratory control support findings of previous research, which suggest a sleep disruption as a model of accelerated ageing. Furthermore, the similar alterations point to a possible mutual mechanism. A possible mechanism put forth in this thesis is the inhibition and/or degeneration of orexin neurons following sleep disruption and in association with advancing age. It is still unclear whether these changes in chemosensitivity with age contribute to the increased prevalence of SDB in older people. It is reasoned that if reductions in central chemosensitivity do contribute to the increased prevalence of the disease, it is most likely via its influences on upper airway tone and arousal thresholds.

7.1.6 Future directions

In light of the findings in this thesis a number areas of further research would be suggested. Firstly, research should investigate further the cause of reduced chemosensitivity in older individuals. The research in this thesis has suggested the increased sleep disruption with age, as one possible mechanism. A simple investigative study assessing the relationship between the degree of sleep disruption and chemosensitivity levels, both central and peripheral, in older individuals would shed light on this hypothesis. A therapeutic investigation attempting to improve the quality of sleep in older individuals and assessing its affect on respiratory chemosensitivity would further test the hypothesis.

On the topic of SDB in the elderly, a study attempting to augment respiratory control during sleep in older individuals with reduced chemosensitivity, could be conducted. This would provide important information on the role of attenuated chemosensitivity in the pathophysiology of apnoeic events in older individuals. Such studies have been previously conducted, however not in older individuals (Stanchina *et al.*, 2002; Giannoni *et al.*, 2010; Xie *et al.*, 2013). Furthermore, age differences in the arousal threshold to hypercapnia would provide further insight into a possible mechanism for increased apnoeas in older individuals.

A study using more reliable techniques should be completed to assess chemosensitivity, during wakefulness and sleep, in narcoleptic patients. In addition to chemosensitivity, the response of upper airway muscles to chemoreceptor stimulation via hypercapnia in these patients would provide further information into the possible role of orexin neurons in the development of SDB. The assessment of cerebrovascular reactivity in narcoleptic patients

would rule out any confounding factors on the assessment of central chemosensitivity, arising from changes in CBF.

7.2 Vascular observations

7.2.1 Brachial vascular function

Previous research has shown reductions in vascular function following a night of total sleep deprivation in shift workers (Amir *et al.*, 2004) and experimental participants (Sauvet *et al.*, 2010). The data presented in this thesis has shown that shortened sleep length over three consecutive night results in a biphasic alteration in vascular function, an observation not reported previously. These findings could have implications in our modern society where sleep is becoming progressively shorter and regarded as less important (Bonnet & Arand, 1995). Alterations in vascular function following reduced sleep length could act as a mechanism for the greater occurrence of cardiovascular disease in those reporting shorter sleep length (Cappuccio *et al.*, 2011). Also, an increased occurrence of sleep disruption in SDB patients could contribute to the impaired vascular function seen in these patients. Another important observation from the data presented in chapter 4 is that acute sleep loss through reduced sleep length across two days is enough to affect vascular function. This suggests that chronic sleep loss is not necessary to cause vascular dysfunction, the loss of sleep over just a few days could have implications for health. Eventful weekends, prolonged working hours and striving for tight deadlines are such examples where acute sleep loss can occur and that are becoming more prevalent in our modern society.

The possible mechanisms for these observed changes in vascular function have been described previously in Chapter 4. However, future research should be directed at these

possible areas of causality, particularly in the recovery of vascular function following a third night of sleep loss. Additionally, more chronic models of controlled sleep loss could be implemented to investigate whether the attenuated response and its recovery to baseline is maintained or if further dysfunction occurs due to augmented sleep loss.

7.2.2 Cerebrovascular function

Research in chapter 5 investigated the impact of sleep fragmentation on cerebrovascular function by assessing cerebrovascular reactivity to a hypercapnic challenge. The primary purpose of which was to assess whether a reduction in central chemosensitivity would occur in conjunction with a counterintuitive reduction in cerebrovascular function. The null finding in cerebrovascular reactivity following sleep fragmentation suggested no paradoxical relationship between CBF and reduced central chemosensitivity following sleep fragmentation. However, it is possible that alterations in reactivity of cerebral blood vessels more local to central chemoreceptive sites within the brain could still occur. Such vessels would include the posterior and basilar cerebral arteries. These arteries can be insonated with TCD, therefore it would be suggested that further research investigating the effect of sleep fragmentation on respiratory chemosensitivity also include these measurements.

The findings also suggest that sleep fragmentation may not play a role in the reported reduction in cerebrovascular reactivity in SDB patients (Qureshi *et al.*, 1999; Durgan & Bryan, 2012). A chronic exposure to intermittent hypercapnia in sleep apnoea patients could also account for the reduced reactivity. However, this debilitative characteristic of cerebrovascular function in SDB patients has been suggested not to occur (Foster *et al.*, 2009; Ryan *et al.*, 2014), as previously described. Nevertheless, additional research should

investigate the effect of chronic intermittent hypercapnia on cerebrovascular reactivity and its possible effect in SDB.

7.2.3 Pulmonary vascular function

Lastly, a further novel finding presented in this thesis is the increase in pulmonary vascular reactivity to hypoxia in older participants. This finding does provide a possible mechanism for the association between advancing age and occurrence of pulmonary hypertension in sufferers of SDB reported by Atwood *et al.* (2004). This is also evidence to suggest that older individuals are at greater risk of developing cardiovascular diseases as a consequence of SDB, than younger individuals.

Also, an increase in pulmonary artery pressure during normoxia was shown in study 4. It is argued that this is a result of a stiffening of the pulmonary vessels with age, which would reduce the compliance of the vessels to blood flow. A reduction in the compliance of the pulmonary circulation with age could have an effect on the exercise capacity of older individuals. Further research should investigate the possibility of the changes in the pulmonary circulation with age being a limiting factor to exercise capacity.

Additionally, future research should be directed towards the possible mechanisms involved in the reported sensitisation of the HPV response in older individuals. These mechanisms have been described in detail in chapter 7. Therapeutic trials involving both anti-oxidant treatment and iron supplementation could shed further light on the potential mechanisms involved and help ameliorate the risks of developing pulmonary hypertension in older individuals.

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Appendix 1 – MFBS model (difference equations)

The set of difference equations shown below is used to solve the two compartment model ventilation presented in the general methods chapter, assuming that $P_{ET}CO_2$ remains the same throughout the breath.

$$(\dot{V}_c)_{n+1} = G_c\left[P_{ET_{CO_2}}\ (t_n - d_c) - B\right] - \left\{G_c\left[P_{ET_{CO_2}}\ (t_n - d_c) - B\right] - (\dot{V}_c)_n\right\} \cdot \exp\left[\frac{t_n - t_{n+1}}{T}\right]$$

$$(\dot{V}_p)_{n+1} = G_p\left[P_{ET_{CO_2}}\ (t_n - d_p) - B\right] - \left\{G_p\left[P_{ET_{CO_2}}\ (t_n - d_p) - B\right] - (\dot{V}_p)_n\right\} \cdot \exp\left[\frac{t_n - t_{n+1}}{T}\right]$$

$$(\dot{V}_E)_{n+1} = (\dot{V}_c)_{n+1} + (\dot{V}_p)_{n+1} + Ct_{n+1}$$

The two compartment model is used assess peripheral and central chemoreflexes, where ($\dot{V}_E$) is total ventilation, ($\dot{V}_c$) and ($\dot{V}_p$) are the central and preiperhal chemoreflex output, respectively. C is a trend term and *t* is time. G_c is the central chemoreflex sensitivity, d_c is the central transport delay time, G_p is the peripheral chemoreflex sensitivity, d_p is the peripheral transport delay time and B is a bias term equivalent to the $P_{ET_{CO_2}}$ for which $\dot{V}_E$ equals zero.

Appendix 2 – Bernoulli's equation

The modified Bernoulli's equation, equation 17 below, is used in Doppler echocardiocariograph to calculate pressure.

$$\Delta P_{mmHg} = 4v^2 \quad \textbf{(17)}$$

Where, ΔP is pressure measured in mmHg and v is velocity.

This equation has been derived from the Bernoulli's original equation, equation 18 below, where pressure is measured in pascals (Pa) and ρ is the fluid density:

$$\Delta P_{Pa} = \frac{1}{2}\rho v^2 \quad \textbf{(18)}$$

$$1mmHg = \frac{1000}{7.5} Pa \quad \textbf{(19)}$$

The original Bernoulli equation was modified to equation 17 using the steps below. This was achieved by converting Pa into mmHg using the conversion formula expressed above, equation 19, and the assumption that the density of blood was $1.067 \times 10^3 kg/m^3$.

$$\Delta P_{Pa} \times \frac{1000}{7.5} = \frac{1}{2}\rho v^2 \Rightarrow \Delta P_{mmHg} = \frac{7.5}{1000} \times \frac{1}{2} \times 1.067 \times 1000 \times v^2$$

$$\Delta P_{mmHg} = \frac{7.5 \times 1.067}{2} \times v^2 \Rightarrow \Delta P_{mmHg} = 4v^2$$

Appendix 3 – HPV model

To assess the SPAP steady state responses to hypoxia and make comparison between the two age groups a model was fitted to the data. The model is given below:

$$y_{n+1} = \left[(1-a)\cdot M_1 + a_1 \cdot \left(M_2 + \frac{(M_1 - M_2)}{(P_1 - P_2)} \cdot \left(P_{O_{2(n)}} - P_2\right)\right)\right] -$$

$$\left\{\left[(1-a_1)\cdot M_1 + a_1 \cdot \left(M_2 + \frac{(M_1 - M_2)}{(P_1 - P_2)} \cdot \left(P_{O_{2(n)}} - P_2\right)\right)\right] - y_n \right\} \cdot exp^{(-0.5/\tau)} \quad \textbf{(20)}$$

Where, M_1 and M_2 are constants of the asymptotes for the baseline euoxic period and the hypoxic stimulus period, respectively. τ represents the time constant for the asymptote. P_1 is the mean O_2 stimulus during the euoxic period and P_2 is the mean stimulus during the hypoxic period. a_1 is a switch that alternates between 0 (euoxia) and 1 (hypoxia) in order to apply the model on the dataset. To solve the equation Matlab software was used.