GLOSSARY OF ABBREVIATIONS

AHI	Apnoea hypopnoea index
AI	Arousal index
BMI	Body mass index
CPAP	Continuous positive airway therapy
CT	Computed tomography
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
$ETCO_2$	End-tidal CO_2
ECG	Electrocardiography
FRC	Functional residual capacity
MRI	Magnetic resonance imaging
non-REM sleep	Non rapid eye movement sleep
OSA	Obstructive sleep apnoea
$PaCO_2$	Arterial CO_2
P_{CRIT}	Upper airway critical closing pressure
PSG	Polysomnography
REM sleep	Rapid eye movement sleep
RV	Residual volume
T_E	Duration of expiration
TP	Tensor palatini muscle

CHAPTER 1. Introduction

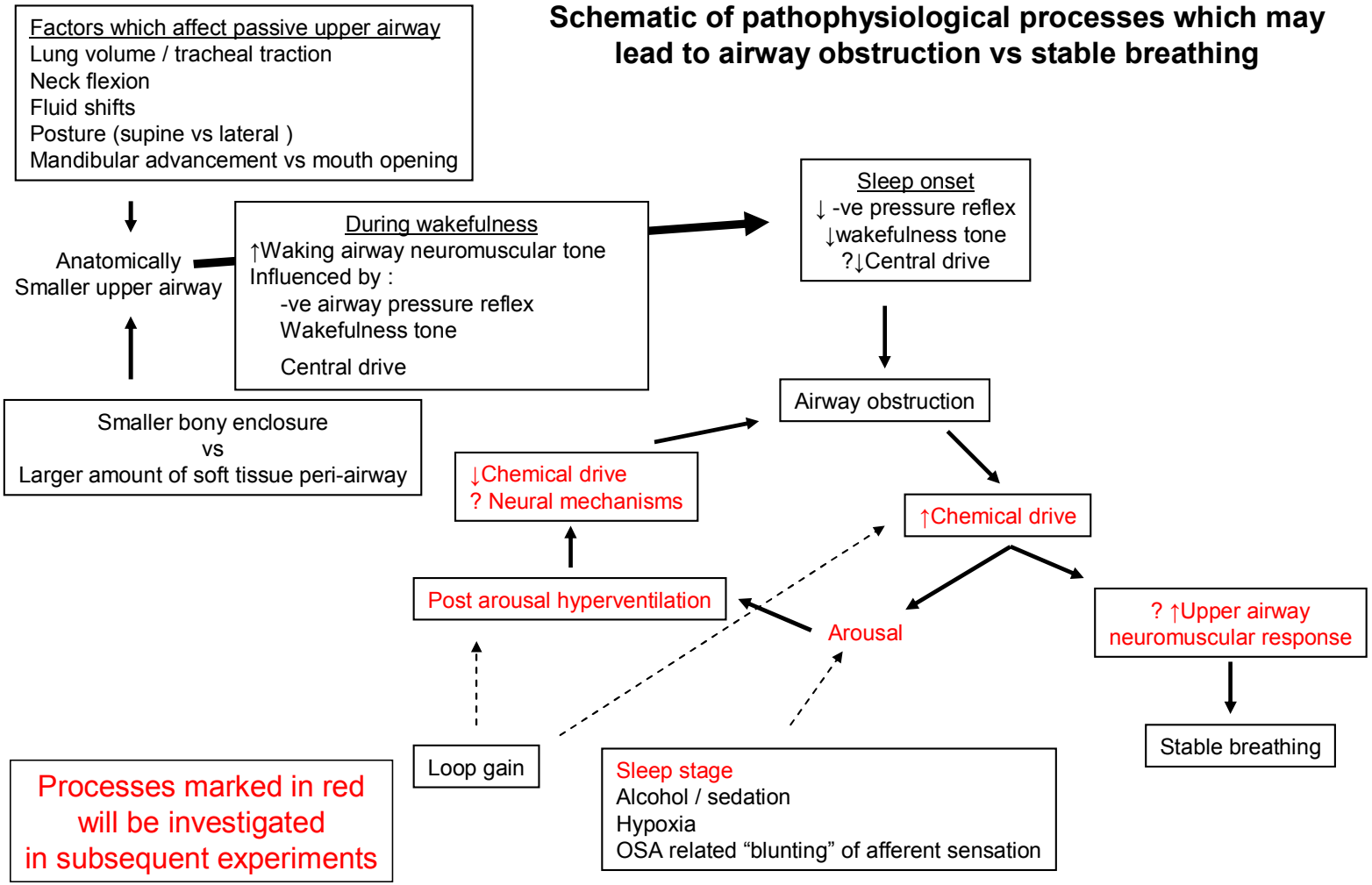
Obstructive sleep apnoea (OSA) is characterized by recurrent complete or partial collapse of the upper airway[1]. These episodes are associated with progressively increasing efforts to breathe due to increasing drive, and frequently culminate in arousal from sleep[2]. These periods are associated with recurrent episodes of hypoxia, hypercapnia, significant fragmentation of sleep and surges in sympathetic tone[3], reduced left ventricular stroke volume[4] and increased cardiac afterload[5].

The community prevalence of OSA syndrome (laboratory evidence of OSA, with associated daytime sleepiness), assessed by polysomnography is thought to be 4% for males and 2% for females[6]. This is likely to have progressively increased since that study in the early nineties, because of the increasing prevalence of obesity[7]. While several methods are utilized to make a diagnosis of OSA, the gold standard test is currently considered an overnight polysomnography (PSG) in a sleep laboratory[8]. This involves measurement of several variables including electroencephalography (EEG), electrocardiography (ECG), electromyography (EMG), electrooculography (EOG), snore sensor, airflow, oximetry, thoracic and abdominal excursion and leg movements among others. The recordings are then analysed by a technician using pre-determined rules for assessing sleep stages[9], arousals[10] and respiratory events[11].

Long term consequences of OSA include reduced quality of life[12], daytime sleepiness, increased risk of motor vehicle[13, 14] or occupational accidents[15], hypertension[16-18], cerebrovascular disease[19-21] and cardiac complications including left ventricular hypertrophy[22], atrial fibrillation[23], and possible nocturnal sudden cardiac death[24]. Although not proven by randomized controlled treatment studies, there is some observational evidence to suggest that long term continuous positive airway pressure (CPAP) therapy is associated with a reduction in overall cardiovascular risk[25, 26].

Major risk factors for OSA are male gender[6], increased body mass index (BMI)[6], neck circumference[6, 27] and age[27, 28]. The pathogenesis of obstructive sleep apnoea in a particular patient involves a complex interplay between several factors including; impaired upper airway anatomy, a reduction in neuromuscular activity of the genioglossus and other upper airway dilator muscles at sleep onset, reflex activation of the genioglossus to negative upper airway pressure, central drive to the upper airway vs respiratory muscles, the respiratory arousal threshold and loop gain, which is an engineering term defined as the ratio of the magnitude of the response to a perturbation relative to the magnitude of the perturbation itself. Loop gain in turn is composed of a number of components; controller gain which reflects ventilatory chemosensitivity to hypoxia / hypercapnia, and plant gain which is the ability of the ventilatory system to ventilate, or clear CO_2. These are discussed in more detail further in this chapter.

Schematic of pathophysiological processes which may lead to airway obstruction vs stable breathing



1.1 Function of the upper airway

The upper airway includes the area from the nose, pharynx and larynx to the extrathoracic trachea. The area of interest as far as the pathology of sleep apnoea is concerned, extends from the nasopharynx to the epiglottis. In humans, the upper airway serves multiple functions: deglutition, speech and respiration. While respiration is possibly best served by a non-collapsible rigid tube, deglutition requires a collapsible muscular tube, with the ability to propel food and liquid boluses to the stomach. Speech requires rapid movements and changes in shape of various parts of the system under fine neuromuscular control. The evolution of the human upper airway has had to serve these multiple needs[29], and it is possible that the development of speech has been the strongest recent evolutionary drive. Various anatomical features of the human upper airway facilitate speech, including shortened maxilla and mandible, a 1:1 ratio of horizontal vs vertical supralaryngeal vocal tract, a descended larynx, shortened soft palate and loss of the “epiglottis / soft palate lock up” found in animals[29]. These features, particularly the shortening of the horizontal component of the upper airway unfortunately also facilitate the development of sleep apnoea[29].

1.2 Muscles of the upper airway

The upper airway has extensive and complex musculature with a mixture of respiratory, voluntary and reflex actions. These muscles have roles in dilating, and / or stiffening the upper airway[30] to facilitate ventilation, in addition to their other roles

in forming and propelling a food bolus, as well as speech. A brief summary of some of these muscles follows.

The upper airway muscles are histologically skeletal muscles. The upper airway muscles start at the nose and mouth. The dilator naris and levator alaeque (referred to as alar nasi muscles) are thought to dilate the nasal passages[31]. Jaw position is largely determined by masseter, medial and lateral pteryoid muscles[32]. Multiple dilator muscles act on the lips, the levator labii superioris, zygomaticus minor and major, levator anguli oris, depressor anguli oris and the lip sphincter orbicularis oris, to name a few[32]. These muscles act to fine tune the size of the mouth orifice. The soft palate is acted on by the tensor veli palatini, levator veli palatini, musculus uvulae, palatoglossus and the palatopharyngeus to coordinate oral vs nasal breathing routes[31]. The superior, middle and inferior constrictor muscles are located on the posterior and lateral walls of the pharynx. While they are predominantly used for swallowing, they do appear to have some respiratory activity[31]. The anterior wall of the upper pharynx is formed by the tongue. The tongue contains muscles classed as intrinsic and extrinsic. Intrinsic muscles are confined to the tongue and act to alter the shape of the tongue. Extrinsic tongue muscles include the genioglossus, hypoglossus, styloglossus and palatoglossus. The hyoid bone forms the anterior wall of the lower pharynx. This muscle is suspended by fascial and muscle attachments. The thyrohyoid, sternohyoid and the omohyoid attach inferiorly to the hyoid[32]. The geniohyoid, mylohyoid, hypoglossal stylohyoid and the digastric muscles attach anteriorly. Activity of these hyoid

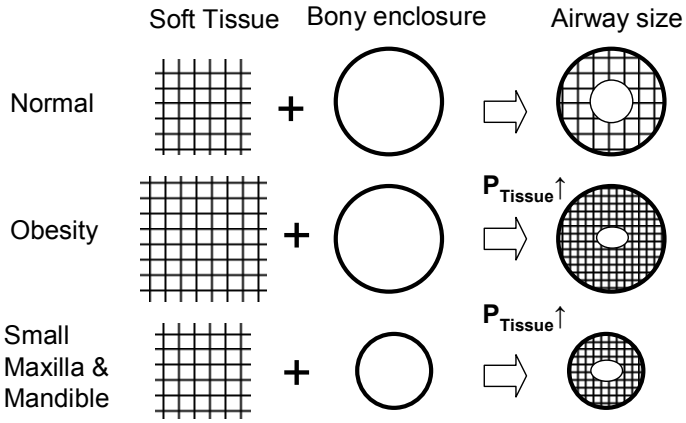
muscles may act to increase upper airway stability. Coordinated activity of these muscles facilitates patency of the upper airway and ventilation[31, 32].

1.3 Upper airway anatomy, impairment in OSA

The size of the upper airway lumen in patients with OSA has been assessed by multiple techniques during wakefulness and sleep, with and without controlling for dynamic lung volume. On clinical examination, patients with OSA have increased narrowing of lateral pharyngeal walls and relative tonsillar enlargement, compared to patients without OSA[33], after controlling for BMI. CT scanning in awake subjects, suggests that the upper airway lumen is significantly smaller in sleep apnoea patients compared to controls[34-36]. Bradley et al[37], using an acoustic reflection technique in awake subjects, noted that OSA patients had significant falls in pharyngeal cross sectional area when exhaling from functional residual capacity (FRC) to residual volume (RV). This parameter, measured by the same technique, was shown to increase following significant weight loss[38]. MRI scanning in awake subjects confirms a smaller upper airway lumen[39, 40]. A new technique using optical coherence tomography in awake patients with sleep apnoea and BMI/age matched controls suggests that patients with sleep apnoea have a smaller velopharyngeal cross sectional area[41]. Pharyngoscopy, performed under general anaesthesia with muscle paralysis in subjects under different levels of CPAP, suggests that patients with sleep apnoea have a smaller maximal area of both oro- and velo-pharyngeal airways compared to weight matched controls[42]. The overall conclusion from these studies is that the upper airway lumen is on average, significantly smaller in patients with OSA compared to controls.

Several factors could account for this. One anatomical model that has been proposed by Watanabe et al[43], is that the mandible and vertebral body of the spinal cord act as a rigid bony enclosure surrounding the upper airway and extraluminal soft tissue. The pressure of soft tissues inside this enclosure, thus acting on the passive collapsible upper airway is determined by the size of the enclosure vs the amount of soft tissue that it contains [43]. This model is shown in figure 1.1.

Figure 1.1 : Schematic for mechanical model of the pharyngeal airway



From Watanabe et al.[43]

Several lines of reasoning support this hypothesis. CT cephalometry suggests that patients with OSA tend to have a relatively retrognathic mandible and increased anteroposterior length discrepancy between maxilla and mandible[44]. While BMI accounts for the largest component of the variability in OSA severity as measured by 2% oxygen dip rate (r^2=0.26), X-ray cephalometric differences in bony facial structures, particularly involving horizontal maxillary length, contribute towards explaining a further 24% of the variance in OSA severity[45]. Sleep apnoea patients have significantly greater deposits of peripharyngeal fat[46, 47]. MRI studies in awake sleep apnoea subjects show increased volumes of peripharyngeal soft tissue structures, including tongue volume and lateral pharyngeal walls[40]. The fact that

these abnormalities are present in non-sleep apnoea siblings of patients with sleep apnoea suggests that they are not simply a consequence of sleep apnoea but might, at least in part, be genetically predetermined[48]. In a group of Japanese patients with OSA and controls with matched craniofacial dimensions, the tongue volume relative to the enclosing mandibular size, rather than tongue size alone, was associated with sleep apnoea severity[49]. While actual tissue pressure has not been measured in humans, these studies suggest that a smaller internal diameter of the upper airway, with attendant risks of OSA is more likely with a smaller bony enclosure and/or increased tissue volume inside this enclosure. In the adult sleep apnoea patient, increased tissue volume is likely to be secondary to accumulation of fat. One caveat associated with the Watanabe model is the fact that the mandible is not a complete enclosure in 3 dimensions, i.e the inferior boundary is open. Theoretically at least, some of the tissue pressure could be dissipated inferiorly.

In the context of this thesis, the term “impaired upper airway anatomy” is meant to suggest the smaller upper airway diameter of OSA patients compared to non-OSA controls, all other factors being equal. As discussed below, this is a somewhat overarching phrase, as the upper airway lumen can be affected by several factors external to the upper airway, including the respiratory cycle. In addition, as will be discussed subsequently, neuromuscular factors although considered under the heading “non-anatomical”, do influence the upper airway lumen.

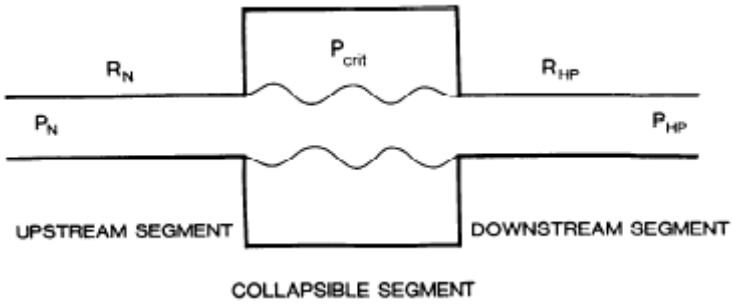
1.4 The passive upper airway during sleep

The upper airway has several biomechanical properties which affect airflow. They include resistance, compliance and collapsibility.

In a rigid tube, the resistance of the tube is inversely proportional to the radius to the 4th power (r^4). As described in chapter 1.3, the upper airway lumen in patients with sleep apnoea is significantly smaller than in non apnoeics. Thus, if the upper airway functions as rigid tube, resistance would be expected to be significantly higher in apnoeics.

However, the upper airway does not function as rigid tube. It is more accurately modelled as a compliant, i.e deformable section of airway, with rigid segments proximal and distal to this, otherwise known as a Starling resistor[50] (see fig 1.2). The compliance of the upper airway (or it's stiffness) has been demonstrated in static models, where the upper airway diameter has been measured as a function of airway pressure in subjects on CPAP. This has been performed under anaesthesia with muscle relaxation[42], as well as sleep, under sedation[51]. The compliance, defined as $\Delta A / \Delta P$ (change in upper airway diameter as a function of airway pressure) fits an exponential function curve, with the highest compliance near the closing pressure (or the pressure at which the upper airway cross sectional area is zero)[42, 51]. The compliance of the upper airway is higher in subjects with sleep apnoea, compared to those without[42].

Figure 1.2 : Schematic representation of the passive upper airway



From Schwartz et al.[52]

R_N = nasal resistance

R_{HP} = hypopharyngeal resistance

P_N = pressure at nose

P_{HP} = hypopharyngeal pressure

P_{CRIT} = critical closing pressure

When the airway pressure in the downstream segment is above that of P_{CRIT}, flow in the airway is characterized by the equation:

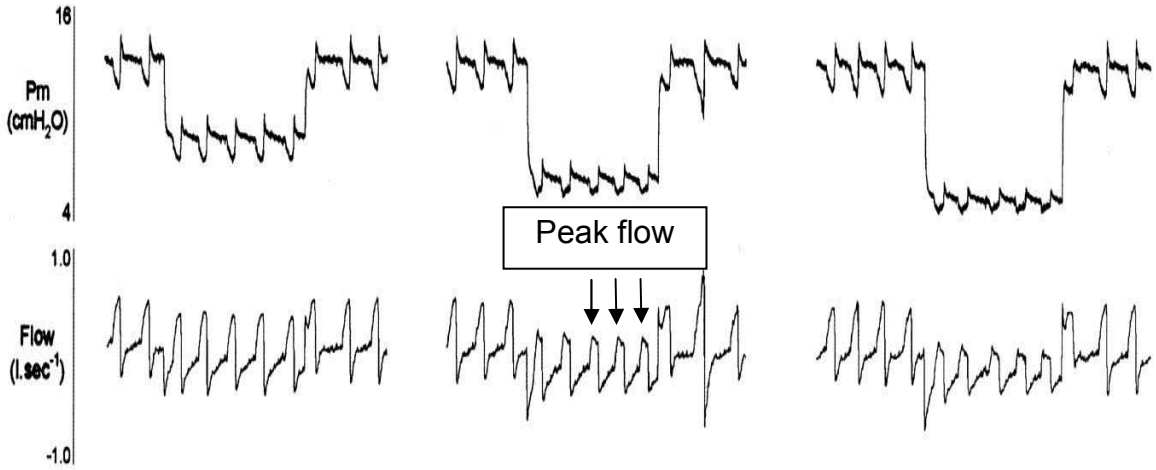
$$\dot{V} = \frac{P_N - P_{HP}}{R_N + R_{HP}}$$

When the airway pressure in the downstream segment drops below the critical closing pressure (P_{CRIT}), flow becomes independent of hypopharyngeal pressure and is characterized by the following equation:

$$\dot{V} = \frac{P_{upstream} - P_{crit}}{R_{upstream}}$$

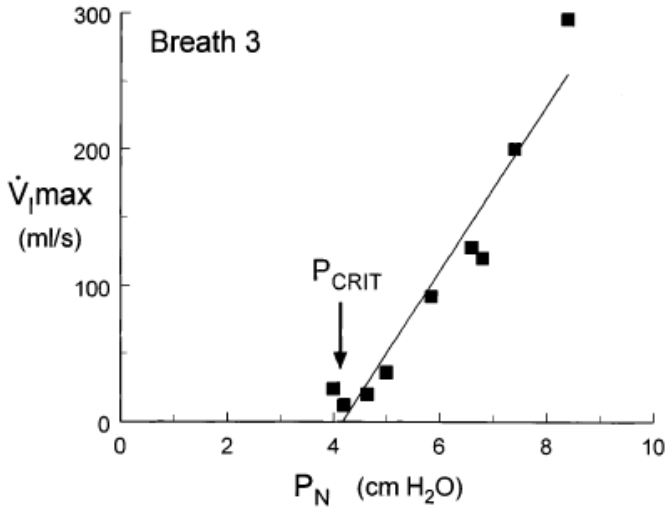
Operationally, P_{CRIT} is determined by rapidly dropping airway pressure from therapeutic CPAP, plotting a least squares regression line of mask pressure against inspiratory peak flow at several levels of sub-therapeutic CPAP pressure and solving for the mask pressure where 0 flow would occur, see figs 1.3 & 1.4. P_{CRIT} has been used as a measure of collapsibility, or the calculated airway pressure at which no inspiratory flow can occur.

Figure 1.3 : Measuring peak flow at different levels of airway pressure



From Eastwood PR et al.[53]

Figure 1.4 : Example measurement of P_{CRIT}



From Schwartz AR et al[54]

Studies using different levels of CPAP plotted against peak inspiratory flow suggest that this upper airway behaviour holds in subjects with and those without sleep apnoea. However the calculated P_{CRIT} is significantly elevated, and often positive in subjects with OSA[55], whereas it tends to be negative (i.e. subatmospheric) in subjects without OSA[56]. The same model does not apply in awake subjects, indeed the upper airway of both sleep apnoea patients and healthy volunteers when awake, is highly resistant to collapse by negative pressure[57]. This suggests that P_{CRIT} is likely to be the result of a combination of the peripharyngeal tissue pressure, mucosal tension within the airway wall, and residual upper airway neuromuscular dilatory activity. It is likely to be influenced by both the actual size of the upper airway orifice, as well as airway compliance, i.e. wall stiffness[30].

The length of the pharyngeal airway may also be an important contributor to collapsibility. One study using finite element modelling of the upper airway suggests that increased upper airway length in males contributes to increased collapsibility[58], potentially helping to explain the male preponderance to OSA.

1.4.1 Factors which influence the passive upper airway during sleep

1.4.1.1 Tracheal traction

Lung inflation is thought to increase upper airway diameter[59]. At least some of this effect is thought to be mediated by increased caudal traction at higher lung volumes. With neuromuscular paralysis in decerebrate cats, caudal traction of the trachea has beneficial effects on P_{CRIT} and peak flow[60]. In an in-vitro (i.e. without any surrounding tissue) preparation of cat upper and lower airway, increasing the longtitudinal tension decreases transmural pressure[61]. Even the tracheal traction caused by paced diaphragmatic breathing can reduce airway resistance (measured as the pressure drop across the upper airway at a constant flow) in anaesthetized tracheostomized dogs[62]. Tracheal traction may reduce upper airway collapsibility by directly acting on the upper airway mucosa, influencing tissue pressure, or both. In an anaesthetized rabbit model, graded tracheal traction reduced the measured tissue pressure, as well as upper airway collapsibility[63]. In a human study where lung volume was increased by means of an extrathoracic negative pressure shell during anaesthesia with muscle paralysis, increased lung volume significantly reduced upper airway closing pressure[64]. Likewise during sleep, the minimum CPAP pressure required to eliminate upper airway flow limitation was shown to be

reduced significantly after increasing lung volume by means of an extrathoracic negative pressure shell[65].

1.4.1.2 Neck flexion

Neck flexion in decerebrate cats increases upper airway collapsibility, an effect thought to be mediated by airway shortening[66]. In anaesthetized, non-paralysed[67] as well as paralysed[68] humans, neck extension has been shown to reduce and neck flexion to increase collapsibility of the upper airway[68]. Although it has not been demonstrated, this effect may be mediated by increased longtitudinal tension along the upper airway mucosa as well as reduced tissue pressure surrounding the collapsible segment of the upper airway[66].

1.4.1.3 Mandibular advancement / mouth opening

An anaesthetized rabbit model suggests that graded mandibular advancement also reduces the measured peripharyngeal tissue pressure and upper airway resistance[69]. Mouth opening also increases upper airway collapsibility in paralysed anaesthetized subjects[68]. In theory, mouth opening causes retrograde movement of the mandible, causing a reduction in size of the bony enclosure.

1.4.1.4 Posture

In paralysed subjects under anaesthesia, the cross sectional area of the upper airway viewed endoscopically is significantly smaller in the supine compared to the

lateral position[70]. However in awake subjects, no difference in the upper airway cross sectional area was noted between the supine and lateral postures using an acoustic reflection technique[71]. Similarly in a recent study using optical coherence tomography in awake controls and age / BMI matched patients with OSA, overall airway cross sectional area was not different between supine and lateral postures. Airway shape however altered from a transversely oriented ellipse in the supine posture, to a more circular shape in the lateral posture[72], which the authors postulate may be more resistant to collapse. Differences in the diagnostic techniques used and sleep vs wakeful state may account for these discrepant results.

1.4.1.5 Fluid shifts

In awake healthy subjects, causing body fluid shifts by applying positive pressure to the thighs and legs via anti-shock trousers produces a small increases in collapsibility (measured by P_{CRIT})[73]. This is associated with a small reduction in upper airway cross-sectional area[74]. While this may contribute to the pathophysiology of OSA in patients with disorders of fluid overload such as cardiac / renal failure, it is unclear if this contributes to OSA severity in OSA sufferers without these conditions.

1.5 Non anatomic factors that potentially influence upper airway function

1.5.1 Upper airway anatomy does not explain OSA variability over the course of the night

While a passive Starling resistor is a useful model of upper airway behaviour, it does not fully describe several observations of OSA. In a study of 106 patients with a wide range of OSA severity, supine P_{CRIT}, a measure of passive collapsibility, only explained 3% of the overall variance in AHI (although no attempt was made to partition the AHI into postural or REM sleep components)[75]. In a study of 82 patients with severe OSA challenged by rapid dial down from therapeutic CPAP, most subjects had periods of stable breathing at loads expected to have produced ventilatory cycling behaviour if there had been no neuromuscular compensation for increased ventilatory load[76], suggesting that these subjects were able to compensate for a deficient anatomy, at least some of the time. Furthermore, OSA clearly does not occur during wakefulness, despite frequently compromised anatomy.

Given that upper airway anatomy is unlikely to change significantly throughout the night in most patients (provided that body posture and the passive factors described above remain unchanged), OSA severity might be expected to be relatively constant throughout the night. This is not in fact the case. Observational studies suggest that OSA tends to be more severe during REM sleep, compared to non-REM sleep[77, 78]. Likewise within non-REM sleep, there is some evidence to

suggest that OSA is more frequent during light sleep compared to slow wave sleep[79, 80]. This has not however been examined in detail in large numbers of subjects. One of the experimental chapters in this thesis explores the relationship between OSA severity and non-REM sleep stage in a large group of patients referred for investigation of OSA.

This overnight variability suggests that non-anatomic factors play a large part in the genesis of OSA. As described previously, the upper airway has complex musculature, the coordinated action of which significantly influences the biomechanical properties of the upper airway.

1.5.1.1 Effect of upper airway muscle activity on upper airway collapsibility / diameter

Increased activity of the upper airway muscles leads to decreased collapsibility of the upper airway. Direct electrical stimulation of the hypoglossal nerve in an isolated decerebrate cat upper airway leads to reduced P_{CRIT} as well as increased peak flow[81]. Likewise direct stimulation of the genioglossus in anaesthetized dogs, leads to reduced upper airway resistance[82]. There is some evidence that electrical stimulation of the genioglossus by a sub-mental electrode in humans, at least partially improves measures of OSA[83, 84]. The upper airway closing pressure in 7 sleeping healthy subjects (measured by the P_{CRIT} technique described above) was (mean±SD) -13.3±3.2 cmH_2O[56]. The closing pressure (determined by extrapolating an exponential curve to solve for an upper airway cross sectional area of 0) in 17 anaesthetized, paralysed healthy subjects was -3.7±3.4 cmH_2O[42]. Although there

were differences in the measurement technique and slight differences in BMI and age, the apparent large difference in closing pressure observed in these two studies is likely to reflect residual neuromuscular activity in the sleeping subjects, not present in anaesthetized/paralysed subjects.

Finally, awake subjects breathing through a tracheal stoma have been shown to have significant increases in their retrolingual airway diameter (measured by lateral neck fluoroscopy), associated with phasic increases in genioglossal EMG, confirming that genioglossal activation increases airway diameter[85]. A recent study using tagged MRI sequences of the tongue, showed that in awake healthy volunteers, the genioglossus moved anteriorly during inspiration, supporting an important contribution of phasic respiratory activation in promoting airway patency[86].

1.5.2 Dependence on upper airway muscle activity in awake OSA subjects

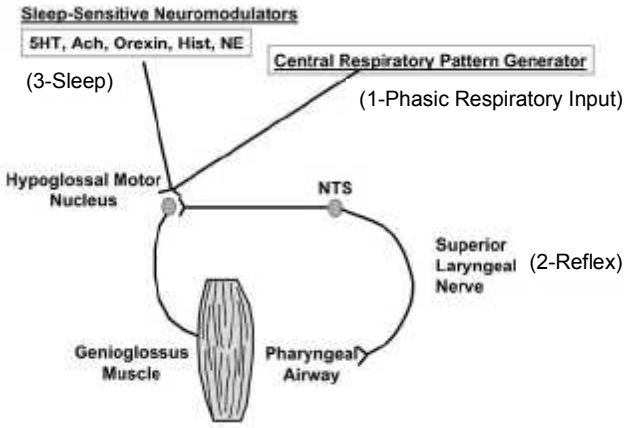
Patients with OSA tend to have a positive P_{CRIT} during sleep, i.e. their upper airway has a passive tendency to collapse at above atmospheric pressures. This tendency is countered during wakefulness by increased upper airway muscle activity. The genioglossus is the most studied of the upper airway muscles. Awake OSA patients have a significantly higher level of baseline phasic and tonic genioglossal EMG activity as a percentage of their voluntary maximum, compared to non-OSA controls[87], even when matched for BMI[88]. This suggests that OSA patients

compensate for an anatomically smaller airway when awake via increased genioglossal tone.

1.5.3 Neural inputs to genioglossus

Upper airway muscles, such as the genioglossus, have 3 primary sources of neural input to increase activity; phasic drive from the respiratory pattern generator, airway pressure mediated reflexes and a 'wakefulness input' (Figure 1.5).

Figure 1.5 : 3 sources of neural input to the genioglossus muscle.



5HT = Serotonin, Ach = acetylcholine, Hist = histamine, NE = norepinephrine, NTS = nucleus tractus solitarius

From White DP[89]

1.5.3.1 Central drive

In awake healthy subjects, increasing central drive by rebreathing CO_2 causes progressive increases in genioglossal EMG and diaphragmatic EMG, with a strong correlation between the two, r=0.96, p<0.001[90]. Ventilatory drive responsiveness to CO_2 is reduced in healthy subjects during sleep compared to wakefulness[91, 92]. Even subjects with a permanent tracheostomy (i.e. eliminating the effect of increasing upper airway resistance during sleep) show reductions in ventilation and an increase in sleeping end-tidal CO_2 from wakefulness[93]. In sleeping healthy volunteers, increasing ventilatory drive by increasing inspired CO_2 maintains total inspiratory lung resistance despite also increasing peak inspiratory flows[94]. Central stimulation by isocapnic hypoxia in sleeping healthy volunteers reduces total inspiratory pulmonary resistance[95]. Although not examining the upper airway muscles specifically, these studies suggest an important contribution to overall ventilation by increasing central drive.

A number of studies suggest direct input from the respiratory central pattern generator to upper airway muscles. The hypoglossal nerve (which supplies the genioglossus muscle) increases activity during hypercapnia in anaesthetized mongrel dogs[96]. Augmentation of genioglossal EMG is noted in tracheostomized rabbits with increased inspired CO_2[97]. In tracheostomized dogs with an isolated upper airway, increasing ventilatory drive by inspiring CO_2 results in increased phasic genioglossal EMG activity[98]. This activity is associated with reduced collapsibility of the upper airway and is abolished by neuromuscular blockade[98]. In an isolated upper airway from decerebrate cats, hypercapnia significantly reduces

collapsibility of the upper airway[99], although this effect is reduced by the application of local anaesthesia to the upper airway. These last 2 studies in animal preparations suggest that central stimulation of genioglossus independently reduces collapsibility of the upper airway. Humans who have a permanent tracheal stoma (and thus no significant upper airway pressure or flow), also demonstrate phasic EMG changes in the genioglossus[100] which increases with increasing drive caused by hypercapnia[101]. In fact, genioglossal activation occurs before the development of negative upper airway pressure or flow, a phenomenon described as pre-activation[102]. This activity is thought to prepare and stiffen the upper airway for impending negative pressure. Upper airway muscles also demonstrate increased EMG activity prior to the diaphragm in tracheostomized dogs[103], tracheostomized rabbits[97], and humans with an intact upper airway[90], prior to the onset of inspiratory flow[104]. This pre-activation is lost with reduced central drive, as occurs with hypocapnia caused by hyperventilating subjects with a negative pressure ventilator[102].

There is some controversy as to how much central drive independently influences upper airway activity in the sleeping healthy human. Clearly, increasing drive to breathe also increases the negative upper airway pressure generated, and the upper airway may be responding to this stimulus, rather than central drive *per se*. Several studies have examined this possibility. In a study of 18 awake healthy volunteers, Shea et al[105] found no significant differences in the genioglossal EMG vs airway pressure relationships elicited by mild hypercapnia (mean±SEM 45.3±2.7 mmHg), isocapnic hypoxia (SaO_2 86.8±0.5%) or mild hypocapnia (34.6±3.4 mmHg)

induced by a negative pressure ventilator. The correlation between negative upper airway pressure and genioglossal EMG remained strong (r=0.87) and unchanged, suggesting that the relationship between airway pressure and genioglossal activation was not modulated significantly at these levels of chemostimulation. Pillar et al[102] however, found that more severe hypocapnia (29.7 ± 0.8 mmHg) caused by negative pressure ventilation in awake OSA patients reduced the slope of the relationship between genioglossal EMG and negative upper airway pressure. Further, pharyngeal resistance at peak flow increased during hypocapnia, suggesting that 'central' and mechanoreceptor influences may have additive effects on upper airway responses. In a further study, Pillar et al[106] found significant increases in genioglossal EMG activity during wakefulness, but not during sleep in 18 healthy volunteers exposed to quite marked hypercapnia (up to ~50 mmHg). Consequently the ventilatory drive contribution to genioglossal activation may be importantly modulated by state.

In another study of sleeping healthy volunteers, Stanchina[107] et al showed that a combination of inspiratory resistive loading (5, 10 and 15 $cmH_2O/L/sec$) and hypercapnia (mean±SEM 49.7±0.6mmHg) led to significant increases in peak genioglossal EMG compared to baseline, whereas equivalent levels of hypercapnia or inspiratory resistive load applied individually did not. This suggests that the combination of resistive load and hypercapnia was needed to elicit a genioglossal response during sleep. Again, there was a robust relationship between the peak negative epiglottic pressure and genioglossal EMG (r^2=0.69). The peak negative

epiglottic pressure reached was also higher during the combination of hypercapnia and resistive load, compared to either stimulus individually.

Finally, Lo et al[108] studied 11 sleeping healthy volunteers, who were commenced on CPAP to minimize negative airway pressure and baseline genioglossal activity and then administered 2 levels of hypercapnia (mean±SEM 47.1±0.7 and 51.6±0.7 mmHg). Significant increases in peak genioglossal EMG were noted at both levels of hypercapnia compared to eucapnia, regardless of whether the subject was on CPAP or not. Although levels of genioglossal activity were lower on CPAP at eucapnia and both levels of hypercapnia, the slope of genioglossal EMG response to CO_2 was not different when on and off CPAP. The authors concluded that hypercapnia has independent effects on peak genioglossal activity, when upper airway negative pressure was minimized. However, the contribution to genioglossal EMG activation from inspiratory decrements in upper airway pressure, inevitably present even in the presence of CPAP, remains unclear.

In summary, the central pattern generator clearly has important inputs to the genioglossus muscle, during wakefulness and sleep. The role central drive plays in maintaining upper airway stability during sleep is unclear. While mild levels of hypo- and hypercapnia do not appear to influence genioglossal activity, more extreme levels importantly modulate the relationship between airway pressure and genioglossal EMG activity when awake. In animal models, central chemostimulation significantly decreases upper airway collapsibility, possibly by modulating reflex upper airway responsiveness to negative airway pressure. In

sleeping healthy humans, while there is some evidence that the EMG response to combined inspiratory resistive load and hypercapnia is higher than either stimulus alone, this may still be on the basis of the higher negative airway pressure generated during combined stimuli. No studies have been performed in OSA patients during sleep to determine if chemo-stimulation increases upper airway inspiratory airflow. One of the experimental chapters in this thesis examines the effect of chemostimulation on upper airway airflow in subjects with moderate to severe sleep apnoea.

1.5.3.2 Negative upper airway pressure mediated reflexes

The presence of a sudden pulse of negative pressure in the upper airway activates the genioglossus in awake subjects[109]. The short latency from stimulus to EMG activation (~35 ms) is much faster than voluntary responses and clearly indicates a reflex origin to the response. The pressures studied range from -2.5 to -35 cmH_2O and are in the range of negative airway pressures reached by patients with severe OSA[110]. This pressure drop is thought to be sensed by superficially located receptors in the upper airway, with afferent neural activity mediated via the superior laryngeal branch of the vagus nerve, to reflexively increase firing of the hypoglossal nerve and increase genioglossal activity[111]. In healthy humans, selective local anaesthesia has shown that nasal mucosal afferents innervated by the trigeminal nerve contribute to the negative pressure reflex as well as oropharyngeal afferents innervated by the glossopharyngeal nerve[112].

. In OSA subjects, the negative pressure reflex is significantly reduced with upper airway local anaesthesia[113], again attesting to the importance of mucosal receptors

in this reflex. However, the genioglossal reflex EMG response to sudden negative upper airway pressure in awake treated sleep apnoea subjects is not different to healthy controls in seated or supine postures[114].

In addition to sudden supraphysiologic pulses of airway pressure, the genioglossal EMG responses to the negative pressures generated during tidal breathing have also been studied. Awake subjects with a tracheostomy demonstrate marked reductions in breath by breath peak and tonic genioglossal EMG when breathing through the tracheal stoma, as opposed to nasal breathing[115]. In awake healthy subjects passively ventilated by a negative pressure ventilator to eliminate central or voluntary respiratory drive and phasic diaphragm EMG, phasic genioglossal EMG activation persists and is strongly correlated with peak negative epiglottic pressure (r=0.97), consistent with negative pressure mediated reflex activation[116]. Using the same model of negative pressure ventilation in awake OSA subjects, Pillar et al[102] showed that local anaesthesia of the upper airway significantly reduced the strength of the relationship between negative airway pressure and genioglossal EMG as well as increasing upper airway resistance, further supporting the importance of local afferent sensation[102].

The genioglossal EMG response to the sudden application of a pulse of negative upper airway pressure has been shown to be significantly attenuated and delayed during sleep compared to wakefulness[117, 118]. More recent evidence suggests that sleep posture may also be important, with an increase in the genioglossal negative pressure reflex response observed in the supine posture compared to the lateral

posture[119]. In 15 healthy volunteers subjected to brief inspiratory resistive loads during wakefulness and non-REM sleep there were strong correlations between epiglottic pressure and genioglossal EMG during wakefulness in the majority of subjects, but the slope of the relationship and strength of the correlation dropped significantly during sleep[120]. Further, following elimination of central drive using negative pressure ventilation, breath by breath peak genioglossal responses to negative airway pressure have been shown to be significantly reduced during theta EEG (i.e. sleep) compared to periods of alpha EEG, i.e. wakefulness[121].

In summary, genioglossal activity is strongly correlated with upper airway negative pressure when awake. Both physiologic and sudden supraphysiologic negative airway pressures result in genioglossal EMG activation. This activity is thought to be reflexively mediated via airway superficial mucosal receptors. Subjects with OSA have a significantly higher peak genioglossal EMG during wakefulness compared to controls. This is thought to be a response to an anatomically smaller upper airway and greater negative airway pressures even when awake. During sleep, the strong correlation between negative airway pressure and genioglossal activity observed during wakefulness is diminished.

1.5.3.3 Wakefulness tone

Transitioning from wakefulness (α EEG) to sleep (θ EEG) is associated with a significant decrease in phasic genioglossal EMG activity in healthy subjects[122]. This is particularly evident in the first two breaths of θ EEG. These changes are associated with significant falls in ventilation and an increase in upper airway

resistance. Subsequently during θ EEG, phasic genioglossal EMG activity increases. The authors suggest that sleep onset is associated with reductions in upper airway muscle activity, however subsequent genioglossal recruitment occurs which may be secondary to increasing ventilatory drive[122]. Similar findings are noted in OSA patients, although baseline genioglossal activity during wakefulness is higher compared to both healthy weight younger men and healthy weight men of similar age[123]. Patients showed greater reductions in genioglossal EMG at sleep onset compared to both control groups, even when CPAP was applied during wakefulness to match upper airway resistance[123]. The genioglossal recruitment noted by the 3rd breath after sleep onset in the previous study was no longer evident, presumably since therapeutic CPAP minimizes the increases in drive following sleep onset. A further study by Lo et al[124], involving 10 healthy volunteers, applied timed mode bi-level positive airway pressure ventilation during transitions from wakefulness to sleep, with the aim of minimising upper airway pressure fluctuations and resistance, as well as eliminating central drive. Significant reductions in genioglossal EMG were noted at sleep onset, strongly suggesting that wakefulness *per se* has independent tonic effects on genioglossal activity. This has been described as the loss of wakefulness tone[125].

1.5.3.4 Other upper airway muscles

Other upper airway muscles have not been as extensively studied as the genioglossus. The alae nasi muscles may act to dilate the flow limiting segment of the nose[31]. They exhibit phasic EMG activity during wakefulness and sleep, starting before inspiratory airflow occurs, indicating input from the central respiratory pattern

generator[104]. Phasic EMG activity is present even in subjects with a permanent tracheal stoma and thus no nasal airflow, with significant increases in activity with hypercapnia[101]. The peak EMG during non-REM sleep is significantly reduced compared to quiet wakefulness, and does not appear to respond to increased resistive load, during either sleep or wakefulness[126].

The tensor palatini (TP) is thought to retract the soft palate from the posterior pharynx during sleep. The TP does not demonstrate phasic increases in EMG activity during inspiration and is therefore described as a tonic muscle[120, 127]. At sleep onset, significant reductions are noted in basal TP activity[122-124]. While the TP does not demonstrate phasic activity during normal breathing, it does demonstrate a reflex EMG response to a sudden pulse of negative airway pressure, similar to the genioglossus. During non-REM sleep the latency to this reflex activity is prolonged and the amplitude of the response is reduced compared to wakefulness[128].

The levator palatini and palatoglossus act antagonistically to lift the palate during oral breathing and pull the palate forward during nasal breathing respectively. Both demonstrate respiratory phasic EMG activity[129]. The peak activity of the levator palatini is reduced, whereas the palatoglossus is more active during nasal breathing. Both muscles demonstrate reflex responses to a pulse of negative airway pressure. Responses are significantly reduced in awake untreated OSA patients compared with awake controls, but are not impaired in CPAP treated sleep

apnoea patients[130]. The negative pressure reflex responses of these muscles have not been studied during sleep.

In summary, non-genioglossal upper airway muscles demonstrate either phasic and/or tonic activity during wakefulness. They all appear to demonstrate reflex EMG activity to negative upper airway pressure. Those muscles which have been studied during sleep demonstrate significant reductions in EMG activity either at sleep onset, and / or during stable sleep.

1.5.4 Summary of airway and ventilatory changes during sleep onset

At the onset of sleep, there are significant changes in the activity of the upper airway muscles and in ventilation. Upper airway resistance increases significantly and overall ventilation is reduced[131, 132]. While part of the reduced ventilation can be ascribed to increased upper airway resistance, even when this is normalised with CPAP[132], or eliminated via tracheostomy[93], reduced ventilation and increased end-tidal CO_2 persist. The ventilatory response to hypercapnia during sleep in healthy subjects is significantly reduced compared to wakefulness[91, 92], although it is not entirely clear if the central drive to upper airway muscles is similarly affected. Upper airway muscles as well as the diaphragm and intercostal muscles show a sudden decline in EMG activity at the wake / sleep transition[122]. This has been thought to be due to loss of the wakefulness drive[123, 124]. OSA patients have a higher waking peak genioglossal activity[88], which falls disproportionately at the onset of sleep compared with young and age matched controls[123].

The reflex mediated response of the upper airway muscles to negative airway pressure is significantly reduced and delayed in healthy subjects during sleep, particularly in the lateral position[117, 118]. Consequently, the very tight correlation between negative airway pressure and genioglossal activity observed during wakefulness is significantly reduced during sleep[120]. While not as well studied, baseline and reflex activity of other upper airway muscles are likely to be reduced during sleep as well. The ability of the upper airway to maintain upper airway tone is therefore likely to be reduced significantly. If the passive tendency for a particular subject's airway is towards collapse at positive airway pressures (i.e. significantly positive P_{CRIT}), without adequate opposing muscle action, the airway is likely to close.

While various neural inputs to upper airway muscles are reduced during sleep, there is evidence nevertheless that the upper airway can respond by increasing muscle activity, particularly in the context of hypercapnia and high negative airway pressure[107].

1.5.5 Neuromuscular compensation in OSA

In addition to impaired upper airway anatomy, patients with OSA may also have impaired neuromuscular compensation responses to challenges to their upper airway function. Patil et al[133] studied 16 subjects with OSA and 16 normal subjects, matched for age, gender and BMI. Passive P_{CRIT} was measured as described previously, averaging values for breaths 2-5 post dialdown from therapeutic CPAP. An 'active' P_{CRIT} was defined in a similar manner from breaths recorded after

reducing CPAP to a subtherapeutic pressure for 10 minutes, to allow time for neuromuscular compensation responses to develop. OSA patients showed more positive passive P_{CRIT} compared to controls. Subjects without OSA were able to significantly reduce their active P_{CRIT} compared to their passive P_{CRIT}, even in controls with a passive P_{CRIT} comparable to that of the OSA group, suggesting impaired neuromuscular compensation for deficient airway anatomy in OSA patients.

A follow up study in 10 OSA patients and 9 age, weight and gender matched healthy controls without OSA showed similar findings of an impaired ability to favourably alter P_{CRIT} over time in OSA patients[134]. Control subjects but not OSA patients demonstrated significantly higher tonic, but not phasic genioglossal EMG during the active (or compensated) dialdown at what was thought to be similar levels of increased upper airway load[134], further supporting deficient neuromuscular compensation responses to prolonged respiratory load in OSA patients.

While the above two studies discuss differences between OSA and non-OSA groups in their ability to compensate for impaired anatomy, Younes[76] suggests that 82% of subjects with severe OSA can adequately compensate for mechanical load at least some of the time. How patients with OSA can achieve periods of stable breathing has not been well described. One of the experimental chapters in this thesis explores differences in upper airway function following complete and partial airway occlusion in stage 2 versus slow wave sleep.

1.5.6 Role of ventilatory control in OSA

The genioglossus and other muscles involved in the maintenance of upper airway tone, as well as those more directly involved in ventilation clearly derive significant input from the respiratory pattern generator. With the loss of the wakefulness drive, control of breathing in humans becomes more dependent on chemical drive[135, 136]. Chemical control of ventilation in humans is tightly regulated by negative feedback loops to maintain appropriate levels of arterial PaO_2 and particularly $PaCO_2$. However, any system that depends on negative feedback loops to maintain a stable outcome has the potential to become unstable. This concept is frequently described by the engineering concept of loop gain[137-139], discussed in more detail below (1.5.6.2 Loop Gain). While much of the experimental work investigating loop gain involves subjects with idiopathic or heart failure associated central sleep apnoea / Cheyne-Stokes breathing, ventilatory control abnormalities are likely to play a significant role in the pathogenesis of OSA as well.

1.5.6.1 Implications of abnormal ventilatory control in OSA

Mathematical modelling of upper airway and ventilatory control suggests that ventilatory control plays a significant part in the genesis of OSA. In an inherently unstable system, increasing upper airway stiffness may simply convert the disorder from obstructive apnoeas to recurrent central apnoeas[140], with persistence of periodic breathing patterns and sleep disturbance.

The likely importance of ventilatory control abnormality as a mechanism for OSA is borne out by clinical studies. Observationally, the pharyngeal airway tends to significantly narrow and often completely collapse during either spontaneous or ventilator induced central apnoeas[141], suggesting a significant overlap between OSA and central apnoeas / periodic breathing disorders. Patients with OSA and heart failure have a significantly prolonged hyperpnoea time, time to peak ventilation and overall cycle duration compared to OSA patients without heart failure, which corresponds to an increased circulatory delay[142]. This strongly suggests an underlying ventilatory control disorder 'sculpting' the pattern of OSA in these patients.

The genioglossal and diaphragmatic EMG in patients with OSA show cyclical fluctuation very similar to Cheyne-Stokes breathing, with upper airway occlusions occurring at the nadir of EMG activity[143]. Some patients with OSA treated with tracheostomy subsequently develop central apnoeas[144]. Further, a minority of patients with OSA treated with CPAP tend to develop mixed and central apnoeas, the recently termed complex sleep apnoea[145], suggesting that in these patients there is an important underlying problem with ventilatory control stability.

In interventional studies, hypoxia during non-REM sleep in humans[146] and animals[147] causes a reduction in the difference between eupnoeic P_ACO_2 and the apnoeic threshold P_ACO_2 (as determined by mechanical hyperventilation) with increased controller gain (see fig 1.7B and loop gain discussion). In healthy volunteers[148] and snorers[149] with elevated upper airways resistance, hypoxia tends

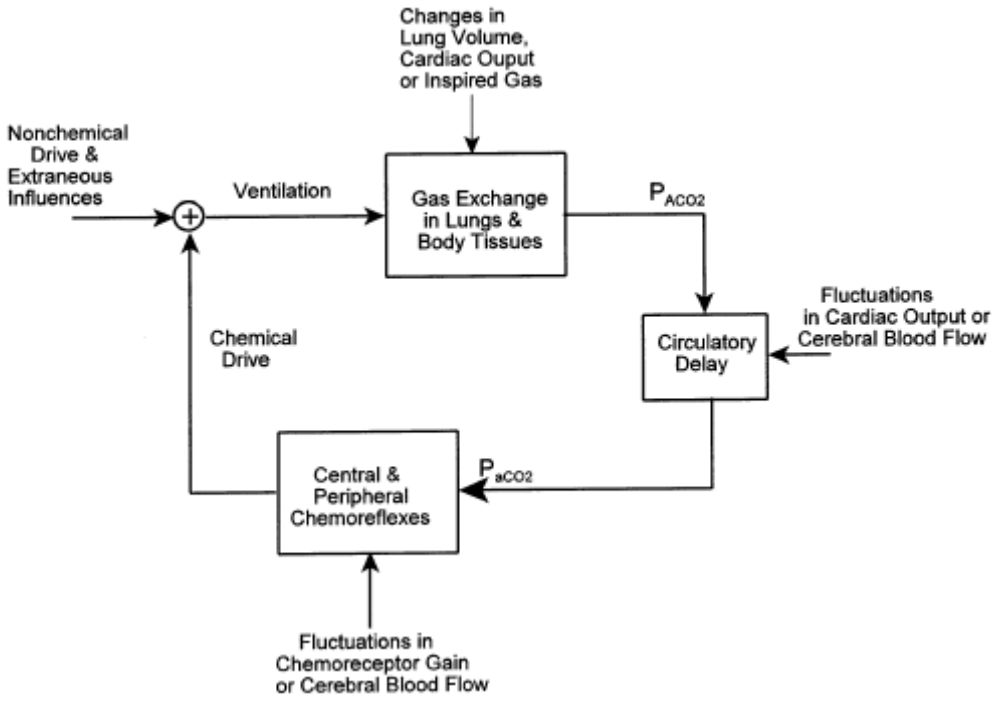
to cause a pattern of periodic breathing with upper airway occlusions or marked increases in upper airway resistance at the nadir of ventilation. Gleeson et al[150] measured the sleeping ventilatory chemosensitivity to CO_2 rebreathing in healthy male subjects. They subsequently induced brief complete upper airway obstruction for 3 breaths. The subjects with higher chemosensitivity tended to have significantly increased ventilation post release of obstruction. This then tended to produce subsequent hypocapnia and prolongation of T_E. The maximum decrease of end-tidal CO_2 following this release was strongly correlated with the hypercapnic response.

In summary, abnormalities in chemical ventilatory drive likely play a substantial part in the genesis of OSA.

1.5.6.2 Loop gain

Loop gain is the ratio of the magnitude of a feedback control systems response to an input disturbance relative to the initial disturbance itself. Together with phase delays inherent in any feedback control loop, loop gain provides a measure of the systems responsiveness to disturbances and propensity for instability. A loop gain equal to or greater than unity indicates a system that mounts a corrective response equivalent or greater than the initial disturbance, which if sufficiently phase delayed, can result in self-sustaining or amplifying feedback disturbances and therefore indicates an inherently unstable system[137-139].

Figure 1.6 : Simplified model of ventilatory control system



From Khoo MC[137]

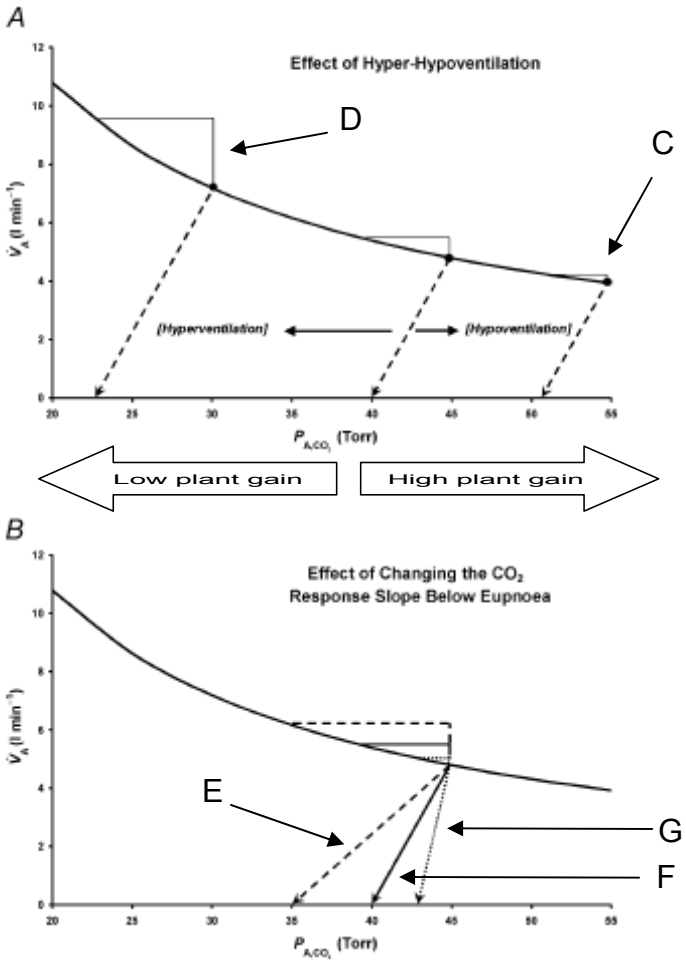
Figure 1.6 represents a simplified model of the chemoreflex control of ventilation. Changes in $PaCO_2$ stimulate chemoreflexes to adjust ventilation to restore $PaCO_2$ towards the original equilibrium level. For example, a brief episode of increased ventilation or hyperpnoea would cause a drop in $PaCO_2$ which would subsequently elicit a reduction in ventilation to restore $PaCO_2$ to the system set-point. However, precise homeostasis would require instantaneous ventilatory corrections to counteract the original hyperpnoea. Inevitable phase lags between the sensor and effector components of the system are present (time taken for blood $PaCO_2$ to be sensed by the carotid body and medullary chemoreceptors), such that the additive effects of disturbances and corrective responses propagate as dampening or potentially self-perpetuating cyclical disturbances over time.

Mathematically, a phase lag of 180 degrees (where the time lag between the sensory disturbance and corrective response are exactly out of phase) promotes the greatest system instability. When loop gain is less than unity (i.e. the size of the response is smaller than the perturbation), and the phase lag is less than 180 degrees, the tendency is for a significant perturbation to produce oscillations that decay back to baseline. When loop gain is greater than 1 (i.e. the size of the response is greater than the perturbation) and the phase lag is 180 degrees, the tendency is for a perturbation to self propagate oscillations around baseline[139].

Loop gain is composed of the product of plant gain and controller gain. In ventilatory terms, plant gain represents the ability of the ventilatory system to ventilate, or clear CO_2 while controller gain is synonymous with chemosensitivity.

Given the potential importance of ventilatory drive inputs to upper airway muscles and therefore upper airway patency and function, factors that influence ventilatory control may directly and/or indirectly impact on the propensity for upper airway collapse.

Figure 1.7: Relationship between ($\dot{V}_A$) and P_{ACO_2} at a fixed resting CO_2 production ($\dot{V}_{CO_2}$).



From Dempsey JA[151]

Both plant gain and controller gain are represented in Figure 1.7A and B respectively, by the isometabolic line representing the alveolar gas equation:

$$P_ACO_2 = \frac{\dot{V}CO_2}{\dot{V}_A}$$

Where P_ACO_2 = alveolar CO_2, $\dot{V}_{CO_2}$ = CO_2 production due to metabolism (in this example assumed to be 250 ml/min) and $\dot{V}_A$= alveolar ventilation.

The isometabolic line is asymptotic in shape. Therefore at conditions of high plant gain (arrow C, Diagram A), assuming that controller gain (dashed line) is constant, 2 factors predispose towards at least central and mixed, and potentially obstructive apnoeas; 1) The horizontal distance from eupnoeic CO_2 to apnoeic threshold CO_2 is significantly reduced. 2) The vertical distance representing the amount of additional ventilation required to reach the apnoeic threshold CO_2 is also markedly reduced. The situation is reversed during conditions of low plant gain (arrow D, Diagram A), where the horizontal distance between the eupnoeic and apnoeic threshold CO_2 is significantly increased, as is the amount of additional ventilation required to achieve the apnoeic threshold CO_2[147, 151]. Plant gain is higher in a hypoventilating, hypercapnic subject, who is thus predisposed towards central apnoea. Sleep onset is associated with reductions in FRC[152, 153], increased upper airway resistance[132] and relative hypercapnia[132, 154],which may theoretically predispose towards higher plant gain.

As shown in Fig 1.7B, changes in controller gain also affect the likelihood of at least central apnoea. At a reduced slope of controller gain (dashed line E), both the horizontal distance representing the difference between the eupnoeic and the apnoeic threshold, as well as the increase in ventilation required to get to that point is significantly elevated, compared to increasing controller gains (arrows F and G). Accordingly patients with higher levels of controller gain tend to be predisposed towards central apnoeas. Sleep onset is associated with a reduced hypercapnic responsiveness (i.e. controller gain) compared to wakefulness[91, 92]. It must be noted however that the hypercapnic response refers to the slope of the line above

eucapnia, which may not be identical to that below eucapnia[151]. A number of studies have assessed the slope of this line by inducing hypocapnia via mechanical hyperventilation in animals[147] and humans[146] during sleep, but these have not compared wakefulness versus sleep. Awake humans are resistant to apnoeas even at significant levels of hypocapnia ($ETCO_2$ levels of ~21 mmHg)[155], suggesting non-linearity below eupnoea when awake.

The overall gain during sleep compared to wakefulness has been suggested to be unchanged in healthy subjects assessed by either CO_2 rebreathing[156] or pseudorandom breath sequences of hyperoxic hypercapnia[157]. This is thought to be due to a reduced controller gain which is counteracted by elevated plant gain, although there appears to be significant inter-individual variation[156]. However, the impact of sleep on plant, controller and overall loop gain in OSA patients is not known[158].

In obese seated awake OSA subjects, the closed loop response to pseudorandom hypercapnic breaths has been to shown to be elevated compared to normal weight, non-OSA subjects[158]. The closed loop response is thought to represent the dynamic interactions of controller and plant gains. Interestingly, the open loop response which represents central dynamic gain, or chemosensitivity, was not different between normal weight controls and OSA subjects. Overall loop gain, assessed by the technique of proportional assist ventilation, has been shown to be significantly higher in sleeping patients with severe OSA, compared with patients with mild OSA[159]. A further study suggested that loop gain is strongly correlated

with AHI, but only in patients with a P_{CRIT} close to atmospheric pressure[160]. Subjects with a negative (less than -1 cmH_2O) or strongly positive P_{CRIT} (greater than 1 cmH_2O) showed no significant correlation between loop gain and OSA severity. The authors concluded that in patients who had airways that were highly resistant to collapse (very negative P_{CRIT}), fluctuations in loop gain were unlikely to lead to airway collapse and thus did not influence sleep apnoea severity. In patients at the other extreme, with very collapsible upper airways, changes in chemical drive may be ineffective, with arousal from sleep required to open the airway. As a result, cycling airway closure and arousals occur, regardless of loop gain. However, patients in between these two extremes, P_{CRIT} -1 to +1 cmH_2O may be more susceptible to loop gain influences on OSA severity.

The relative contribution of central vs peripheral (i.e. carotid) chemoreceptors in the control of breathing in humans is currently uncertain. However, intact isolated carotid body studies in animals suggest that peripheral chemoreceptors may play a substantial role in sleep apnoea, acting as fast sensors to brief drops in CO_2[161]. Changes in cerebral perfusion with changes in arterial pCO_2 and hypoxia may also act to alter the central responses to these stimuli. Patients with heart failure and central sleep apnoea show reduced cerebral vaso-reactivity to CO_2[162]. The authors suggest that a high $PaCO_2$ causes cerebral vasodilatation and low $PaCO_2$ vasoconstriction. High blood flow at the central chemoreceptor thus causes less of an increase in H^+, whereas less blood flow during hypocapnia causes less of a decrease in H^+. A reactive cerebral perfusion system would thus ‘buffer’ changes in

arterial CO_2. Reduced cerebral vasoreactivity therefore leads to a more unstable ventilatory control system.

In summary, abnormalities in loop gain appear to be a prominent contributor to both central sleep apnoea syndromes and disorders of periodic breathing associated with heart failure. There are theoretical reasons why loop gain may also play a part in OSA, particularly in certain subsets of patients.

1.5.7 Role of arousals in the pathophysiology of OSA

Arousals from sleep frequently accompany both partial and complete airway occlusion[1, 163]. Since occlusions frequently terminate with an arousal, they have been thought of as a protective response to an occluded airway[1]. More recently however, it has been proposed that arousals may in fact contribute to unstable breathing[163].

1.5.7.1 Causes of respiratory arousal from sleep

A respiratory arousal from sleep is believed to occur at a particular level of respiratory drive, or effort to breathe[164], as normally measured by either oesophageal or epiglottic pressure. Within an individual, this appears to occur regardless of what causes the increase in drive, either chemostimuli such as hypercapnia or hypoxia, or resistive upper airway load[164]. A number of studies have explored the effect of various chemostimuli on the increasing respiratory drive that occurs post upper airway occlusion. Hypercapnia reduces the time to arousal

following complete upper airway occlusion[165]. This appears to be due to an increasing rate of drive augmentation post occlusion with no actual change in the level of drive at which arousal occurs (i.e. arousal threshold)[165]. Conversely, hyperoxia increases the time to arousal, apparently due to a slower increase in respiratory drive post upper airway obstruction[166]. Both these studies support a relatively consistent level of respiratory drive at which an individual arouses in non-REM sleep[165, 166], but with considerable inter-individual differences[164]. Combined with pre-arousal neurocompensatory and/or post-arousal overshoot-undershoot ventilatory responses, such differences in arousal threshold could potentially influence the propensity for OSA itself.

Sensory information arising from mechanoreceptors in the upper airway may play a significant part in respiratory arousal from sleep. Topical upper airway anaesthesia causes an increase in the maximum negative oesophageal pressure at which arousal occurs[167].

Chemoreceptor inputs may also directly contribute to arousal rather than their effects acting via increases in respiratory drive alone. Subjects with neurologically complete C3 spinal injuries who are on chronic nocturnal ventilatory support arouse consistently when $ETCO_2$ is increased by inhaling CO_2[168], despite being unable to increase ventilatory output. In decerebrate cats, which have had both carotid sinus nerves and vagi cut, rhythmic midbrain firing occurs in time with phrenic nerve output (which has a significantly higher frequency than expected from central chemoreceptor equilibration to chemostimuli). This phenomenon persisted when

respiratory drive was increased by electrical stimulation of the carotid nerve or by inducing hypercapnia. Severing the spinal cord at C1-2 did not influence the midbrain respiratory modulated activity. The authors suggest that the midbrain activity may be a projection or corollary discharge from brainstem respiratory centres and may be part of a pathway carrying respiratory sensory traffic directly from brainstem to cortex[169].

One proposed model by Berry et al[110] suggests that both sensory stimuli from the lungs / mechanoreceptors, as well as the corollary discharge from the brainstem respiratory centres, itself receiving afferents from peripheral and central chemoreceptors are likely to be important in respiratory arousal. The relative contribution of these two mechanisms to respiratory related arousal is currently unknown.

1.5.7.2 Effect of sleep stage on respiratory arousal

The arousal threshold for many stimuli varies across the night. The arousal probability to auditory stimuli is significantly reduced during slow wave sleep compared to light sleep[170]. The respiratory arousal threshold (or maximum negative oesophageal pressure pre-arousal) is also known to be elevated during slow wave sleep[171] and varies cyclically through the night with the delta power of sleep[172]. Two other studies support this conclusion, although they did not measure the arousal threshold *per se* it was found that the probability to arouse during a fixed inspiratory resistive load was significantly reduced during slow wave sleep[173, 174]. The

mechanism for the sleep stage differences in arousal threshold and probability is currently unknown.

1.5.7.3 Effects of OSA on respiratory arousal

Several studies suggest that subjects with untreated OSA have significantly elevated arousal thresholds[175] (as measured by the maximum negative oesophageal pressure), compared with normal controls[165, 171, 176]. A number of theories have been proposed to explain this observation. OSA is associated with recurrent episodes of hypoxia and sleep fragmentation. Sustained hypoxia has been shown in healthy volunteers to increase the arousal threshold to both external resistive load and upper airway occlusion[177]. While not a true model for OSA, sleep deprivation increases the arousal threshold to complete airway occlusion during non-REM sleep in tracheostomised dogs[178]. Sleep fragmentation induced by auditory tones for 2 nights prior to the study night, reduces the probability of arousal to external resistive loads in stage 2 sleep[173]. These results suggest that a general decrease in cortical responsiveness to respiratory stimuli is likely a consequence of the hypoxic and sleep fragmentation effects of OSA.

Another theory is that OSA is associated with sleep specific blunting of the cortical response to upper airway sensation. Patients with mild OSA have both a reduced probability of K complex elicitation to mid inspiratory occlusion, as well as reduced amplitude of the N550 respiratory evoked potential during sleep[179, 180]. The earlier components of awake respiratory evoked potentials, suggesting receipt of afferent sensory information, show no significant difference from controls[179], or only minor

differences thought to be associated with reduced attention / increased sleepiness in the OSA group[180]. This would imply that mechanoreceptor function is preserved, at least in patients with mild OSA, and that impaired cortical responses are sleep specific. Further, compared to controls, patients with OSA showed reduced respiratory evoked potentials during stage 2 sleep, but not auditory evoked potentials, implying that blunted cortical responses are specific also to respiratory stimuli[179]. This may reflect habituation to a previously repetitively presented stimulus, or an underlying abnormality in cortical processing of upper airway sensory signals. No studies have compared pre-treated to treated OSA patients to determine if these sensory effects are reversed with treatment.

OSA is also often associated with significant upper airway oedema[181] and inflammation[182], due to repetitive snoring trauma which may cause dysfunction of the upper airway mechanoreceptors and reduced upper airway sensation[183, 184]. This may impair afferent sensory input from these receptors, thus the increased arousal threshold in OSA patients may not necessarily be due to an abnormality of central nervous system processing of respiratory afferent input, but the level of sensory input provided by upper airway mechanoreceptors. Local anaesthesia of the upper airway increases the duration of apnoea as well as the maximum negative upper airway pressure prior to arousal, suggesting that this is a plausible explanation[167].

The maximum negative oesophageal pressure pre-arousal progressively reduces following therapy with CPAP[185, 186] and interestingly, increases again when CPAP is

withdrawn for 3 days[187]. CPAP therapy reduces upper airway oedema[167] and at least partially improves upper airway sensation[183]. However, CPAP treatment also improves the recurrent hypoxia as well as sleep fragmentation associated with OSA[188], thus it is unclear by which mechanism arousal threshold improves.

1.5.7.4 Consequences of arousal on ventilatory control

Restoration of airflow post-apnoea is associated often with a burst of genioglossal EMG and generalized EEG evidence of arousal[1]. Acoustic tone-induced arousals are also associated with markedly increased activity of genioglossal and levator palatini activity[189]. Consequently, arousals have been thought to be required to open the airway following an upper airway obstruction[1, 190]. More recently however, Younes[163] suggested that subjects with OSA can in fact open the airway without arousal at least some of the time, and that arousals are incidental phenomena which occur coincidentally around the time of airway opening, but with no systematic temporal relationship[163]. The author further suggested that the flow response post airway opening was already excessive without arousal, corresponding to 180±148% of the initial decline in ventilation. With the presence of arousals, this was even higher (267±154%) and more likely to lead to subsequent reductions in chemical drive and further cycling behaviour of the upper airway.

Arousal from sleep is associated with a rapid significant increase in ventilation in both healthy subjects[191, 192] and patients with OSA[193]. This may be due to homeostatic mechanisms such as the sudden restoration of waking chemosensitivity[91, 92], as well as the removal of sleep induced increases in upper

airway resistance. This ventilatory response is greater in males than females[191], and when upper airway resistance is higher prior to the onset of arousal[193]. There may also be a waking cardiorespiratory 'reflex', regardless of the underlying upper airway and drive conditions[194]. Healthy subjects mechanically ventilated and on CPAP, thus eliminating the sleep induced hypercapnia and increases in upper airway resistance, still demonstrate brisk increases in cardiorespiratory activity with arousal[194]. One small study in healthy humans who had their $ETCO_2$ clamped by a proportional rebreathing device, suggested that the ventilatory response to arousal was independent of the pre-arousal $ETCO_2$[195]. A further study involving combined hypercapnic and hypoxic stimulation in healthy volunteers suggested no further increase in the post-arousal hyperventilation compared to normoxia[196]. In tracheostomised sleeping dogs, significant increases in peak flow and ventilation were noted post tone-induced arousal, despite changing $FiCO_2$ such that awake and sleeping $ETCO_2$ were matched[197]. The authors felt that >50% of the ventilatory response was due to a 'reflex' response to arousal. It is unclear regarding the proportions of reflex vs homeostatic mechanisms contributing to the post arousal increase in ventilation and both may be important.

While in patients with central sleep apnoea or Cheyne–Stokes respiration, arousals have been shown to be associated with recurrent ventilatory cycling[198], this has not convincingly been shown to be the case in patients with obstructive sleep apnoea.

1.5.7.5 Mathematical modelling of the effects of arousal

As discussed previously, the presence of wakefulness provides a tonic input to both ventilatory drive as well as genioglossal muscle activity[122]. This has been modelled as a non-chemical component of ventilatory control[137]. The periodic loss of this input (with subsequent transient restoration following arousal) causes a significant perturbation in the overall control of ventilation[138]. A low arousal threshold can therefore transform an inherently more chemically stable system with low loop gain, into an unstable one, characterised by recurrent ventilatory cycling and arousals[137]. This occurs in the form of central apnoeas if the airway is modelled with high compliance, or OSA if modelled with low compliance[140].

1.5.7.6 Summary of arousal influences in OSA

In summary, arousal has been considered to be both a necessary response to terminate at least some obstructive apnoeas, as well as coincidental and unnecessary to restore airflow in many respiratory events, with post-arousal hyperventilation potentially exacerbating ventilatory instability. Arousal threshold is increased during slow wave sleep. Further, patients with OSA have an increased arousal threshold compared to non-OSA subjects, either due to abnormal central processing of upper airway stimuli or mechanoreceptor dysfunction. If a respiratory arousal from sleep is a protective response to airflow obstruction, then clearly this increased threshold may contribute to the pathogenesis of OSA, possibly by prolonging apnoeic periods. If arousal is an event which may worsen OSA, then

decreased arousal propensity may in fact be beneficial and may represent a partial adaptation to OSA.

1.6 Summary and aims of thesis

An exploration of non-anatomical factors influencing OSA severity forms the basis for the experimental chapters in this doctoral thesis.

One of the key observations supporting the importance of non-anatomic factors is the significant overnight variability in the severity of OSA, despite fixed upper airway anatomy. This has not been confirmed in a large group of subjects with adequate control for posture. Chapter 2 documents the variability of OSA severity across sleep stages and postures in a large group of patients. The hypothesis tested is that OSA frequency reduces significantly from stage 2 to slow wave sleep in a large number of subjects despite stable posture. This is followed by an exploration of airway function and arousal propensity comparing stage 2 vs. slow wave sleep (chapter 5), which may potentially explain a key component of overnight variability in OSA severity. The hypotheses explored in this chapter are that, basic airway function and compensatory ventilatory responses are not different between light and slow wave sleep in patients with OSA, however there are significant differences in arousability.

Abnormalities in ventilatory control may contribute to periodic breathing disorders. Particularly following arousal from sleep, a brief period of hyperventilation occurs,

which is thought to lead to a period of reduced ventilatory and upper airway drive. This may lead to central apnoeas in susceptible patients, and possibly predispose towards upper airway collapse or OSA, in patients with a collapsible upper airway. To explore the potential role of changing CO_2 levels on upper airway and ventilatory function during sleep, a CO_2 stabilising or 'clamp' device was developed and tested to enable the provision of positive airway pressure and by proportional rebreathing, the maintenance of relatively constant end-tidal CO_2 despite significant hyperventilation. This device was initially tested during wakefulness in healthy volunteers in chapter 4, exploring the hypothesis that end tidal CO_2 can be maintained with minimal change, despite significant voluntary hyperventilation while maintaining a positive airway pressure. Using this device, the effects of maintaining a mild degree of hypercapnia during partial airway obstruction and preventing hypocapnia post arousal were subsequently studied in OSA patients during sleep. The hypotheses of this chapter are that airway function post dialdown from therapeutic CPAP and arousal can be improved by clamping and maintaining a mild level of hypercapnia, compared to control.

CHAPTER 2. Marked reduction in obstructive sleep apnoea severity in slow wave sleep

2.1 Introduction

Obstructive sleep apnoea (OSA) is a common disorder. 24% of adult males and 9% of adult females experience at least 5 events of disordered breathing per hour of sleep[6]. The diagnosis and severity of obstructive sleep apnoea is typically determined from the apnoea hypopnoea index (AHI)[11], a measure of the total number of complete or partial upper airway obstruction (apnoea or hypopnoea) events lasting 10-sec or more, divided by total sleep time. AHI is known to be strongly influenced by factors such as sleep posture[77, 199, 200], head position[68, 201] and REM sleep[77, 200] and in clinical studies OSA severity frequently changes substantially over the course of a night, even in severely affected patients. It has also been widely accepted that obstructive sleep apnoea improves during slow wave sleep (SWS). However, with the exception of a few preliminary reports based on small patient samples[79, 80, 202], there appears to be remarkably little published evidence available to assess the magnitude and prevalence of this effect.

If improvements in sleep disordered breathing in SWS amongst OSA patients are substantiated as being both common and marked it may be a phenomenon worthy of further investigation, for while much has been learned about the risk factors for OSA and OSA pathogenesis, only a relatively small degree of the variance in OSA severity is currently explained by these various mechanisms[89]. It seems likely that non-anatomical factors mediating upper airway and respiratory control stability,

such as ventilatory chemosensitivity, plant gain control factors, arousal threshold and the magnitude of post-arousal ventilatory overshoot-undershoot responses[89], would be involved in SWS-mediated improvements in sleep apnoea severity. Ultimately, better knowledge of the extent and mechanisms of SWS-mediated improvements in OSA could lead to new therapeutic approaches for this disorder.

Therefore, the purpose of this study was to investigate in detail, the effect of non-REM sleep stages on the frequency of respiratory and arousal events in a large cohort of patients referred for the investigation of possible OSA.

2.2 Methods

2.2.1 Patients

The study was approved by the Repatriation General Hospital research and ethics committee. De-identified full night diagnostic polysomnography studies of all patients investigated in the Adelaide Institute for Sleep Health laboratory during a 3 month period between January and March 2005 were retrospectively analysed in detail.

2.2.2 Polysomnography

All studies were recorded and primarily analysed using a Compumedics E-series system and software (ProFusion, Compumedics Inc, Melbourne Australia) in an attended laboratory setting. EEG was recorded using electrodes at C3/A2 and C4/A1 scalp locations. Eye movements were measured via 2 channel electrooculography (left and right EOG). ECG, submental chin EMG, leg movements, snoring and posture were also continuously recorded. Nasal pressure cannulae were used to record airflow, thoracoabdominal bands and an uncalibrated sum signal used to assess ventilatory effort. Finger pulse oximetry was used to determine arterial blood oxygen saturation. Sleep recordings were analysed by an accredited sleep technologist using 30-sec epochs and Rechtshaffen and Kales criteria for staging sleep[9]. Arousals and respiratory events were scored according to ASDA[10] and 1999 AASM criteria[11], (specifically that the respiratory event lasts longer than 10 seconds and consists of at least a 50% reduction from baseline in a valid breathing measurement, or a clear reduction in amplitude associated with a >3% desaturation, or arousal.)

2.2.3 Analysis

For each 30-sec epoch within each sleep study, sleep posture was categorised according to prone, lateral (left and right lateral combined) or supine, and the total number of respiratory and arousal events determined. Total respiratory (apnoeas plus hypopnoeas) and arousal event rates irrespective of posture and in supine and lateral postures separately were determined within each sleep stage (non-REM 1-4 and REM) as the total number of events divided by total sleep time for each posture and stage combination for which there was at least 5-min (10 epochs) of sleep in total. A minimum of 5 minutes within each posture and/or stage was chosen to reduce the impact of more extreme event rates estimated from short periods (i.e. poor event rate resolution). Based on previous normative data published by our group using American Academy of Sleep Medicine 1999 criteria[11], total sleep AHI ≥15 /hr was considered the cut off for the diagnosis of OSA[203]. The sample was divided on this basis into OSA and non-OSA groups. To exclude central sleep apnoea, the total sleep central apnoea index was calculated and patients with ≥ 5 central events per hour were excluded from the analysis.

2.2.4 Statistics

Sleep stage (1-4 and REM) and posture (supine vs. lateral) effects in OSA versus non-OSA groups were examined using linear mixed model analysis using an autoregressive covariance structure (SPSS version 16, SPSS Inc, Illinois). In the event of significant mixed model effects, relevant post-hoc comparisons were performed using Student's t-tests with Dunn-Sidak correction for multiple comparisons[204]. The strength of relationships between respiratory and arousal

event rates in non-REM sleep in the supine and lateral postures in the OSA and non-OSA groups were examined on a within subject basis for each individual in which 3 or all 4 non-REM stages were available for analysis. Effect sizes for posture and stage effects were estimated from within subject differences in lateral versus supine values in stage 2 sleep and differences between stage 2 and SWS in the supine posture respectively. All data are presented as mean ± SEM. p-values <0.05 were considered significant.

2.3 Results

Two hundred and fifty three overnight diagnostic studies were performed during the time period of interest. 14 patients had a central apnoea index >5 /hr and were excluded from the main analysis. 171 of the remaining patients had an AHI ≥15/hr and 68 had an AHI <15/hr. Baseline demographic and sleep study data for the groups are summarised in table 2.1. The patient population was typical of diagnostic referrals primarily for evaluation of OSA and was comprised predominantly of males who were middle-aged and overweight to obese. Approximately 70% of patients received a positive diagnosis for OSA. This group was significantly older, comprised relatively more males and had a higher BMI than the non-OSA group.

There was no difference in total sleep time between OSA and non-OSA patients (Table 2.2). OSA and non-OSA patients spent similar proportions of total sleep time in lateral, (60.8±2.1 vs. 66.2±3.2% p=0.159) and supine sleep (42.3±2.3 vs. 35.6±3.6% p=0.119) respectively. As expected, OSA patients had significantly more stage 1 and less stage 4 sleep compared to the non-OSA group both in absolute terms (group x stage effect p=0.037) and as a proportion of total sleep time (Table 2.2). OSA patients took significantly longer to attain the first epoch of slow wave (39.3±3.5 vs. 25.6±2.6 mins, p=0.005) and REM sleep (147.4±6.1 vs. 123.3±6.8 mins, p=0.015) following sleep onset compared to the non-OSA group.

Changes in AHI and AI as a function of sleep stage and OSA diagnosis are shown in Figure 2.1A and further separated as a function of supine versus lateral postures

in Figure 2.1B. There was a striking non-REM sleep stage dependence in the frequency of respiratory (stage effect $p<0.001$) and arousal events ($p<0.001$), with a progressive reduction in event rates from stage 1-4 and an intermediate level in REM sleep. Compared to stage 2, the REM AHI was ~40% higher (effect size 0.55), while SWS AHI was ~50% less (effect size 0.94). The reduction in respiratory and arousal event rates was noted in both OSA patients and non-OSA patients. Total sleep event rates were weighted towards stage 2 values, consistent with the predominance of stage 2 sleep. The ratio of respiratory events to arousal events also showed strong stage ($p<0.001$) and group ($p<0.001$) effects. In REM sleep, respiratory events occurred considerably more frequently relative to arousal events (AHI/AI ratio 3.9±0.2, compared to non-REM sleep 1.2±0.2, $p<0.001$) but remained essentially unchanged within non-REM sleep. The frequency of arousals exceeded the frequency of respiratory events in all non-REM sleep stages in non-OSA patients, whereas the opposite was true for OSA patients, (AHI/AI 0.7±0.3 vs. 1.7±0.2, $p<0.001$). The respiratory event rate was strongly correlated with the arousal index, (overall r^2 0.82±0.02).

As shown in Figure 2.1B posture had substantial effects on respiratory and to a lesser degree arousal event rates in both OSA and non-OSA patient groups. Respiratory event rates in the lateral postures were in the order of 50-60% of supine values in both groups ($p<0.001$), while arousal event rates were in the order of 60-80% of supine values in lateral postures, ($p<0.001$). Thus respiratory event frequency in the supine posture was higher, relative to arousal frequency, ($p=0.018$). However, a similar pattern of decline in respiratory and arousal frequencies were noted from stage 1 to 4 non-REM sleep.

Sleep stage effects were large and of similar or greater magnitude when compared to posture effects. In the OSA patient group, the effect size of the difference between stage 2 and slow wave sleep (stages 3 and 4 combined) was 1.1 for AHI and 1.4 for AI, while that of the difference between supine and lateral postures in stage 2 sleep was 0.8 for AHI and 0.7 for AI. The corresponding effect sizes in the non-OSA group were weaker; 0.6, 1, 0.6 and 0.3 respectively.

Most patients attained relatively low event rates in slow wave sleep. For example, 82% of OSA patients exhibited <15 respiratory events/hr and 57% < 5 events/hr in stage 4 sleep.

Table 2.1 – Patient characteristics

	N (%Total)	N (%Males)	Age (yr)	BMI ($kg \cdot m^{-2}$)	AHI (/hr)	AI (/hr)	CAI (/hr)
non-OSA (AHI<15 /hr)	68 (27%)	36 (53%)	47.5 ± 1.9	28.9 ± 0.8	9.3 ± 0.4	14.3 ± 1.0	0.2 ± 0.1
OSA (AHI≥15 /hr)	171 (68%)	120 (70%)*	54.1 ± 1.1*	31.8 ± 0.5*	37.7 ± 1.6*	23.6 ± 0.9*	0.8 ± 0.1*
CSA (CAI≥5 /hr)	14 (6%)	13 (93%)*	53.9 ± 5.2	32.2 ± 1.4	54.3 ± 6.3*	27.2 ± 4.8*	16.8 ± 3.9*

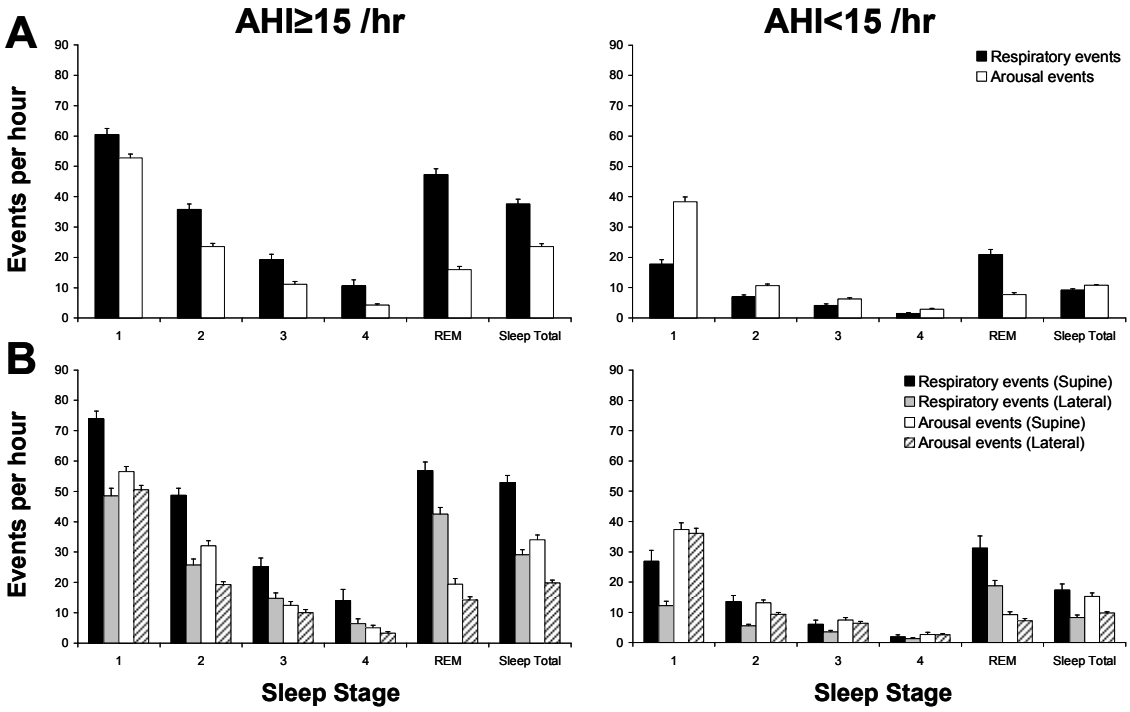
BMI: body mass index, AHI: Apnoea-hypopnoea index, AI: arousal index, CAI: Central apnoea index. Values are numbers and (percentage) of patients and mean ± SEM. * indicates $p<0.05$ versus the non-OSA group.

Table 2.2 – Time spent in each sleep stage

	non-OSA (AHI<15)		OSA (AHI≥15)	
Stage	min	%	min	%
1	33.9±4.2	10.4±1.2	45.4±2.3*	15.3±0.8*
2	153.6±7.2	47.4±1.5	148.1±3.7	47.8±0.9
3	50.3±3.3	16.5±1.1	50.2±2.4	16.0±0.6
4	37.0±3.4	11.7±1.1	22.3±2.0*	7.2±0.7*
REM	54.3±3.6	16.2±0.8	50.6±2.0	15.7±0.5
Total Sleep	316.2±10.1	100	310.1±5.7	100

Values are time (minutes) spent in each sleep stage and percentage of total sleep time, mean ±SEM. * indicates $p<0.05$ vs. non-OSA group

Figure 2.1: Respiratory and arousal event frequencies as a function of sleep stage



(A) All postures combined and (B) lateral and supine postures alone in OSA (AHI ≥15 /hr, n=171) and non-OSA (AHI<15 /hr, n=68) patients. Values are mean ± SEM.

2.4 Discussion

This is the first large systematic observational study of the effects of non-REM sleep stage on OSA severity. OSA patients demonstrated significantly reduced stage 4 and increased stage 1 sleep, and a significantly longer latency to achieve slow wave sleep compared to patients without OSA. There was striking non-REM sleep stage dependence in OSA severity in both OSA patients and non-OSA patients, with progressive reductions in respiratory and arousal event rates from stage 1 to stage 4 sleep, that were systematically improved during lateral sleep. Respiratory and arousal event rates were strongly associated throughout non-REM sleep.

Previous studies of sleep-stage dependence of OSA severity have generally focussed on REM versus non-REM sleep comparisons and shown elevated AHI in REM compared with non-REM[77, 200], while ignoring the changes in AHI within non-REM sleep. The present study, conducted in a large clinic patient population, has shown a marked reduction in AHI during SWS compared with stages 1 and 2 non-REM sleep. These findings are similar to those in previous preliminary reports[79, 80, 202] but extend these earlier observations by showing that the SWS effects are of a similar or greater magnitude than posture effects and that most patients, even those with moderate to severe OSA, can achieve low respiratory event rates in slow wave sleep. The magnitude of the improvement we observed amongst OSA patients in AHI during SWS versus stage 2 is similar to the *deterioration* in AHI observed in REM sleep compared with stage 2 sleep.

There are several possible reasons for the improvement in OSA during SWS. One possibility is that the upper airway becomes more neuromechanically stable with the onset of slow wave sleep. Alternatively, upper airway function could improve for some other reason, allowing patients to progress to deeper sleep. There is some evidence that the upper airway is better able to resist collapse during induced airway occlusion in SWS[190] but this finding was not replicated when passive airway function was measured by the P_{CRIT} technique[205]. In healthy volunteers, upper airway resistance is thought to be significantly higher during SWS than light sleep[132], despite genioglossal EMG activity being higher in this state[206] suggesting that if anything, the upper airway is neuromechanically disadvantaged in SWS.

An alternate reason for OSA improvement with increasing non-REM sleep depth may relate to sleep stage modulation of arousal propensity, and potentially to sleep stage modulation of post-arousal ventilatory responses. Poor airway function frequently triggers arousal and there is emerging evidence that arousal responses themselves promote conditions that predispose the airway to re-collapse[163]. The arousal index was strongly non-REM sleep stage dependent with a consistent relationship between arousal and respiratory indexes. Respiratory arousals from non-REM sleep are thought to occur when progressively increasing respiratory effort or drive reaches a particular effort threshold[164]. This threshold is significantly higher during SWS compared to light sleep[171 172]. Arousal frequency following respiratory loading is reduced during SWS in healthy subjects[173, 174]. Studies have also shown much higher thresholds to non-respiratory stimuli during SWS compared to stage 2 sleep[170, 207]. Thus, reduced propensity to arouse from sleep during SWS may be an important cause for improvement in OSA.

Although protective upper airway dilatory responses are thought to be impaired during sleep[117, 120], the upper airway does have the ability to respond to increasingly negative upper airway pressure and chemostimuli such as hypoxia and hypercapnia[107, 108], possibly in combination. Reduced arousal propensity during SWS may permit upper airway neuromuscular drive (chemo and/or mechanoreceptor mediated) to achieve sufficient activity to allow steady-state ventilation compatible with sustained sleep. Arousals from sleep may in fact promote ongoing cyclical breathing via post-arousal hyperventilation and a subsequent state of reduced drive[191], which may favour airway collapse. Theoretical models of OSA predict that a low arousal threshold predisposes to cyclical breathing[138], although this remains to be shown in OSA patients.

2.4.1 Methodological considerations

There are a number of limitations of this study. Given its observational nature, the causes of improved AHI during slow wave sleep remain speculative. Respiratory and arousal events were scored according to AASM and ASDA criteria respectively. Consequently, sub-criterion events, either respiratory or arousal were not considered. However, given that the same criteria were applied across all sleep stages by a single technician within each patient, altered classification criteria and inter-patient scoring differences are unlikely to substantially influence the main within patient findings of this study. Our non-OSA group comprised of clinic referred patients and may not be a “normal” group. Nevertheless, progressively lower respiratory and arousal event rates across non-REM sleep stages, regardless of

sleep posture, in non-OSA and OSA patients remains consistent with important non-REM sleep stage effects on OSA severity, regardless of overall OSA severity. It seems that regardless of the level of underlying anatomical compromise and propensity for upper airway collapse, most people can overcome this in SWS and achieve normal or near normal respiratory and arousal event frequencies.

2.4.2 Summary and implications

We conclude that both OSA and non OSA subjects demonstrate marked reductions in respiratory and arousal event rates during slow wave compared to light non-REM and REM sleep. Further investigation into potential differences in upper airway behaviour and arousal responses to ventilatory challenges in light compared to SWS are warranted, and may lead to an improved understanding of the pathophysiology of OSA and ultimately, to improvements in therapy for this disorder.

CHAPTER 3. A simple circuit to simultaneously provide continuous positive airway pressure and "clamp" end-tidal CO_2 during hyperventilation

3.1 Introduction

CO_2 disturbances and abnormalities in ventilatory control potentially contribute as key pathogenic mechanisms in both central and obstructive forms of sleep apnoea. Patients with heart failure, where central sleep apnoea is particularly prevalent[208, 209], frequently present with Cheyne-Stokes breathing, a cyclical pattern of progressively increasing ventilation frequently leading to an arousal, followed by progressive decreases in ventilation leading to a central apnoea. This disorder can be associated with significant sleep fragmentation[208], periods of hypoxia[208] and daytime sleepiness[210]. These patients are thought to have a sleeping eupnoeic pCO_2 closer to their apnoeic threshold[211], as well as an elevated ventilatory response to CO_2[212, 213]. Consequently, perturbations in ventilation, such as with a spontaneous arousal may cause the patient's pCO_2 to drop below their apnoeic threshold, leading to apnoea[198], a subsequent period of hyperventilation and consequently a self sustaining cyclical pattern of breathing. A prolonged circulation time sculpts the duration of the hyperpnoeic period and thus cycle length, but does not appear to be required to initiate the cyclical breathing[214]. Many patients with heart failure have overlapping obstructive and central sleep apnoea[215].

There is increasing evidence that the causes of obstructive sleep apnoea are multifactorial[76] and that ventilatory instability may play a key role in some patients

[160] in whom periods of upper airway collapse may coincide with periods of low ventilatory drive[141].

Inhalation of low dose CO_2 has been shown to alleviate Cheyne-Stokes breathing[216, 217], idiopathic central sleep apnoea[218] and mixed obstructive / central sleep apnoea refractory to other forms of therapy[219]. Given that obstructive and central apnoeas frequently coexist, a device incorporating CPAP to splint open the upper airway to treat obstructive sleep apnoea, combined with a mechanism to maintain pCO_2 at eucapnic levels during post apnoeic hyperventilation, may provide superior control of disordered breathing in patients with mixed or potentially even predominantly obstructive disease.

Sommer et al[220] have previously described a simple breathing circuit designed to minimize changes in alveolar ventilation and end-tidal CO_2 during hyperpnoea. The beauty of this system is its simplicity and the passive nature of $ETCO_2$ control. That is, no active control circuits are required to deliver varying concentrations of CO_2 to the airway. One drawback however, is that an external source of CO_2 is required, thus somewhat limiting its clinical and practical utility in unsupervised settings. This design was modified by Banzett et al[221], essentially replacing the externally sourced CO_2 with the subjects' own expirate. However, neither of these circuits allow for the simultaneous provision of CPAP. In this study, further modifications to the latter device were developed and tested to allow the circuit to provide variable CPAP, without influencing the circuit's ability to "clamp" $ETCO_2$, i.e. maintain end-tidal CO_2 despite hyperventilation.

The purposes of this study were to 1) validate that $ETCO_2$ could be maintained during hyperventilation via a circuit allowing simultaneous delivery of CPAP (nominal pressures of 5 and 10 cmH_2O), and 2) to guide selection of an appropriate fresh gas inflow rate to most effectively 'clamp' $ETCO_2$.

3.2 Methods

3.2.1 Circuit design

The circuit is shown schematically in Figure 3.1. Fresh gas was supplied from a compressed variable flow source to fill a collapsible bag enclosed in a rigid container. A standard CPAP pump, connected to the inspiratory and expiratory limbs of the circuit via a T-piece, allowed pressurisation of the circuit. The subject inspired gas from the collapsible bag via inspiratory tubing, through a low resistance one way valve and into a facemask. The subject expired through a low resistance expiratory valve into large volume tubing acting as a deadspace reservoir. The expiratory valve incorporated a low-pressure pop-valve that allowed inflow when a threshold level of negative inspiratory pressure was exceeded. The distal end of the deadspace reservoir was connected to an expiratory port and, via the T-piece, to the CPAP pump.

When the subject's ventilation was less than or equal to the fresh gas flow (FGF), the subject did not collapse the bag, breathing completely from the inspiratory limb and out into the deadspace reservoir.

When ventilation exceeded FGF, the initial portion of the breath was inspired from the inspiratory limb and fresh gas. At this point, the bag collapsed and a more negative pressure developed in the mask, overcoming the bidirectional expiratory valve pressure and allowing inspiratory flow through the expiratory valve. The remainder of the breath was therefore comprised of deadspace gas from the expiratory limb of the circuit. Since the deadspace gas has a pCO_2 close to that of mixed venous blood, this gas would not participate further in gas exchange, thus effectively clamping the subject's $PaCO_2$ at close to eucapnic $P_{ET}CO_2$, independent of the level of hyperventilation.

For the purposes of this experiment, the fresh gas provided was medical grade air, with the flow determined using a flow meter.

3.2.2 Subjects

Eight healthy subjects were recruited by advertisement at a local University following approval from the Repatriation General Hospital Research and Ethics committee. All subjects gave written informed consent. The subjects were all non smokers and were not taking any medications at the time of the study. Five males and three females were recruited with a (mean $\pm$ SEM) age of 21.8 $\pm$ 0.9 years.

3.2.3 Experimental protocol

Each subject was studied during control and clamped conditions. During the control condition, subjects breathed via standard 20.5 mm internal diameter corrugated tubing (Resmed, North Ryde, NSW, Australia) and nasal mask (ComfortGel^{TM} Philips-Respironics, Murrysville, PA) connected to a CPAP pump (Rescare, North

Ryde, NSW, Australia). A pneumotachometer (Hans Rudolph Inc, Kansas City, USA) was inserted between the mask and the expiratory port to allow measurement of inspiratory and expiratory flow. Each subject was asked to breathe through their nose and their mouth was taped to prevent leak. Subjects sat in front of a computer screen, displaying tidal volume in real time. An initial 10 minute period of acclimatisation to breathing at 5 or 10 cmH_2O CPAP was followed by 1 minute interventions during which the subject was asked to hyperventilate at 200% and 400% of their baseline tidal volume, while maintaining breathing frequency at the baseline level. Between interventions, the subject returned to baseline breathing for 3 minutes. The display screen was only switched on during interventions. The order of breathing interventions and CPAP pressures was randomised.

The circuit was constructed such that the collapsible bag, fresh gas inflow meter and CPAP pump were in an adjacent room, with an intervening soundproof wall. The baseline FGF was determined as the minimum flow required to allow the collapsible bag to visually collapse at end inspiration, without allowing deadspace rebreathing, during the initial 10 minute period of acclimatisation. To assess the effect of a reduced gas inflow rate consistent with correction of mild hyperventilation during wakefulness combined with the normal drop in ventilation at sleep onset, CO_2 clamping was performed at 2 different levels of FGF; 100% and 80% of baseline FGF.

3.2.4 Measurements

Inspiratory volume was determined by integration of the pneumotachometer flow signal. Mask CO_2 was determined by sampling the expirate (POET II 602-3, Criticare systems, Waukesha, WI, USA) through a length of Nafion tubing (Permapure NJ) to prevent condensation. A perforated tube was threaded outside the nasal mask and connected to a CO_2 analyser to act as a leak detector (POET II 602-3, Criticare systems, Waukesha, WI, USA). Mask pressure was determined continuously by pressure transducer (Becton Dickinson, Franklin Lakes, NJ, USA). All signals were recorded via a computerised data acquisition system (DI720, DataQ Instruments, Akron, OH, USA) at 200Hz per channel. Breath timing (inspiratory, expiratory and total breath time), inspiratory tidal volume (V_T), minute ventilation ($\dot{V}$), actual CPAP level (mask pressure at end expiration), inspiratory circuit resistance (slope of the flow vs. mask pressure throughout inspiration) and $ETCO_2$ (peak expiratory CO_2) were determined breath-by-breath using custom software developed in our laboratory. For each variable, breath-by-breath measurements were averaged over the final 30-sec of the pre-intervention baseline and the last 30-sec of each 1-min intervention period.

3.2.5 Statistics

Analysis of variance for repeated measures was performed to compare changes in $ETCO_2$ from baseline between the different conditions (5 vs. 10 cmH_2O CPAP, level of clamping; CPAP circuit only, 100% FGF and 80% FGF). SPSS version 12.1 was used for statistical analysis. Significant ANOVA effects were explored using post-hoc Student's *t*-tests corrected for multiple comparisons using the Dunn-Sidak

procedure[204]. Dunn-Sidak corrected Student's *t*-tests were also performed to test for significant changes in end-tidal CO_2 between the preceding baseline and each intervention condition. Data are expressed as means ± SEM, p values <0.05 were considered significant.

3.3 Results

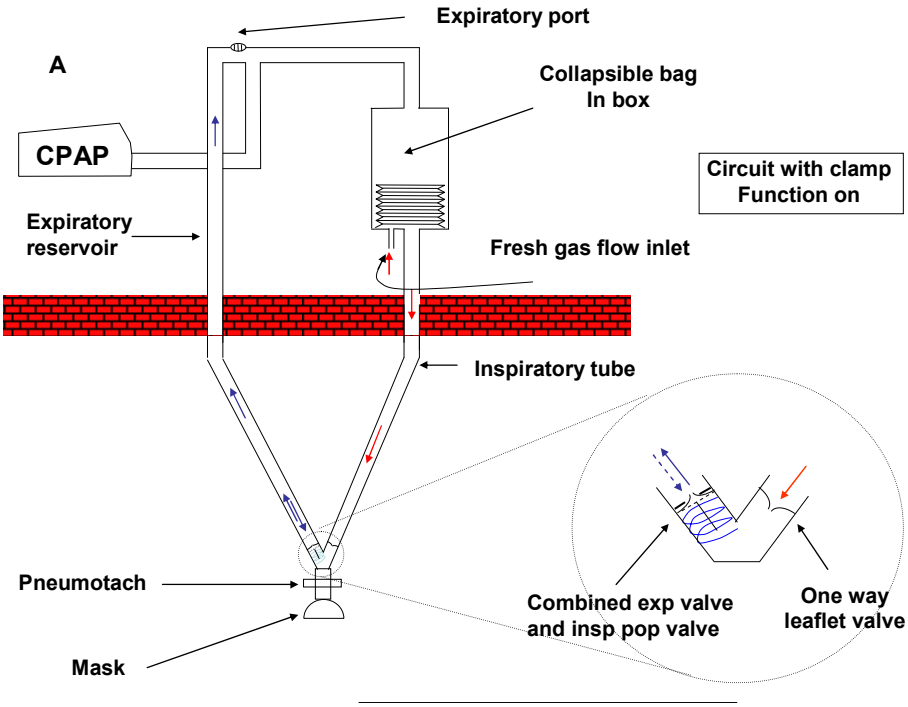
As requested, subjects achieved voluntary hyperventilation at close to the target levels: 202.8 ± 5.7% of baseline for 200% target ventilation and 326.6 ± 16.5% for 400% target hyperventilation. This was achieved almost entirely via increases in tidal volume, with no significant change in breathing frequency (p=0.09). Absolute values for VT increased from 0.58 ± 0.02L baseline to 1.16 ± 0.41 and 2.14 ± 0.1L for 200% and 400% ventilation respectively. Measured CPAP was 4.4 ± 0.1 cmH_2O and 9.1 ± 0.1 cmH_2O for the nominal 5 and 10 cmH_2O CPAP conditions respectively.

Hyperventilation on CPAP without CO_2 clamping produced marked ventilation-dependent reductions in $ETCO_2$ in the order of 6-10 mmHg (p<0.01, Figure 3.2). In contrast, $ETCO_2$ reductions were significantly attenuated with CO_2 clamping, with significantly greater attenuation with 80% versus 100% FGF (FGF effect p<0.001) and greater inflow-dependent attenuation with the 400% versus 200% level of hyperventilation (FGF x ventilation p=0.001). With the exception of the 5 cmH_2O, 100% FGF condition, hyperventilation produced only small (≤~2 mmHg) and non-significant decrements in $ETCO_2$ with CO_2 clamping at 100% or 80% FGF. Whilst there was no significant effect of CPAP level on $ETCO_2$ changes, there appeared to be overall trends for smaller decrements in $ETCO_2$ at 10 vs. 5 cmH_2O CPAP during

clamping (pressure effect p=0.097) and a pressure x ventilation interaction (p=0.06). Figure 3.3 shows an example of one subject hyperventilating at 10 cmH_2O CPAP pressure on and off the clamp circuit.

Figure 3.4 shows the effects of circuit type and level of ventilation on inspiratory resistance. There were significant increases in inspiratory resistance from baseline, when using the clamp device compared to the CPAP circuit alone, $p<0.001$. The level of ventilation was also associated with increased resistance ($p<0.001$) with a significant interaction between the two ($p<0.001$). The highest resistance was noted during the 80% clamp condition at 400% level of ventilation. The level of CPAP had no effect on inspiratory resistance (p=0.996).

Figure 3.1 : Schematic of CO_2 clamping circuit with and without CO_2 clamp



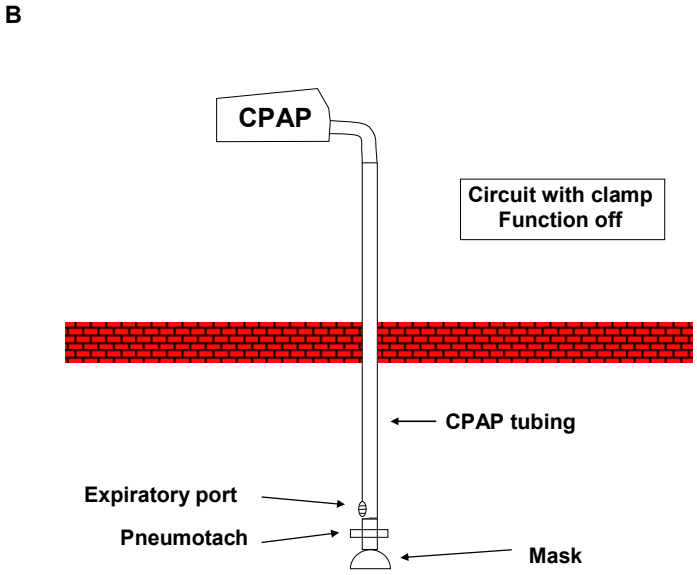
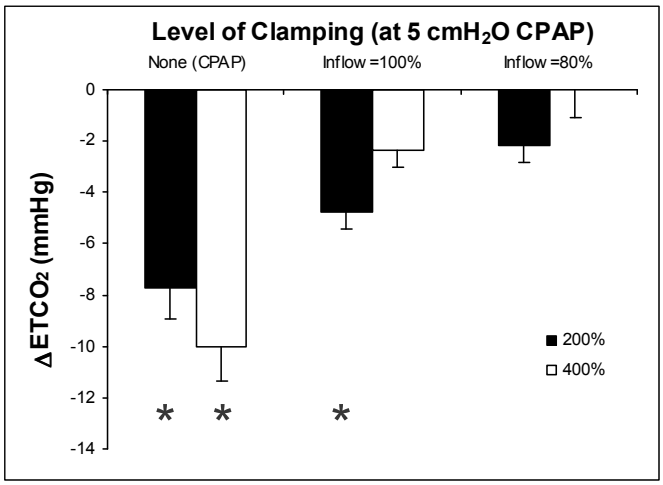
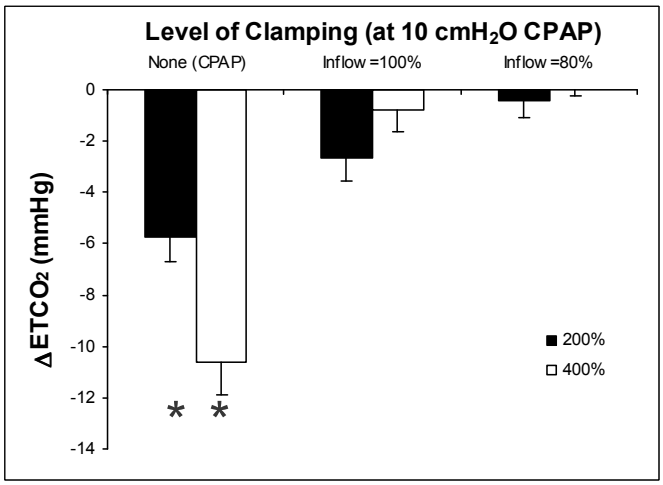


Figure 3.2: Change in end-tidal CO_2 during different conditions

A



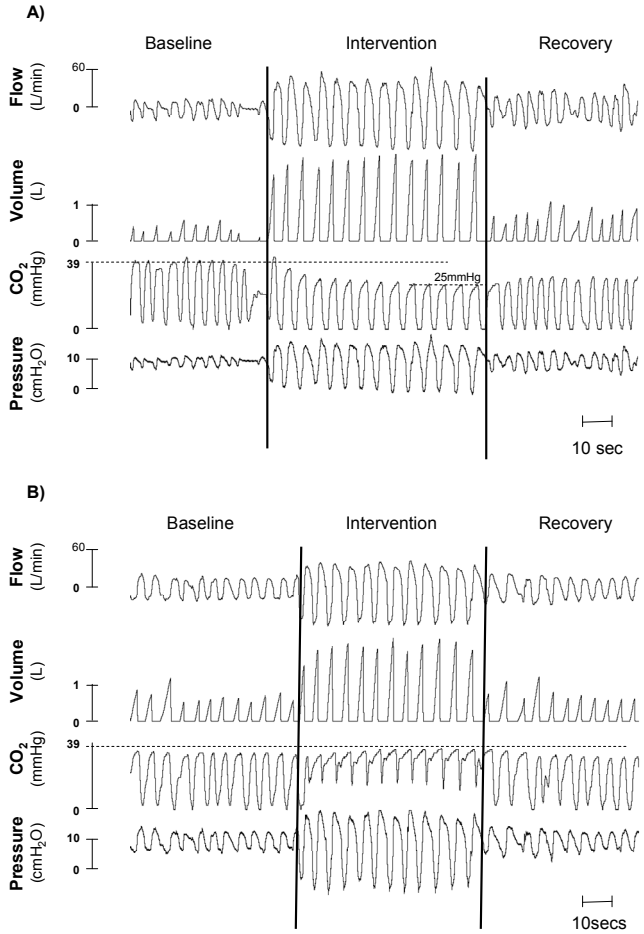
B



Levels of $ETCO_2$ falls for the conditions of; CPAP circuit alone, clamp circuit at 100% FGF and 80% FGF, during 2 different levels of hyperventilation (200,400% of baseline ventilation) at 5 cmH_2O (A) and 10cmH_2O (B) respectively. n=8

* = interventions during which there were significant drops in $ETCO_2$ compared to baseline, $p<0.05$

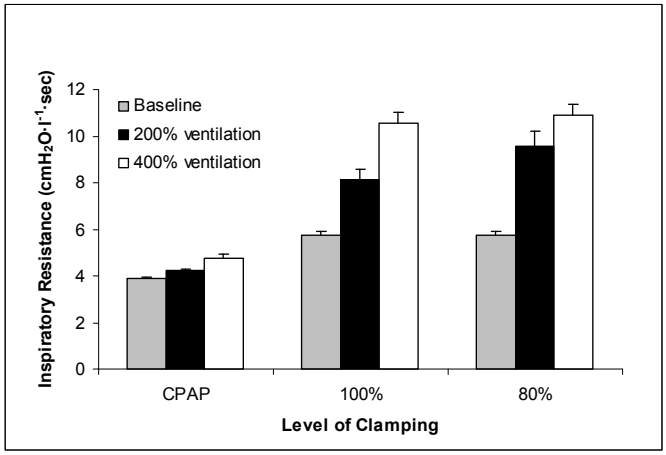
Figure 3.3: Example of one subject breathing via CPAP + clamp circuit vs. on CPAP only



A) One subject on 10cm H_2O CPAP, hyperventilating at 400% baseline ventilation, breathing through CPAP circuit alone, with significant drop in $ETCO_2$

B) Same subject breathing at same ventilation level and airway pressure through clamp circuit, no significant change in $ETCO_2$

Figure 3.4: Inspiratory circuit resistance under different ventilation and clamping conditions



Inspiratory resistance for pre-intervention baseline, hyperventilation at 200% and 400% of baseline, during conditions of CPAP circuit alone, clamp circuit at 100% FGF and 80% FGF. Results for both 5 and 10cm CPAP have been combined in this figure.

3.4 Discussion

This circuit was able to maintain end-tidal CO_2 despite substantial voluntary hyperventilation in awake subjects, and was able to be pressurised independently of the level of fresh gas inflow without interfering with end-tidal CO_2 clamping efficacy. Trends for CPAP pressure dependant differences in hyperventilation-induced $ETCO_2$ decrements between 5 and 10 cmH_2O are likely explained by a greater lung volume with the higher pressure.

This circuit is a modification of the Banzett design[221], which has been used previously in experimental protocols both in awake[222] and sleeping[195] subjects to maintain end-tidal CO_2 during either hypoxia or auditory tone induced arousal from sleep in healthy subjects. If similar studies of physiological arousal were to be attempted in subjects with sleep disordered breathing, they would likely be limited by recurrent upper airway obstruction and consequent sleep fragmentation. The incorporation of continuous positive airway pressure to the Banzett circuit to stabilise the upper airway potentially enables future studies of the role of $ETCO_2$ in cardiorespiratory responses to arousal to be performed in these patients.

However, the greatest potential application for this device is for the treatment of patients with mixed obstructive and central sleep apnoea in whom central events are likely a manifestation of unstable respiratory control that is poorly treated with CPAP alone[219]. Patients with heart failure particularly, have high incidences of both obstructive and central sleep apnoea[208, 209] and are therefore most likely to benefit from this treatment.

Treatment with CPAP alone for patients with central sleep apnoea is usually suboptimal, the sleep disordered breathing being rarely normalised[223, 224]. A long term study using CPAP in patients with central sleep apnoea[225] also found no significant improvements in long term outcomes such as survival and/or cardiac transplantation[225]. A proportional assist ventilator device (CS Auto, Resmed) has been shown to be much more effective than CPAP in treating Cheyne-Stokes respiration in the short term[226, 227]. However the cost of this device is high and prohibits general use. A device that applies positive airway pressure as well as stabilising respiratory control by preventing post-apnoea hyperventilation / hypocapnia may have considerable therapeutic utility[219]. The device described by Thomas et al[219] uses stored CO_2 in a cylinder and a sophisticated detection and servo-controlled system for minimising hypocapnia. Our device is much simpler and uses the subject's own expired breath for clamping CO_2 at eucapnic levels during periods of hyperventilation.

There are some limitations to this device and experimental circuit. The inspiratory resistance was high and became more elevated with higher levels of clamping. While the CPAP tubing and pneumotachometer device no doubt contribute to this, the biggest contributor is likely the clamp device itself. During clamping, the two way expiratory pop-valve has to be overcome to enable re-breathing from the deadspace chamber, however this acts as an inspiratory resistive load. The requirements to miniaturise the device and reduce circuit resistance to improve patient tolerance are challenges that will need to be addressed before the device is likely to be generally applicable in the clinical setting.

3.4.1 Conclusion

A circuit which passively maintains end-tidal CO_2 despite significant voluntary hyperventilation and which is able to be pressurised independently was successfully developed and tested. This circuit is therefore appropriate for use in experimental protocols designed to control and/or manipulate CO_2 in patients with sleep disordered breathing where a stable end-tidal CO_2 is required along with splinting of the upper airway. Potential clinical uses could involve treating patients with a combination of obstructive and central sleep apnoea. However, increased inspiratory resistance during hyperventilation may potentially limit tolerance and efficacy during sleep.

CHAPTER 4. The influence of CO_2 on upper airway and ventilatory function during sleep in patients with obstructive sleep apnoea

4.1 Introduction

There is increasing recognition that both anatomical and non-anatomical factors play a role in the pathogenesis of obstructive sleep apnoea (OSA)[89, 228]. Patients with OSA characteristically have an anatomically smaller upper airway lumen than non-apnoeics[39, 41, 42]. While anatomy may be compromised further with changes in head position[67, 68] and body posture[70], such factors alone cannot fully account for substantial changes in sleep apnoea severity across the night. Our group and others have shown that OSA frequency improves significantly during slow wave sleep (SWS)[79, 80, 202, 229] and is worse in REM[200] and stage 1 sleep[229]. Younes[76], showed that even patients with severe OSA show periods of stable breathing at least some of the time, despite mechanical loads that would be expected to cause cyclical breathing. Clearly, basic upper airway anatomical deficiencies persist, with overnight variability in sleep apnoea severity likely indicative of important ventilatory and/or upper airway neuromuscular control influences changing over time. The overall aim of these experiments was to determine the effect of relatively small perturbations in $PaCO_2$ above and below eucapnic levels on upper airway stability during sleep in OSA patients.

During periods of flow limitation in sleep the upper airway is traditionally modelled as a Starling resistor, with rigid segments upstream and downstream of a

collapsible segment[52, 54, 56], which then exhibits inspiratory flow limitation independent of downstream pressure and consequently ventilatory drive. While clearly a very useful model, upper airway neuromuscular activity is known to be importantly modulated by ventilatory drive inputs to upper airway muscles[105, 108], and negative intraluminal pressure mediated reflexes[102, 105, 120]. Consequently, neuromuscular activation during periods of airway obstruction has the potential to alter upper airway airflow mechanics from that of a 'pure' Starling resister. In an animal model of an isolated upper airway decreased upper airway collapsibility was observed during hypercapnia suggesting that ventilatory drive inputs to dilator muscles are important[98]. In healthy sleeping humans, the data are somewhat conflicting. In one study exogenous CO_2 administration to increase end-tidal CO_2 to approximately 50 mmHg did not appear to influence genioglossal activity significantly[106]. Similar findings were reported by the same group in another study although, when combined with inspiratory resistive loading, moderate hypercapnia (5-6 mmHg above eucapnic levels) did appear to have an independent stimulatory effect on genioglossal activity[107]. A subsequent study by the same group in healthy volunteers showed a small absolute increase in peak genioglossal activity (9-12% of maximum EMG possible) with 5 to 10mmHg rise in end-tidal CO_2[108]. The stimulatory effect was similar on and off CPAP suggesting that hypercapnia-induced increases in EMG activity were not mediated indirectly via enhanced respiratory pump muscle drive and negative airway pressure reflexes. Together these studies suggest that moderate hypercapnia, particularly in the presence of increased upper airway loading, can augment pharyngeal dilator muscle activity during sleep in humans. However, the functional effect of these relatively small increases in EMG activity on the airway is unclear. Further, these results in young

healthy subjects may not be directly applicable to patients with sleep apnoea who may have a reduced ability to mount a compensatory neuromuscular response to airway collapse, compared to healthy controls[134]. Therefore, the first aim of this study was to determine whether mild hypercapnia would prevent or mitigate the effects on ventilation of sudden, partial upper airway obstruction in sleeping OSA patients.

Another cause of ventilatory instability in OSA that may be importantly influenced by fluctuations in arterial CO_2 partial pressure is arousal. Arousal from sleep is associated with significant hyperventilation[191, 193] which is immediately followed by ventilatory undershoot or hypoventilation[163]. Airway collapsibility is increased during periods of low ventilatory drive in OSA[141]. Hypoventilation after arousal-induced hyperventilation might be a direct consequence of the hyperventilation (i.e. neural reflex inhibition)[230] or withdrawal of homeostatic stimuli (development of hypocapnia[231]). Post-arousal hypocapnia may therefore critically influence the propensity for airway recollapse and therefore respiratory event frequency in OSA. The second aim of this study therefore was to determine whether clamping CO_2 levels would ameliorate post-arousal hypoventilation during experimentally induced partial upper airway obstruction during sleep in OSA.

4.2 Methods

4.2.1 Patients

The study was approved by the Repatriation General Hospital Research and Ethics Committee and 10 subjects with moderate to severe OSA were recruited after

providing written informed consent. Patients were identified by retrospective review of diagnostic polysomnography studies showing OSA with an overall Apnoea-Hypopnoea Index (AHI) >30/hr. To recruit patients who may be able to compensate for an unfavourable upper anatomy at least some of the time, patients were required to show AHI improvement during SWS (at least 40% less than the AHI during stage 1/ 2 non-REM sleep in the same posture) with at least 5 minutes of SWS available for assessment. Given that subjects were required to sleep comfortably with positive airway pressure, patients were required to have been on CPAP therapy for a minimum of 3 months, with a stated subjective compliance of at least 4 hours usage every night. Patients were excluded from participation if they were on medications potentially affecting respiration and/or causing sedation such as opiates or benzodiazepines. Subjects with other sleep disorders, circadian rhythm abnormalities, pre-menopausal women and post-menopausal women on hormone replacement therapy were also excluded, given the possible effects of hormonal influences on upper airway function[232].

4.2.2 Equipment

The breathing circuit (Figure 4.1) was designed to allow delivery of CPAP at the same time as end-tidal 'CO_2 clamping' via delivery of a fixed flow rate of fresh gas as the inspirate, with any additional ventilatory demand met via rebreathing from the expiratory limb of the circuit. This was a modification of an existing circuit[221]. A modified CPAP device, able to rapidly switch between therapeutic and sub-therapeutic airway pressures was connected via a T-piece to the inspiratory and expiratory limbs of the circuit. On the inspiratory side, a box containing a bag filled via compressed medical air at a flow rate close to the patients' minute ventilation

allowed delivery of fresh inspirate under CPAP conditions. Patients breathed via a nasal mask (Comfortgel, Philips-Respironics, Murrysville, PA) attached to a custom-made breathing valve containing a low-resistance valve (Hans Rudolph, Shawnee, KS, USA) on the inspiratory side and a lightly spring-loaded expiratory valve permitting retrograde flow when inspiratory flow exceeded flow delivery from the inspiratory limb of the circuit. A small leak near the T-piece served as the final expiratory path. A 3-way tap and by-pass containing a one-way valve served to allow more conventional CPAP delivery during periods without end-tidal CO_2 clamping. Flow was measured via 2 separate pneumotachographs on each limb of the circuit.

EEG (C3/A2 and C4/A1 locations), ECG and bilateral submental EMG and EOG were recorded. Continuous SaO_2 measurement was performed (Novametrix Oxypleth, Soma Technology, CT). The expirate was sampled at the mask to determine the end-tidal CO_2 (Capstar-100, CWE Inc, PA) via tubing incorporating 30 cm of Nafion tubing (Permapure, NJ) to prevent condensation from blocking the sample line. A perforated tube was threaded around the nasal mask and connected to a CO_2 analyser to act as a qualitative leak detector (POET II 602-3, Criticare Systems, Waukesha, WI, USA). A solid state pressure transducer (Spectramed DTX, Oxnard, USA) was used to measure mask pressure. Abdominal and thoracic excursions were measured (Thoracoabdominal bands, Compumedics, Abbottsford, Victoria, Australia) as was body position.

Data were acquired using 2 recording systems. One system (Compumedics S series, Abbottsford, Victoria, Australia) was used for recording EEG, EOG, EMG,

ECG, SaO_2, body position and an event channel. Sleep staging and scoring of arousals was performed using this system. A second system (WinDaq, DataQ instruments Inc, OH) was used to record inspiratory and expiratory flow, mask pressure, end-tidal CO_2, SaO_2 and the mask leak signal, all sampled at 200Hz. An event mark was placed simultaneously on both systems, prior to each intervention to link both systems in time.

4.2.3 Protocol

Patients were advised to attend the laboratory 1 hour before their usual bedtime, having abstained from caffeine and alcohol for 24 hours. Patients were instrumented as described above and their mouths taped to prevent leak. Patients slept with 1 pillow and wherever possible in the supine posture. Patients commenced CPAP at their established therapeutic pressure, 1-2 cm above the pressure where inspiratory flow limitation was abolished. This pressure was maintained for the night as the baseline pressure. Brief dialdowns were used to select a sub-therapeutic pressure that produced significant flow limitation, with~50% reduction in peak flow. This pressure was subsequently used as the dialdown pressure for the remainder of the night. Throughout the night, 2 minute dialdown interventions were delivered following 30 second periods of stable non-REM sleep. Three different types of interventions were performed in order to examine the effect of a brief period of hypercapnic stimulation prior to challenging the upper airway vs. a prolonged period of stimulation before and during the challenge on post-dialdown ventilatory and upper airway responses. These were; 1) No hypercapnia/CO_2 clamping during the baseline or dialdown periods (control), 2) CO_2 clamping during the 30 second baseline period alone (baseline only

clamping) and 3) CO_2 clamping throughout the baseline and dialdown periods (CO_2 clamp). CO_2 clamping with mild hypercapnia was achieved via a fresh gas inflow rate of 80% baseline minute ventilation. Interventions were performed in random order throughout the night, allowing at least 1 minute between interventions on therapeutic CPAP to allow re-establishment of stable ventilation in sleep.

4.2.4 Analysis

Sleep staging and arousal scoring was performed by a technician blinded to the respiratory signals, using standard rules[10, 233]. Given sleep stage effects on arousal threshold and possibly ventilatory variables, analysis was limited to interventions performed during Stage 2 non-REM sleep. During CO_2 clamping, inspiration could occur from both the inspiratory and expiratory limbs of the circuit. Consequently, inspiratory flow was calculated from the sum of the inspiratory signals from both pneumotachometers to give total inspiratory flow. Ventilatory parameters including inspiratory, expiratory and total breath time (T_I, T_E and T_{TOT}), tidal volume (V_T), peak inspiratory flow (PIF), minute ventilation (V_I), end-tidal CO_2 ($ETCO_2$), mask pressure at zero flow prior to inspiration (Pmask baseline) and nadir inspiratory pressure (minInsp Pmask) were calculated breath-by-breath using custom analysis software. Total circuit inspiratory resistance was calculated by measuring the slope of mask pressure against total inspiratory flow. Time to arousal (TTA) was determined as the time from dialdown to the onset of EEG arousal. Breath-by-breath variables from replicate interventions were averaged within subjects for the 5 baseline breaths prior to each dialdown, the first 5 post-dial down breaths pre-arousal, and for the last 2 breaths before and first 5 breaths after EEG arousal. For

the purpose of this analysis, breaths were considered pre-arousal if inspiration was fully completed prior to the onset of EEG arousal.

4.2.5 Statistics

Linear mixed model analysis with an autoregressive covariate structure was used to examine the influence of intervention type and breath number on ventilatory responses to dialdowns and arousal (SPSS version 14, SPSS, Inc, Chicago, IL, USA). Significant intervention, breath number and interaction effects were further explored via selected post-hoc contrasts corrected for multiple comparisons using the Dunn Sidak procedure[204]. Cox regression survival analysis with robust standard errors (Stata version 10, Statacorp, Texas) was used to examine the effect of intervention type on time to arousal. All data are presented as group means ± SEM, $p<0.05$ was considered significant.

4.3 Results

Ten patients successfully completed the protocol. While posture was fixed throughout all interventions within each patient, 1 patient was unable to sleep in the supine posture. Data from the left lateral posture only were included for this individual. No interventions had to be excluded secondary to mask leak. Approximately 5.4 ± 0.9 control, 6.0 ± 1.1 Baseline only clamp and 6.4 ± 1.3 CO_2 clamp replicate trials were performed per subject.

Patient characteristics were as follows: 9/10 males, overall AHI 54.9 ± 4.8 /hr, SWS AHI 18.3 ± 3.3 /hr, Age 58.7 ± 2.4 yr, BMI 33.7 ± 1.8 kg/m².

Fig 4.2 shows examples from the three different types of intervention for one subject. No rebreathing occurs during baseline or dialdown periods in the control intervention. CO_2 rebreathing occurs during the baseline period only in the "baseline only clamping" intervention. CO_2 rebreathing occurs during both baseline and dialdown periods in the "CO_2 clamp" intervention.

Table 4.1 summarises ventilatory measurements during the 30 second pre-dialdown period in the three conditions. By design, end-tidal CO_2 was elevated in the two hypercapnia conditions. As a consequence, peak inspiratory flow, breath volume and minute ventilation were significantly increased with greater inspiratory decrements in mask pressure but with no significant change in breath timing. Baseline mask pressure was slightly but significantly elevated in the hypercapnia conditions. Similarly, there was a very small but consistent decrease in SaO_2 in the CO_2 clamp condition. Inspiratory circuit resistance was also significantly increased with hypercapnia.

Following dialdown from therapeutic CPAP, CO_2 remained relatively stable and elevated in the CO_2 clamp condition, rapidly rose then transiently declined over time in the control condition, and showed a very similar pattern of decline in the baseline only hypercapnia condition (Fig 4.3A, condition effect, $p<0.001$; condition x breath interaction, $p=0.001$; control vs. baseline only hypercapnia, $p=0.995$). There were no differences in dialdown pressures between conditions ($p=0.465$, Fig 4.3B). Dialdown caused a rapid decline in ventilation and PIF (Figures 4.3C,D), $p<0.001$, but with significantly higher post-dial down minute ventilation and peak inspiratory flow throughout the baseline only clamp and sustained CO_2 clamp conditions

compared to control, (condition effects p=0.023 and <0.001 for minute ventilation and peak flow respectively).

Dialdown from therapeutic CPAP consistently led to quite rapid arousal with no significant differences in arousal probability between conditions (Fig 4.4). Compared to the control condition, the hazard ratios for arousal in the baseline only clamp and sustained CO_2 clamp conditions were 1.03 (p=0.910) and 1.21 (p=0.320) respectively.

End-tidal CO_2 after arousal was higher in the sustained clamping condition, compared to control (p<0.001) and baseline only hypercapnia conditions (p<0.001), and there was a condition x breath number interaction effect (p=0.012, Fig 4.5A). Immediately post-arousal, there was a significant increase in ventilation in all three conditions followed by a transient decline (p<0.001, Fig 4.5B). Prevention of hyperventilation-induced relative hypocapnia in the sustained CO_2 clamp condition led to significantly elevated post-arousal ventilation compared to the control and baseline hypercapnia conditions (condition effect p=0.001, no significant interactions).

Table 4.1. Ventilatory variables in the 30 second baseline period prior to each dialdown intervention, (on therapeutic CPAP)

		Condition		
	Control	**Baseline only clamp**	**CO_2 Clamp**	**Condition effect p-value**
TI (s)	1.9 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	0.594
TE (s)	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	0.935
TTOT (s)	4.2 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	0.321
FB ($min^{-1)}$	14.6 ± 0.7	14.9 ± 0.7	14.7 ± 0.7	0.184
$PETCO_2$ (mmHg)	41.5 ± 0.9	45.5 ± 0.8*	45.2 ± 0.8*	<0.001
SaO_2 (%)	96.7 ± 0.2	96.5 ± 0.2*	96.4 ± 0.2*	0.003
VTi (L)	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.041
VI (L/min)	8.5 ± 0.3	10.2 ± 1.1*	9.9 ± 0.9	0.003
PIF (L/min)	29.1 ± 1.4	39.6 ± 2.6*	38.6 ± 2.4*	<0.001
Pmask baseline (cmH_2O)	13.1 ± 0.7	13.3 ± 0.8*	13.3 ± 0.7*	<0.001
minInsp Pmask(cmH_2O)	11.0 ± 0.7	9.7 ± 0.8*	9.7 ± 0.7*	<0.001
Rcircuit	4.0 ± 0.1	5.0 ± 0.2*	5.1 ± 0.1*	<0.001

* = $p<0.05$ versus control. n=10.TI = Inspiratory time, TE = Expiratory time, TTot = Duration of ventilatory cycle, FB = breath frequency, $PETCO_2$ = End tidal CO_2, SaO_2 = Oxygen saturation, VTi = Inspiratory tidal volume, VI = minute ventilation, PIF = peak inspiratory flow (non flow limited), Pmask = end expiratory mask pressure, minInspPmask = lowest inspiratory mask pressure, Rcircuit = Inspiratory circuit resistance.

Figure 4.1: Schematic of breathing circuit with clamp function on and off

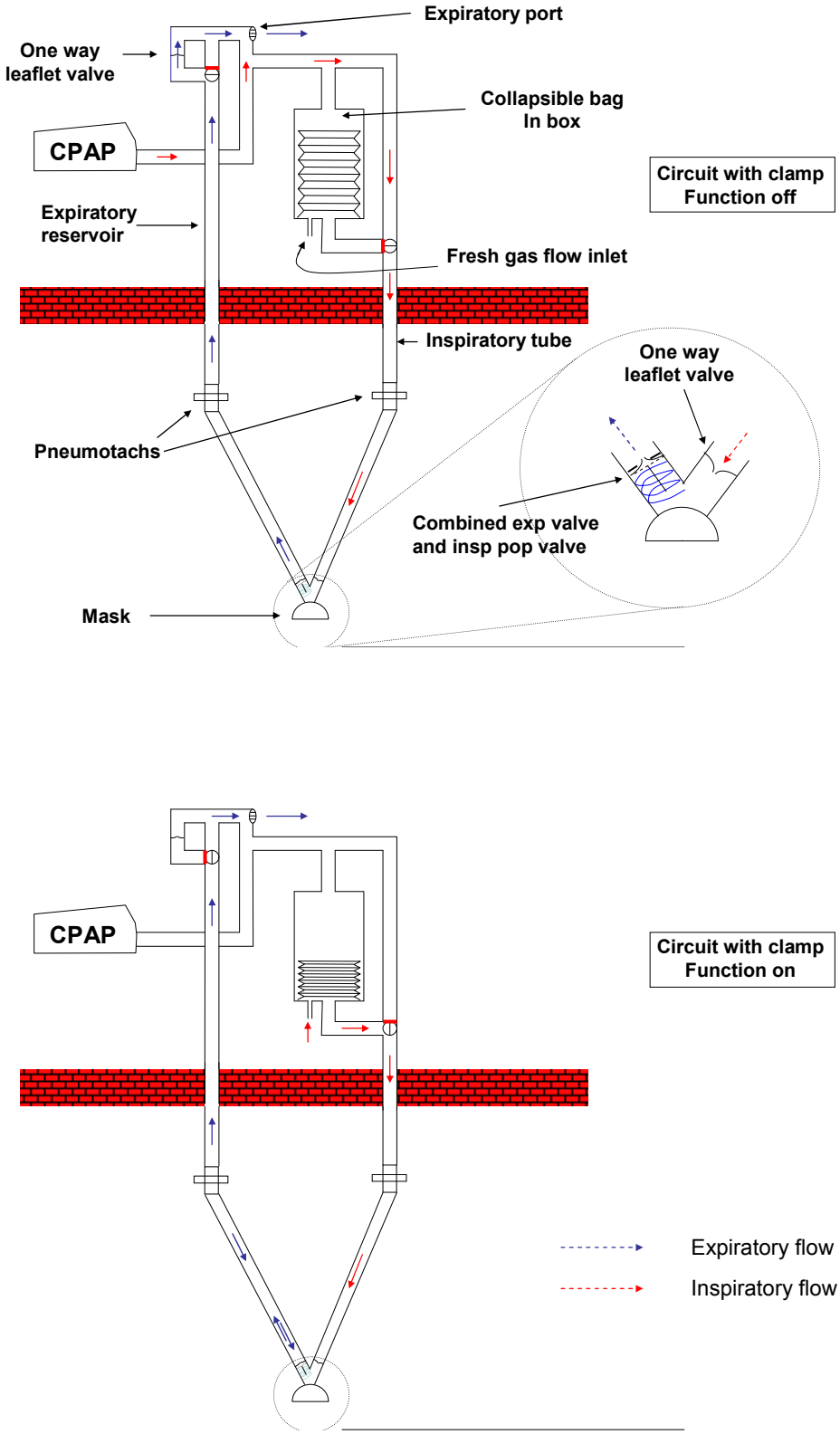


Figure 4.2: Examples of 3 different interventions in one subject, pre and post dialdown

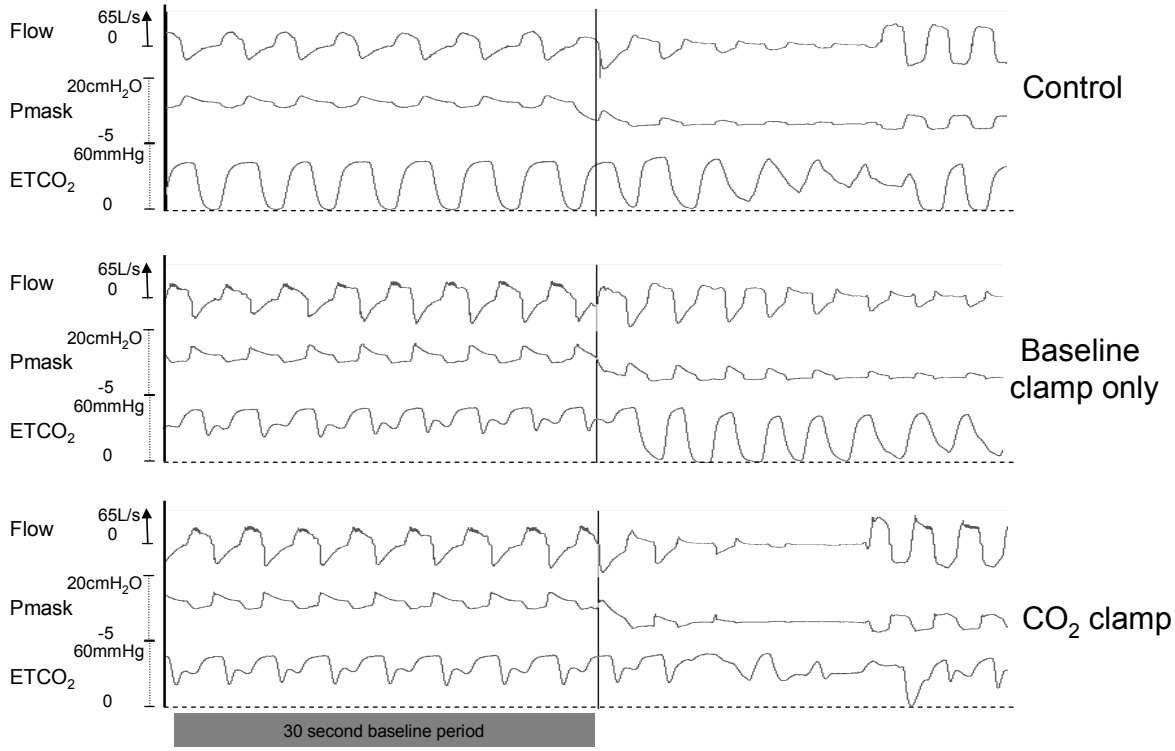
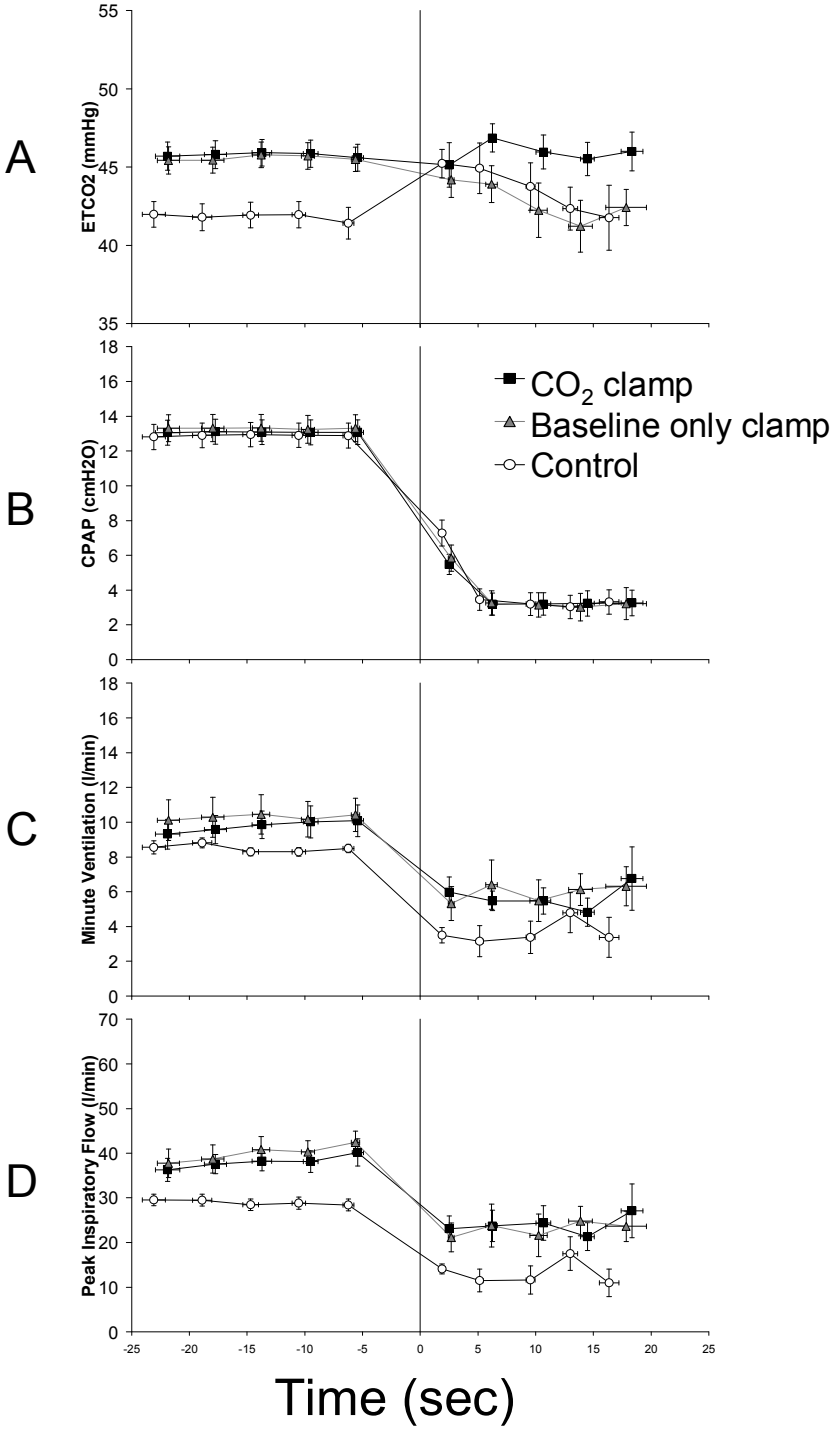
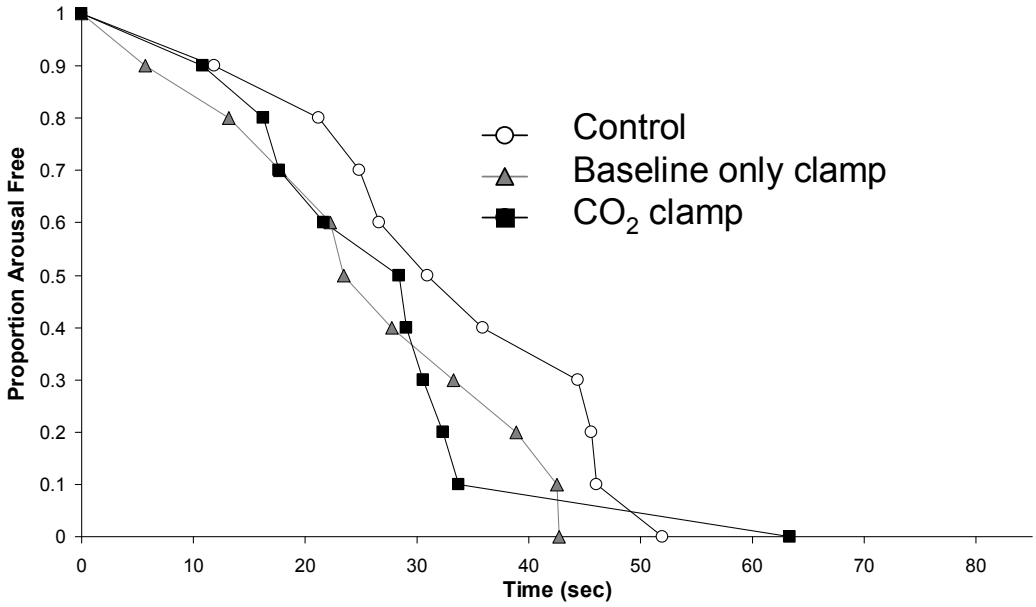


Figure 4.3: Ventilatory variables pre and post dialdown



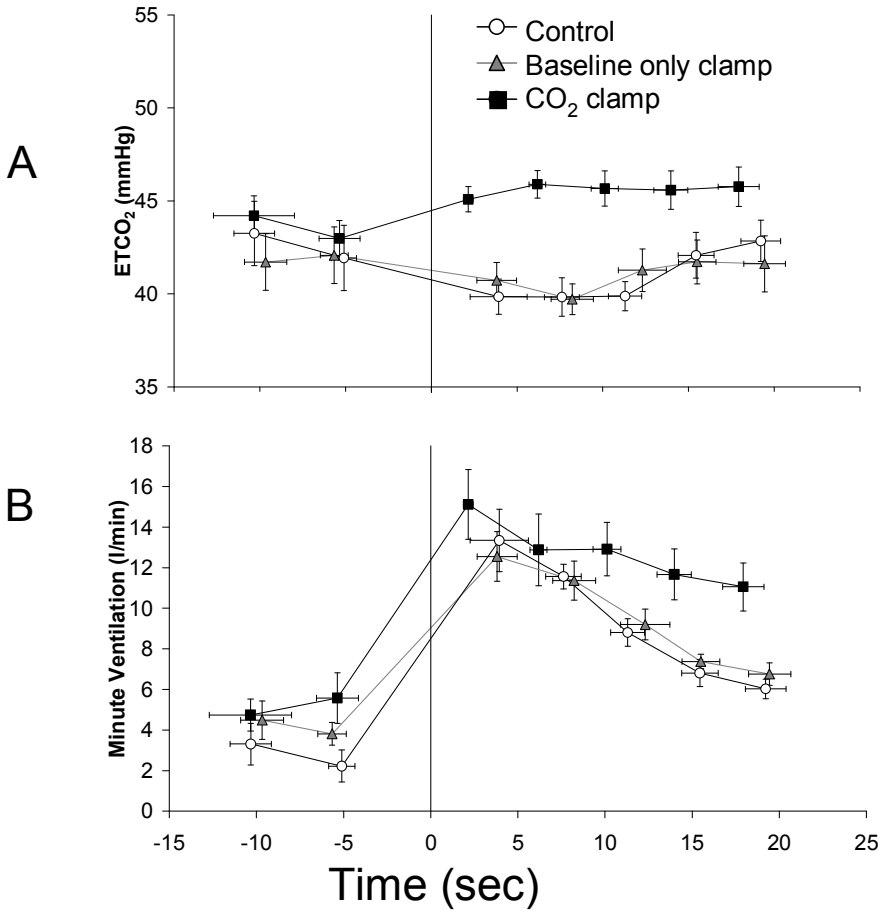
A, B, C, D represents end-tidal CO_2, CPAP level, minute ventilation and peak inspiratory flow respectively for five breaths pre- and post-dialdown under 3 different conditions; control, baseline clamp only, and CO_2 clamp. Vertical line represents onset of dialdown.

Figure 4.4: Survival to arousal post dialdown



The proportion of patients remaining arousal free as a function of post dial down time, averaged for replicate trials within each patient and condition.

Figure 4.5: Ventilatory variables pre and post arousal from sleep



A, B represent end-tidal CO_2 and minute ventilation respectively, for 2 breaths pre and 5 breaths post arousal under three different conditions; control, baseline only clamp and CO_2 clamp. Vertical line represents onset of arousal.

4.4 Discussion

There are two main findings in this study of the role of CO_2 in stabilising upper airway function in OSA patients during Stage 2 non-REM sleep. Firstly, during conditions of moderate-severe airflow limitation induced by sub-therapeutic CPAP, experimentally-induced mild hypercapnia increased inspiratory airflow by approximately 70% compared to eucapnic conditions. This suggests that even mild hypercapnia (i.e. within the range previously measured during sleep in normal subjects and snorers) has the potential to improve airway function in OSA. Secondly, following arousals induced by acute partial upper airway obstruction (i.e. CPAP dialdowns to sub-therapeutic levels), prevention of post-arousal hypocapnia by clamping end-tidal CO_2 significantly attenuated the post-arousal ventilatory decline. This suggests that hypocapnia caused by transient arousal-induced hyperventilation importantly contributes to subsequent hypoventilation and ventilatory undershoot and potentially therefore to a pattern of recurrent cyclical upper airway obstruction during non-REM sleep.

Previous studies in sleeping humans have suggested that in the flow limited state, the upper airway acts as a Starling resistor[52, 54, 56]. In a Starling resistor, when the pressure downstream to a collapsible segment is less than the critical closing pressure of the collapsible segment, flow becomes independent of the difference between nasal and supraglottic pressure, and dependent on the difference between the upstream pressure and the critical closing pressure, defined as the upstream pressure associated with total airway collapse and no airflow. An isolated dog upper airway model suggests that hypercapnia leads to decreased collapsibility of

the upper airway, an effect lost with muscle paralysis[98] consistent with an upper airway neuromuscular activation effect of hypercapnia.

There is some evidence that following immediate reductions in airway pressure from therapeutic CPAP, peak phasic and tonic genioglossal EMG activity progressively increase within the first few breaths post-dialdown[54]Counter intuitively however P_{CRIT}, a measure of passive collapsibility appears to simultaneously increase. Furthermore during REM sleep, peak phasic and tonic genioglossal activity are markedly reduced compared to non-REM sleep but with no difference in P_{CRIT} between these sleep stages[54]. These data suggest that increased genioglossus EMG activity is not necessarily sufficient to improve airway collapsibility. While several human studies have examined the genioglossal EMG response to hypercapnia[90, 102, 105, 107, 108, 120], the functional outcome of this increased activity in terms of maintaining airflow or ventilation is not known. Patients with sleep apnoea may have reduced ability to increase neuromuscular activity of the upper airway compared to controls[134]. However, this group has not been studied in terms of their airway response to hypercapnia. This current study extends the current literature by measuring the ability of mild hypercapnia to augment ventilation and peak inspiratory flow, and not simply upper airway dilator muscle EMG activity, in the presence of significant flow limitation, and by studying these effects in OSA subjects.

Despite the fact that the degree of hypercapnia in this study was mild, (~46mmHg during the pre-dialdown period, compared to other studies where 5 to 10 mmHg increases in end tidal CO_2 were utilized[106-108]),significant increases in peak flow

(~70%) and ventilation (~50%) occurred during flow limited breathing post dialdown in the clamped conditions, compared to control. Hypercapnia increases the drive to breathe and hence negative airway pressure in the context of airway occlusion[165]. The upper airway during sleep is thought to respond neuromuscularly to a combination of hypercapnia and negative airway pressure[107]. Thus in this case, it is not clear whether the improved airway function is solely on the basis of increased drive to upper airway muscles, or alternatively a reflex response to more negative airway pressures, in combination with hypercapnia.

We did not find that arousal propensity or timing was different between conditions, and all subjects aroused at least once during each 2 minute intervention. While this finding appears to contrast with a previous study showing a reduction in time to arousal following airway occlusion with hypercapnia[165], it may reflect milder hypercapnia and the non-occlusive stimulus of this study leading to more consistent development of the ventilatory drive stimulus most likely underpinning the majority of respiratory arousals[164].

In agreement with previous studies[191, 193], arousal from non-REM sleep was associated with substantial increases in ventilation that subsequently impacted on CO_2 homeostasis. In the CO_2 clamp condition, ventilation remained significantly elevated, closer to the pre-dialdown baseline level of ventilation and with a slower rate of post-arousal ventilatory decline. In the baseline only clamp condition, clamping had ceased prior to arousal, hence the results mirrored the control condition. Given evidence that low ventilatory drive states may promote airway collapse[141] and data from this study showing improved airflow with elevated CO_2,

attenuated ventilatory undershoot may help suppress the development of subsequent obstructive events.

Use of CO_2 clamping to prevent hypocapnia and maintain a mild degree of hypercapnia may also be potentially clinically useful in patients who exhibit abnormal ventilatory control and are poorly controlled on CPAP alone, such as patients with mixed obstructive and central apnoeas or complex sleep apnoea[234].

4.4.1 Methodological considerations

There are several limitations to this study. Minor differences in ventilatory variables were noted between conditions in the pre-dialdown period. Oxygen saturation was slightly lower in the CO_2 clamped state, presumably indicative of mild re-breathing. The CPAP pressure was fractionally but significantly elevated during baseline in the CO_2 clamped conditions and circuit resistance was significantly elevated. It is therefore possible that some of the condition effects noted post-dialdown may not be due entirely to the clamping of CO_2 *per se* but to other, pre-dialdown differences between conditions. However, given the small magnitude of these differences (oxygen saturations and inspiratory circuit resistance) between conditions, and the absence of any post-dialdown differences in CPAP, these small pre-dialdown differences appear unlikely to importantly influence the main findings. If anything, increased upstream circuit resistance should reduce peak flow and ventilation in the hypercapnic conditions, (the opposite of which was observed). The subjects in this study were selected to have significant reductions in OSA severity spontaneously during slow wave sleep. If these improvements during slow wave sleep occurred because of sleep stage induced differences in chemical drive /

upper airway responsiveness, these results may therefore not be able to be extrapolated to all OSA patients, during all stages of non-REM sleep. Likewise, it may not be possible to extend these results to REM sleep as particularly during phasic REM sleep, there may be significant reductions in responsiveness to chemical drive[235], as well as marked reductions in phasic genioglossal activity[236].

This study was designed to examine the acute effects of mild hypercapnia on ventilatory responses to a single dialdown pressure. While effects on airway collapsibility (e.g. P_{CRIT}) remain unknown, greater airflow at the same dialdown pressure clearly indicate improved airway function and imply either a more negative P_{CRIT} and/or steeper slope of the airway pressure versus peak inspiratory flow relationship.

Given the already bulky apparatus, to enable sleep onset to occur, no genioglossal EMG or upper airway pressure measurements were made. They are likely to have provided useful information re airway muscle activity and drive.

4.4.2 Summary and conclusions

In summary, mild hypercapnia improves peak flow and ventilation during periods of acute airflow limitation following rapid dialdown from therapeutic CPAP. In addition, clamping end-tidal CO_2 to prevent post-arousal hypocapnia attenuates ventilatory undershoot with arousal.

CHAPTER 5. Upper airway function and arousability to ventilatory challenge in slow wave versus stage 2 sleep in obstructive sleep apnoea

5.1 Introduction

In an observational study in patients with obstructive sleep apnoea (OSA), presented in Chapter 2, it was shown that the frequency of obstructive events is markedly reduced during slow wave sleep (SWS) compared to lighter non-REM sleep[229]. There are two main possible reasons for this observation: a) upper airway stability is permissive of SWS and/or b) SWS is conducive to upper airway stability. The first hypothesis proposes that development of upper airway and ventilatory stability allow progression into SWS, which would otherwise be more difficult with continued disordered breathing. The second hypothesis proposes that some feature of SWS *per se* stabilises upper airway function, allowing relative ventilatory and sleep stability. This could be due to an increased arousal threshold to respiratory stimuli[171, 172], which may simply allow for more time to stiffen and/or dilate the obstructed upper airway via mechano / chemo-receptor mediated stimuli[107, 108]. Alternatively, upper airway tone may be modulated by sleep stage, rendering the upper airway fundamentally more resistant to collapse or contributing to more responsive neuromuscular compensation to challenged upper airway function in SWS versus lighter sleep. It is also possible that as a result of an increased arousal threshold in SWS there are fewer arousals overall, and thus less tendency to post-arousal overshoot-undershoot[163] in respiratory drive that may help perpetuate cyclical airway collapse.

There are conflicting data regarding sleep stage specific differences in upper airway function. Upper airway resistance has been shown to be elevated during SWS[132]. When assessed by an upper airway occlusion technique, Issa et al[190] found that the upper airway in OSA patients was more resistant to collapse during SWS compared to stage 1 and 2 sleep. In apparent contrast, passive upper airway function assessed via the critical closing pressure (P_{CRIT}) technique does not appear to improve in SWS[205]. Increased genioglossus EMG activity during SWS compared to light sleep[206] could indicate improved upper airway drive and/or control, or simply reflect higher overall ventilatory drive associated with increased upper airway resistance[132]. In healthy volunteers, the hypercapnic ventilatory response[91] and the increase in respiratory effort following complete upper airway occlusion do not appear to be different between light and SWS[171]. These responses have not been examined in OSA patients, in whom there are pronounced sleep-stage differences in upper airway behaviour.

Given strong associations between respiratory and arousal events in non-REM sleep[229], known effects of sleep depth on arousal propensity[170, 171] and some evidence that ventilatory compensation mechanisms can overcome periods of airway obstruction without arousal[163], it was postulated that reduced arousability to challenged airway function is most likely the dominant mechanism for OSA improvement during SWS. However, improved upper airway function and/or ventilatory compensation responses in SWS remain possible. Consequently, the purpose of this study was to challenge at graded levels of partial and complete occlusion, upper airway function during stage 2 versus SWS in OSA patients to test the hypotheses that; 1) basic airway function and 2) compensatory ventilatory drive

and output responses are similar, but 3) delayed arousal via an increased arousal threshold permits greater ventilatory drive and compensation before arousal in SWS compared to stage 2 sleep.

5.2 Methods

5.2.1 Subjects

The Repatriation General Hospital Research and Ethics committee approved the study and 14 patients with moderate to severe OSA participated after providing written informed consent. To maximize the likelihood of finding differences in airway function between stage 2 and SWS, retrospective review of diagnostic polysomnography studies was used to select patients with predominantly obstructive events who showed significant improvement in SWS. Patients were considered eligible if they had a clinical diagnosis of OSA with an apnoea-hypopnoea index (AHI) > 30 /hr, showed at least a 40% improvement in AHI during SWS compared to stages 1-2 non-REM in the same posture, and with at least 5 minutes of SWS for assessment. Patients were required to have been on CPAP therapy for a minimum of 3 months, with compliance of at least 4 hours usage every night. Patients were excluded from participation if they were on medications which potentially affect respiration and/or cause sedation such as benzodiazepines or opiates. Pre-menopausal women and post-menopausal women on hormone replacement therapy were excluded, given possible effects on ventilation and upper airway function by the menstrual cycle and hormone replacement[232]. Patients with other sleep disorders and circadian rhythm abnormalities were also excluded.

5.2.2 Equipment

A P_{CRIT} research system (Philips-Respironics, Murrysville, PA) able to rapidly switch between 2 different airway pressures was attached via T-piece to the inspiratory and expiratory limbs of the breathing circuit. A computer controlled rapidly inflatable balloon occlusion valve was placed upstream from a low resistance pneumotachograph (Jaeger PT36) on the inspiratory limb and was attached via a 2 way inspiratory-expiratory breathing valve (Hans Rudolph, Series 2600) connected to a nasal mask (ComfortGel^{TM}, Philips-Respironics, Murrysville, PA) via large bore tubing (34 mm ID Clean Bor, Vacu Med, Ventura, Ca). On the expiratory side, corrugated CPAP tubing (ID 20.5 mm) was connected to an expiratory port connected to the T piece. Consequently, the inspiratory and expiratory limbs of the circuit received the same delivered pressure and the upper airway could be occluded under positive pressure conditions, see fig 5.1.

Sleep signals consisted of EEG recorded at C3/A2 and C4/A1 scalp locations, bilateral EOG, submental EMG, ECG and SaO_2 measured via finger pulse-oximetry (Novametrix Oxypleth, Soma Technology, CT). The expirate was sampled at the mask to determine the end-tidal CO_2 ($ETCO_2$ Capstar-100, CWE Inc, PA) via tubing incorporating 30 cm of Nafion tubing (Permapure, NJ) to prevent condensation from blocking the sample line. A perforated tube was threaded around the nasal mask and connected to a CO_2 analyser to act as a qualitative leak detector (POET II 602-3, Criticare Systems, Waukesha, WI, USA). Epiglottic pressure was measured by a catheter (2.1 mm OD, Microtube Extensions, Sydney, Australia) that was advanced under direct visualisation 1-2 cm below the tongue base, after decongestion of both nostrils (Oxymetazoline 0.05%) and lubrication with 2% Lignocaine gel.

Approximately 10, 1 mm diameter holes were cut radially around the distal 1 cm tip of the catheter. Catheter patency was maintained using low flow (1-2 ml/min) air perfusion. Pressure transducers (MP45, Validyne Engineering, Northridge, CA) were used to measure mask and epiglottic pressures.

Data were acquired using 2 recording systems. One system (Compumedics S series, Abbottsford, Victoria, Australia) was used for recording EEG, EOG, submental EMG and body position. Sleep staging and arousal scoring was performed using this system. A second system (WinDaq, DataQ instruments inc, OH) was used to record inspiratory and expiratory flow, epiglottic and mask pressures, $ETCO_2$, SaO_2, the mask leak signal and ECG, all sampled at 200Hz. An event mark generated from a common source was placed simultaneously on both systems prior to each intervention to link both systems in time.

5.2.3 Protocol

Patients were asked to attend the laboratory 1 hour prior to their usual bedtime, having abstained from alcohol and caffeine for 24 hours. Patients were instrumented as described above and asked to sleep only in the supine posture, with 1 pillow. The mouth was taped to prevent mouth leak.

CPAP was commenced initially at the patient's documented therapeutic pressure and increased if required, 1-2 cmH_2O above the point where any visible inspiratory flow limitation was abolished. This pressure was maintained as the baseline pressure for the duration of the study. 3 sub-therapeutic pressures were determined during a brief assessment period prior to commencement of the study

proper. These pressures were chosen as approximately 75, 50 and 25% on a scale from flow limitation first being observed, to the development of frank apnoeas. Once determined, these dialdown pressures remained fixed throughout the remaining study. In addition, brief upper airway occlusion was performed by inflation of the balloon valve during stable baseline pressure conditions.

Interventions were grouped into blocks of 4 (25, 50, 75, occlusion) with the order of interventions randomized within each block. A 30 second baseline period of arousal free sleep was required prior to each intervention. Both dialdowns and occlusions were performed until EEG/EMG evidence of arousal was observed or for a maximum of 2 minutes (dialdowns only), after which CPAP was returned to the baseline pressure. Intervention blocks continued throughout the night.

5.2.4 Data analysis

An experienced technician blinded to all other channels except EEG, EMG and EOG performed sleep staging and arousal scoring according to conventional standards[9,10]. Interventions scored to have commenced following at least 30-sec stable stage 2 or SWS without arousal underwent further analysis. The remainder (stage 1 or REM, or with arousal within the baseline period) were excluded.

Breath timing (inspiratory, TI; expiratory, TE; and total breath time, TTot), inspiratory tidal volume (V_T), minute ventilation ($\dot{V}_I$), peak inspiratory flow (PIF), CPAP level (mask pressure at end expiration) and $ETCO_2$ were determined breath-by-breath throughout the 30-sec baseline before each intervention and up to the

onset of EEG defined arousal or 120-sec (whichever came first), using custom software developed in our laboratory. The ∆Pepi (a measure of inspiratory drive) was determined as the difference between the epiglottic pressure at breath onset and the nadir of epiglottic pressure for each breath as previously described[171]. For upper airway balloon occlusions, time to arousal (TTA) was determined as the time from the first negative deflection in epiglottic pressure up to the point of EEG defined arousal. Maximum ∆Pepi was defined as the ∆Pepi for the last completed inspiratory effort prior to arousal and was used to assess ventilatory arousal threshold[164].

Breath-by-breath measurements in each 30-sec baseline period were averaged within each trial and averaged across all replicate trials within each patient for each intervention and sleep stage. Throughout each intervention, measurements were obtained from all completed inspiratory efforts commencing after the onset of the intervention and up to the point of EEG defined arousal. Consequently, breaths with an arousal occurring within the inspiratory time were excluded.

To summarise the overall pattern of ventilatory response to dialdown interventions, ventilatory measurements from the first 5 and last 3 arousal free inspiratory efforts were averaged across all replicate trials within each patient for each dial-down pressure in stage 2 and SWS. Given a variable and often short latency to arousal, particularly under the more severe dialdown conditions, breaths potentially contributed to both the first and last breath periods. Early dialdown and pre-arousal upper airway function was assessed from the PIF vs. CPAP relationship on the third and second to last dial-down breath respectively. Linear regression analysis

was performed within subject to calculate the X intercept. The ventilatory drive response to occlusion was assessed from the linear regression slope of the ∆Pepi versus the corresponding time relationship across each occluded effort before arousal.

5.2.5 Statistical analysis

Differences in ventilatory parameters at baseline and between breaths sleep stages and dialdown pressures were examined via mixed model analysis, using an autoregressive covariate structure and separate random effects intercepts for each patient (SPSS version 14, SPSS Inc, Illinois). Mixed model analysis was also used to examine sleep stage and intervention effects on TTA, arousal threshold (Maximum ∆Pepi), the PIF vs. CPAP relationship (with CPAP as a covariate) on the third and second to last pre-arousal dialdown breath, and the ventilatory drive response to occlusion. Arousal free survival time was examined using Cox regression with robust standard errors, with sleep stage and dialdown level as covariates (Stata version 10, StataCorp, Texas). All data are presented as means ± SEM, $p<0.05$ was considered significant.

5.3 Results

Four patients had insufficient sleep or no slow wave sleep and their results were not analysed. The remaining 10 patients were: 8/10 male, age 57.4 ± 1.5 yr, BMI 32.7 ± 1.9 $kg \cdot m^{-2}$, overall AHI 51.5 ± 5.4 /hr, SWS AHI 19.0 ± 4.6 /hr. One patient was only able to sleep in an oblique lateral posture. Data from this patient was

included from this posture alone. Total sleep time was 288.0±16.2 min, with 172.4±11.8 mins in stage 2 sleep and 39.3±8.2 mins in SWS. There were 367 dialdown and 106 occlusion trials available for analysis. There were approximately 10±1 dialdown trials at each pressure and 7±1 occlusion trials per patient in stage 2, and 3±1 dialdown trials at each pressure and 4±1 occlusion trials per patient in SWS. Most dialdown trials (275/367) and all occlusion trials were associated with an arousal within the 120 sec intervention.

Pre-intervention ventilatory variables (on therapeutic CPAP) in stage 2 versus SWS are shown in Table 5.1. With the exception of a small but significant increase in breathing frequency due to shortened expiratory time in SWS compared to stage 2, there were no other differences between stages in any of the remaining ventilatory parameters that were measured.

Post-dialdown arousal probability and timing were strongly related to sleep stage and dialdown pressure. The proportion of patients remaining arousal free as a function of post-dialdown time averaged for replicate trials within each patient and condition are shown in Figure 5.2A. Cox regression survival analysis showed that both dialdown pressure and sleep stage significantly influenced time to arousal. The arousal hazard ratio for slow wave sleep compared to stage 2 sleep was 0.65±0.1, $p=0.006$. The hazard ratios for arousal for 50% and 75% compared to 25% dialdowns were 0.51±0.07 and 0.17±0.03 respectively (both $p<0.001$).

CPAP and ventilatory changes in the first 5 and last 3 breaths during each of the dialdown interventions are shown in Figure 5.2B-E. By design, dialdown pressures

were significantly different between each of the interventions ($p<0.001$). However, there was no stage or breath number main or interaction effect to indicate any differences between sleep stages. Dialdown from therapeutic CPAP caused substantial pressure dependent decrements in $\dot{V}I$ (Fig 5.2C) and PIF (Fig 5.2D), due to decreased tidal volume (all $p<0.001$) and an initial drop in inspiratory time on the first breath, followed on subsequent breaths by significant inspiratory prolongation ($p<0.001$) and expiratory shortening ($p=0.037$), but with no net increase in breathing frequency. There were rapid and progressive increases in ventilatory drive (ΔPepi, Fig 5.2E, breath effect $p<0.001$) particularly in the more severe dialdown conditions (dialdown pressure x breath effect $p<0.001$). However, despite these substantial (approximately 3-7 fold) increases in ventilatory drive, there were only marginal improvements in peak inspiratory flow and ventilation up to the penultimate breath, with no significant main or interaction effects of sleep stage in any variable.

Peak flow plotted as a function of CPAP pressure in the third and penultimate post-dialdown breaths in stage 2 and SWS are shown in Figure 5.3. There was no significant stage, breath number or interaction effect to indicate any differences or improvement in upper airway function over time in either stage 2 or SWS. Calculated X intercepts for breath 3 were, (stage 2; 0.41±1.30, SWS; 0.77±1.82) and breath -2 (stage 2; 0.45±1.76 SWS 2.13±0.88). No significant differences were noted between breaths ($p=0.363$) or stage ($p=0.587$).

The ventilatory drive response to upper airway occlusion trials in slow wave compared to stage 2 sleep are shown in Figure 5.4. In SWS compared to stage 2

sleep, the maximum ΔPepi prior to arousal was significantly more negative (-28.7 ± 2.7 vs. -20.3 ± 1.6 cmH_2O, stage effect $p<0.001$), occurred significantly later (20.5 ± 2.7 vs. 16.1 ± 2.0 sec, $p=0.023$) and with a significantly prolonged time to arousal (23.0 ± 2.6 vs. 17.1 ± 1.7 sec, $p=0.008$). In addition, there was a significant stage by breath number interaction effect ($p<0.001$), and steeper linear regression slope of ΔPepi versus breath time (-1.0 ± 0.2 vs. -0.6 ± 0.1 $cmH_2O \cdot sec^{-1}$, $p=0.019$, $r^2=0.9$ and 0.86 respectively) suggesting brisker ventilatory responsiveness in SWS compared to stage 2 sleep.

Table 5.1 – Pre-intervention ventilatory variables on therapeutic CPAP during stage and SWS

	Stage 2	SWS
PIF (l/min)	26.9 ± 1.9	27.5 ± 1.7
VTi (l)	0.56 ± 0.03	0.56 ± 0.03
VI (l/min)	7.9 ± 0.4	8.0 ± 0.4
TI (sec)	1.8 ± 0.1	1.8 ± 0.1
TE (sec)	2.5 ± 0.1	2.4 ± 0.1*
TTOT (sec)	4.3 ± 0.1	4.2 ± 0.1*
FB (b/min)	14.2 ± 0.5	14.5 ± 0.6*
∆Pepi (cmH_2O)	-2.4 ± 0.4	-2.2 ± 0.4
SaO_2 (%)	95.8 ± 0.3	95.8 ± 0.3
$ETCO_2$ (mmHg)	42.0 ± 0.9	42.0 ± 0.9
CPAP (cmH_2O)	11.0 ± 0.8	11.0 ± 0.8

Values are mean±SEM. * indicates $p<0.05$, stage 2 vs. SWS, n=10.

PIF: Peak Inspiratory flow (non flow limited), VTi: Inspiratory tidal volume, VI: minute ventilation, TI: Inspiratory time, TE: Expiratory time, Ttot: Ventilatory cycle duration, FB: Breath frequency, ∆Pepi: Difference between epiglottic pressure at breath onset and nadir, SaO_2: Oxygen saturation, $ETCO_2$: End tidal CO_2, CPAP: End expiratory mask pressure

Figure 5.1: Schematic of breathing circuit

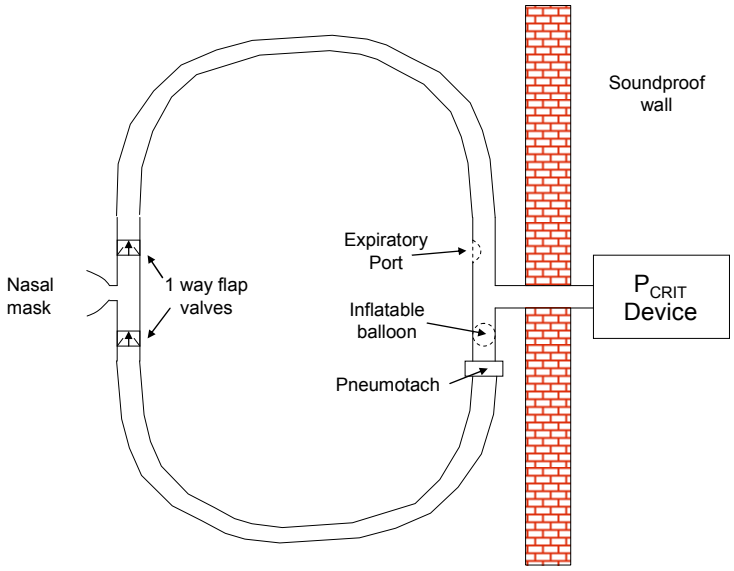
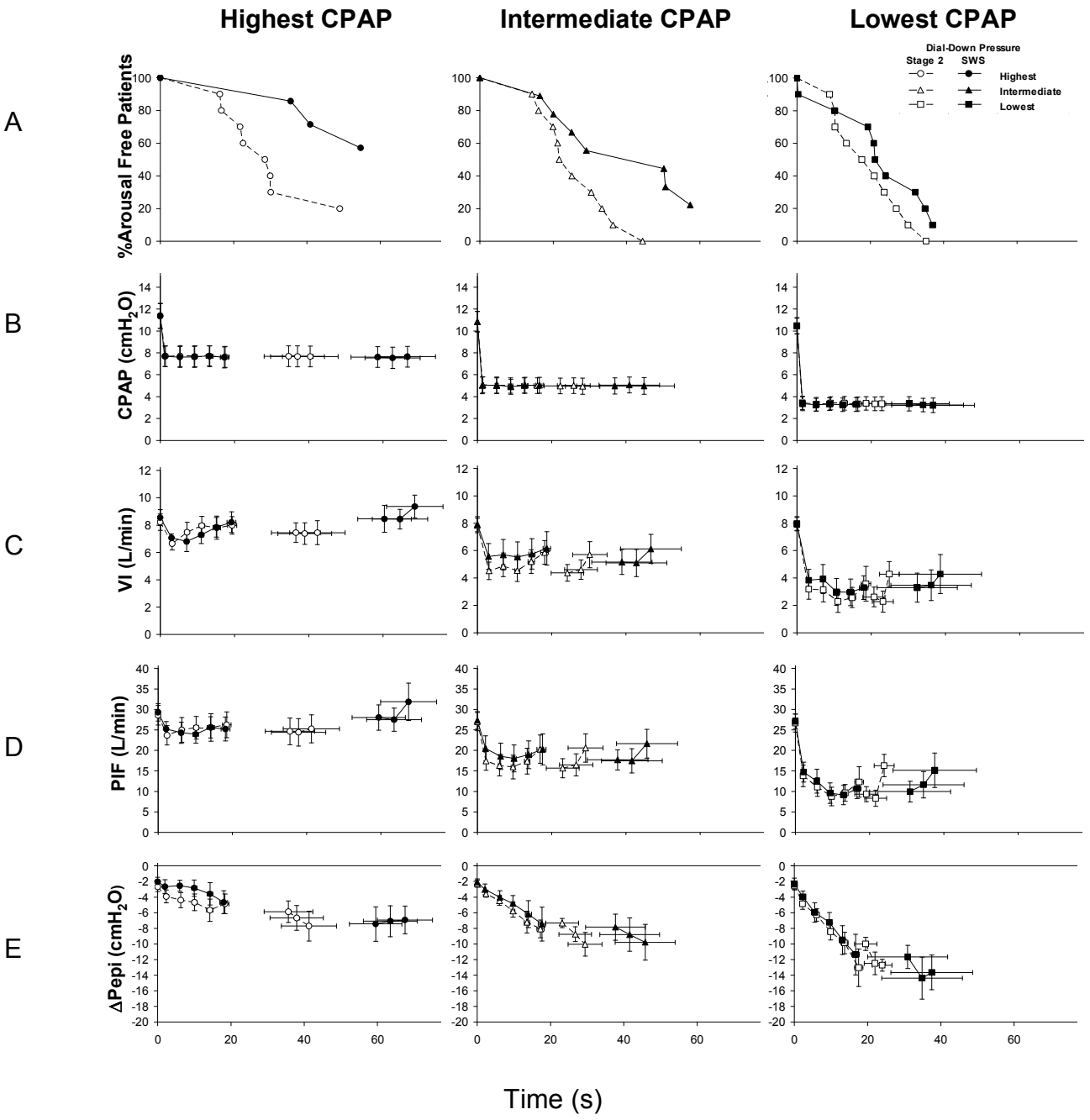
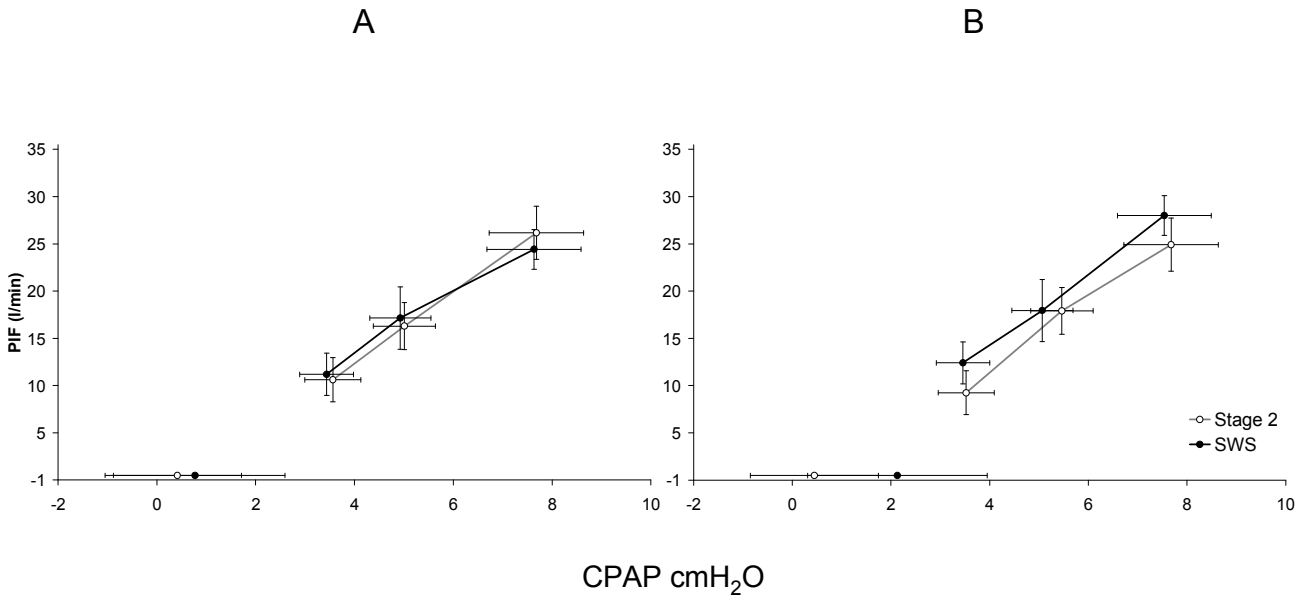


Figure 5.2: Ventilatory variables and arousal propensity post dialdown



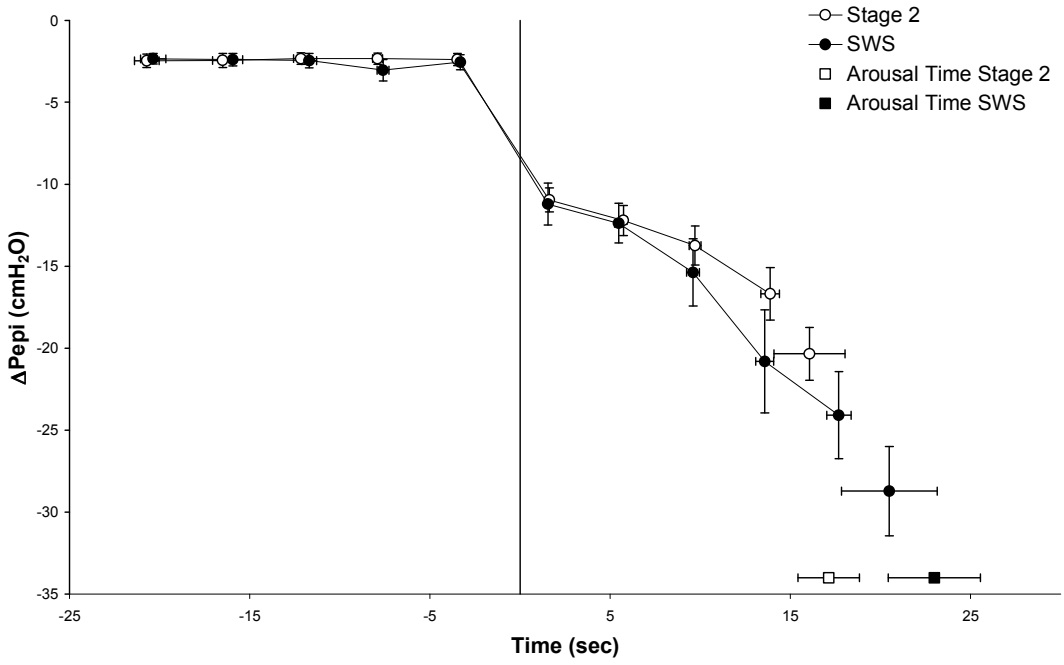
A represents the proportion of patients remaining arousal free as a function of post dial down time averaged for replicate trials within each patient and condition. B, C, D, E represent CPAP levels, $\dot{V}$, PIF and ΔPepi respectively, for breaths 0 (baseline), 1-5 post dialdown and -3, -2, -1 pre end of intervention, for the three different dialdown pressures, over time.

Figure 5.3: Peak inspiratory flow vs. airway pressure



Peak inspiratory flow vs. airway pressure for A) breath 3 post-dialdown and B) the second to last breath prior to the end of the dialdown intervention. Calculated X intercepts are shown as isolated points. Values are mean±SEM.

Figure 5.4: peak epiglottic pressure before and during airway occlusion



Drive (∆Pepi) pre and during balloon airway occlusion on a breath by breath basis, over time. Time of arousal during SWS vs. stage 2 sleep and the final breath pre-arousal are shown as isolated points. Values are mean±SEM.

5.4 Discussion

The key finding of this study was that despite rapid augmentation of inspiratory effort there was no significant ventilatory recovery to partial airway occlusion in either slow wave or stage 2 sleep. Similar and largely ineffectual increases in ventilatory effort with continued flow limitation persisted for longer in slow wave compared to stage 2 sleep. Arousal was delayed and arousal probability was reduced during SWS. There was no evidence to support that upper airway function was intrinsically improved in SWS compared to stage 2 sleep, either early in airway challenge, or in response to augmented ventilatory efforts prior to arousal.

Fixed anatomical factors are thought to contribute to the severity of sleep apnoea. Patients with OSA tend to have an anatomically smaller upper airway measured by MRI[237], or pharyngoscopy[238], compared to controls. Sleep apnoea severity however can vary across the night. Some of this variability may be due to alterations in upper airway anatomy caused by changes in body[199, 200, 205] or head posture[239]. Most patients with sleep apnoea achieve periods of stable sleep, even when controlling for positional effects[76]. We have previously shown that even in patients with severe sleep apnoea, the frequency of respiratory events is significantly reduced during SWS[229]. The current study was designed to investigate possible mechanisms underpinning this phenomenon by exploring differences in airway function and arousal propensity between SWS and stage 2 sleep.

Passive airway function, measured from the airflow response to rapid dialdown from therapeutic CPAP[54, 240, 241] was not different between stage 2 and SWS. This

concords with the findings of Penzel et al[205], who assessed airway function via measurements of P_{CRIT}, but appears to contrast with the findings of Issa et al[190], who found significantly decreased upper airway collapsibility during slow wave compared to light sleep using an upper airway occlusion technique. While the reasons for this difference are unclear and may reflect methodological differences, we did observe faster increments in ventilatory drive during occlusion, but not dialdown trials that may help explain these discrepant findings.

Patil et al[133] suggested that in addition to abnormalities in passive airway function, OSA patients may have impaired neuromuscular compensation responses to airway challenge compared to non-OSA controls. Similarly, apparent improvements in OSA during SWS could reflect either more rapid and effective neuromuscular compensation responses, or greater tolerance to increased ventilatory drive during slow wave compared to lighter sleep[171, 172], allowing for greater improvements in upper airway function and ventilation via increasing negative upper airway pressure and chemostimulation[107] that are more likely to trigger arousal during lighter sleep. Apart from more rapid ventilatory drive augmentation to airway occlusion, we found no evidence to support improved ventilatory compensation during SWS compared to stage 2 sleep. These findings suggest that OSA patients essentially tolerate a higher drive state for longer during SWS, with minimal improvements in airflow or ventilation until arousal.

Substantial sleep stage effects on arousal probability and timing have been shown previously with both respiratory[171, 172] and non-respiratory stimuli[170, 207]. OSA patients have a higher arousal threshold compared to non-OSA controls[110]. It is

unclear whether these differences are due to chronic sleep fragmentation[173], recurrent hypoxia[177], and/or an underlying neurological abnormality. These changes are at least partially reversible following CPAP therapy[185] and recur following withdrawal of CPAP[187]. Afifi et al[179] noted that patients with mild untreated OSA exhibited fewer and lower amplitude K complexes to inspiratory effort related but not auditory stimuli, compared with healthy controls during stage 2 sleep. This was despite no significant differences in either auditory or respiratory related evoked potentials between groups during wakefulness, suggesting that there was significant sleep specific cortical dampening to respiratory stimuli in OSA patients, rather than a generalized sensory dampening or mechanoreceptor dysfunction[179]. It is not clear if this effect may be adaptive or mal-adaptive as it may reduce the frequency of respiratory arousals, but possibly at the cost of prolonged periods of hypoventilation and increased ventilatory drive.

Ventilatory control stability is dependent on negative feedback loops and is frequently described using the engineering concept of loop gain[242]. Loop gain describes the propensity of the ventilatory control system to develop periodic fluctuations in output, a key characteristic of sleep apnoea. Elevated loop gain assessed by proportional assist ventilation appears to be significantly correlated with OSA severity[243], particularly in patients with a near atmospheric pharyngeal closing pressure[244]. In the current study, the increase in ventilatory drive post-complete airway occlusion was used as a marker of chemosensitivity. Patients demonstrated more rapid drive augmentation responses during SWS which, within the limitations of this technique, suggest increased chemosensitivity during SWS. This has not been described previously. A similar technique used in healthy

volunteers suggested no significant differences between stage 2 and SWS in the ventilatory drive response to occlusion[171]. The increased drive responses observed in SWS post-occlusion were not apparent in non-occlusive dialdown stimuli, and there was no further evidence of important differences in ventilatory compensation between SWS and stage 2 sleep.

During the baseline period, there were some minor but significant breath timing differences with shorter expiratory time and increased breathing frequency in SWS compared to stage 2 sleep. These did not result in any ventilatory differences and there were no post-dialdown sleep stage effects in these variables to support key differences in breath timing between the sleep stages.

5.4.1 Methodological limitations

There are a number of limitations to this study. Arousals from sleep were scored according to conventional criteria[10], thus the impact of sub-criterion arousals is unknown. By design, this study investigated respiratory variables only until arousal. We have thus not investigated the effects of the ventilatory response to arousal itself which may further contribute to ongoing cyclical breathing by causing hyperventilation, followed by a period of hypoventilation[191] that may render the airway more prone to re-collapse, and/or more frequently meet the accepted criteria for respiratory event scoring. Reduced arousal propensity to respiratory challenge during SWS would reduce the frequency of post-arousal hypoventilation and therefore potentially explain an important component of the reduced respiratory event frequency in SWS. It is currently unknown if post-arousal ventilatory

responses are modulated by sleep stage, potentially further contributing to the reduced respiratory event frequency in SWS.

There are a number of caveats to our technique of measuring chemosensitivity post-airway occlusion. With an occluded airway, $ETCO_2$ cannot be measured. Conceivably, if there are differences in metabolic rate or respiratory quotient between stage 2 and SWS, the rise in mixed venous CO_2 post-occlusion may be different, with changes in drive therefore not necessarily indicating differences in chemosensitivity *per se*. However, metabolic differences appear unlikely to explain these findings. White et al[245] showed no significant differences between stage 2 vs. SWS in metabolic rate in healthy volunteers. Fontvieille et al[246] studied healthy volunteers and obese subjects and showed that the metabolic rate in stage 3 sleep was slightly but significantly lower than stage 2 sleep, with no significant differences in the respiratory quotient. One further potentially confounding issue is that inspiratory muscles demonstrate short latency reflexes, which may further modulate inspiratory drive[247]. These reflex responses may also exhibit sleep stage effects and therefore contribute to sleep stages differences in ventilatory drive responses.

Our method of calculating the X intercept (or P_{CRIT}) of the peak flow vs. airway pressure relationship was different to previously described methods. Previous studies measured peak flow at multiple airway pressures[240] to plot at least 10 different points along this line[205], particularly getting close to the airway pressure at which no flow occurred. In the interests of acquiring replicate measurements, we choose only 3 different airway pressures, which were not necessarily close to the point of actual zero flow. Consequently the confidence intervals for the X intercept

were quite wide. These results are used to complement the analysis of the measured airway pressure vs. peak flow relationships showing no stage or breath effect.

Finally, this was a small study in selected patients who had SWS during their diagnostic polysomnography even when they had severe untreated OSA. It may not be possible to generalize these results to all patients with OSA. Patients with an extremely collapsible upper airway for example may frequently not achieve SWS, and may require recurrent arousals from sleep to protect ventilation. Likewise, given that we did not study normal subjects, we can not completely exclude that there is a sleep state effect on airway function in subjects without sleep apnoea. For example, patients with sleep apnoea may have a dysfunction of upper airway function / control[134] which transcends sleep stage, and thus minimizes interstage differences. However, we have previously shown that in the majority of patients with and without OSA, respiratory event frequency reduces during SWS compared to stage 2 sleep[229]. Given the similar rates of improvement, it seems unlikely that patients with and without OSA have different mechanisms for the SWS improvement.

5.4.2 Summary and conclusions

In summary, this study found no evidence to support improved upper airway airflow mechanics or ventilatory compensation responses to ventilatory challenges in SWS compared to stage 2 sleep in a group of OSA patients known to exhibit OSA improvement in SWS. Following rapid dialdown from therapeutic CPAP, there was

rapid augmentation of ventilatory drive but this did not translate into significant improvements in flow or ventilation up to the point of arousal in either SWS or stage 2 sleep. However, patients were significantly more likely to arouse and aroused earlier during stage 2 sleep compared to SWS and during more severe ventilatory challenges. Similarly, with upper airway occlusion, arousal threshold and the time to arousal were significantly greater during SWS compared to stage 2 sleep. There was also more rapid ventilatory drive augmentation in SWS that was not apparent post dialdown. It therefore appears that patients are more likely to 'tolerate' reduced ventilation during SWS for longer without arousing, but with no major differences in the ability for ventilatory compensation during SWS compared to stage 2 sleep. Consequently, differences in arousal propensity and potentially arousal responses to respiratory stimuli remain as factors more likely to account for substantial reductions in OSA frequency during SWS.

CHAPTER 6. Summary and conclusions

Anatomical abnormalities resulting in a smaller upper airway diameter are an important determinant of obstructive sleep apnoea[39, 41]. However, non-anatomical factors are increasingly thought to play an important part in the pathogenesis of OSA[76], the exploration of which formed the experimental chapters in this doctoral thesis[76].

Upper airway anatomy can be altered by posture[70] and neck position[68]. However, even when controlling for these factors, significant variability in OSA severity occurs throughout the night. For example, OSA is generally considered to be more severe during REM sleep[77] and to improve in severity during slow wave sleep[80, 202], potentially on the basis of changes in ventilatory and/or neuromuscular control changing over the course of sleep. Passive P_{CRIT} is thought to be a marker of upper airway collapsibility/anatomy. While subjects with OSA do tend to have a more positive P_{CRIT} compared to non-OSA patients, in a large group of subjects with a range of AHIs, very little of the variance in AHI could be explained by the P_{CRIT}[75]. This would again suggest an important contribution to OSA severity by non-anatomic factors. To assess the importance of non-REM sleep stages on OSA severity, the first experimental chapter (Chapter 2) explored the prevalence and magnitude of OSA severity changes across sleep stages in a large group of OSA patients while controlling for posture. A detailed retrospective analysis was performed on 253 diagnostic polysomnographies. After excluding patients with

significant central sleep apnoea, 171 patients were classified as having significant sleep apnoea and 68 patients as non-OSA on the basis of an AHI ≥ 15 or <15/hour respectively. Both patients with and without OSA demonstrated significant reductions in respiratory event and arousal frequencies from stage 1 to 4 non-REM sleep, with REM sleep at an intermediate level. Posture also had a large effect, with reduced AHI in the lateral compared to the supine posture. However a similar pattern of reduction in OSA frequency from stage 1 to 4 non-REM sleep was apparent in both postures, and the effect size of the difference between stage 2 and slow wave sleep was comparable to the posture effect size. The ratio between respiratory events and arousals was maintained throughout the non-REM sleep stages, suggestive of a strong relationship between arousal and respiratory event frequencies.

The cause of reduced OSA event frequencies during slow wave sleep shown in the observational study presented in Chapter 2 is unclear. While there was a strong association between arousal and respiratory events, this could indicate an incidental relationship from a common cause or indicate a causal relationship in either direction; i.e. increased frequency of hypopnoeas could lead to increased arousal frequencies[1] or alternatively, higher arousal frequencies due to reduced sleep depth could lead to increased airway instability. The immediate post-arousal period is characterized by significant hyperventilation[191, 193] and a degree of hypocapnia. In susceptible subjects, this may lead to reduced upper airway drive and a tendency to airway recollapse[141]. Upper airway neuromuscular tone is thought to respond to a combination of increased resistive load and hypercapnia

related inputs[107]. It is possible that by not arousing during slow wave sleep, the upper airway is given time to augment its mechanical properties, whereas during lighter sleep patients simply arouse from sleep. It is also possible that there are significant differences in upper airway collapsibility or neuromechanical responsiveness due to sleep stage influences *per se*. If an increased arousal threshold leads to increased airway stability, use of sedating agents such as gamma-hydroxybutyrate[248] may be beneficial treatments for OSA. Alternatively, if arousal is simply delayed, permitting longer apnoeas, increased hypoventilation and prolonged periods of increased drive and negative intrathoracic pressures, this is likely to be detrimental, even though the RDI which simply measures the frequency of respiratory events over time may be lower. Mild hypercapnia and arousal related changes in CO_2 may also play a significant role in modulating ventilatory and upper airway stability in sleep. The remaining experimental Chapters set out to answer a number of these questions.

To facilitate the assessment of CO_2 influences within the physiological range encountered in OSA during sleep, we developed a device which would provide positive airway pressure and by proportional rebreathing, maintain stable end-tidal CO_2 during periods of hyperventilation. In the experiments described in Chapter 3, this device was tested in healthy volunteers during brief periods of voluntary hyperventilation during wakefulness at 2 levels of 'clamping' function and 2 levels of airway pressure in randomised order. Compared to similar levels of CPAP alone, the clamp device successfully prevented significant drops in end-tidal CO_2 even with marked hyperventilation. However, elevated levels of inspiratory resistance

were observed, particularly during higher levels of ventilation. Potential uses for this device include experiments requiring end-tidal CO_2 manipulation in conjunction with maintenance of positive airway pressure. The device could potentially also be used in patients with Cheyne-Stokes breathing[217], as well as OSA patients not well controlled on CPAP, the newly termed complex sleep apnoea[234]. Significant limitations of the device include the need to minimize circuit resistance and tolerability. This device was used in the experiments described in Chapter 4 to examine the influence of mild stable hypercapnia on upper airway function before and immediately after arousal during periods of experimentally induced airflow limitation.

During sleep, the upper airway is modelled as a Starling resistor[52, 54, 56]. In a Starling resistor, flow is determined by the gradient between the upstream pressure and P_{CRIT} (the pressure at which the airway collapses), and is independent of downstream pressure/ventilatory drive. However, upper airway neuromuscular activity is modulated by both ventilatory drive inputs[108] and negative upper airway pressure reflexes[115]. This activation has the potential to alter airflow mechanics from a 'pure' Starling resistor. In an animal study, hypercapnia has been shown to decrease upper airway collapsibility[98]. In humans the situation is not as clear. There is some evidence from healthy volunteers, that genioglossal EMG activity increases in response to significant levels of hypercapnia[108], particularly in combination with inspiratory resistive load[107], although this was not shown in another study[106]. The functional effect of increased upper airway EMG activity in terms of airflow is not clear. Further, since patients with sleep apnoea may have impaired neuromuscular

compensation responses[134], it may not be possible to extrapolate results in healthy volunteers to patients with OSA. Post-arousal from sleep, there is a period of significant hyperventilation and relative hypocapnia[191, 193]. This may lead to reduced upper airway drive and consequent airway instability in susceptible patients[141]. The experiments described in Chapter 4 set out to explore the functional effect of mild hypercapnia in patients with severe sleep apnoea during induced partial airway occlusion and post arousal.

10 subjects with severe OSA underwent rapid dialdowns to one sub-therapeutic pressure for one minute following 30 seconds of baseline breathing under 3 different conditions in randomised order; Control (no CO_2 clamping), baseline only clamp and CO_2 clamping throughout the baseline and dialdown periods. Following dialdown from therapeutic CPAP, there were significant elevations in peak flow and ventilation in the clamped conditions compared to control, suggesting that even in patients with severe sleep apnoea, mild sustained hypercapnia and increased ventilatory drive *before* partial airway collapse significantly improve airway function and ventilatory output during a subsequent challenge to airway function. Arousal propensity was not different between conditions. Post-arousal, end-tidal CO_2 and ventilation remained elevated in the CO_2 clamp condition, but declined in the control and baseline only clamp conditions, supporting that maintenance of end-tidal CO_2 post-arousal may prevent ventilatory undershoot and potentially reduce the propensity for airway re-collapse. Some potential limitations of this study include the fact that since only one dialdown pressure was measured, it is unclear whether airway collapsibility (as measured by P_{CRIT}) was altered by mild

hypercapnia. Measurement of supraglottic airway pressure may have also provided further information regarding overall airway resistance.

To investigate potential key reasons for OSA improvement in SWS, in the experiments described in Chapter 5, 10 patients with severe OSA who demonstrated OSA improvement during slow wave sleep underwent repeated ventilatory challenges (dialdowns from therapeutic CPAP and occlusion) until arousal or a maximum of 2 minutes. Interventions were presented and compared between stage 2 and slow wave sleep. Post–dialdown, there were marked reductions in peak flow and ventilation. However, despite significant increases in ventilatory drive, there were minimal improvements in flow or ventilation up until the point of arousal and/or the end of the intervention in either stage 2 or slow wave sleep. The relationship between peak flow and airway pressures in the third and penultimate breath, as a measure of “passive” and “pre arousal” airway function, showed no significant differences between breaths or sleep stages. However, time to arousal was significantly longer and arousal probability was significantly lower during slow wave sleep and with less severe dialdown pressures. During airway occlusions, arousals occurred significantly later and at more negative epiglottic pressures in slow wave compared to stage 2 sleep. Inspiratory effort developed more rapidly, suggestive of increased chemosensitivity during slow wave sleep. Consequently, although there may be a heightened ventilatory drive response during complete airway occlusion, there appeared to be no differences in either passive airway function or neuro-mechanical compensation to airway challenge during slow wave compared to stage 2 sleep, at least up to the point of arousal.

The apparent improvement in OSA frequency during slow wave sleep noted in the first experiment (chapter 2) may therefore simply reflect greater tolerance of relative hypoventilation and increased drive. One of the implications of this study is that use of potentially sedating medications to increase the arousal threshold (at least in patients with severe OSA) is not likely to be physiologically beneficial in terms of ventilation / oxygenation, although may reduce the measured respiratory disturbance index.

By design, the study presented in Chapter 5 explored ventilatory function only up to the point of arousal. If the ventilatory response post-arousal contributes to subsequent periods of ventilatory instability[141], a lower likelihood of arousal during slow wave sleep would facilitate fewer subsequent events. Further, it is possible that the ventilatory response itself may be altered by sleep stage. Future studies to address these questions are warranted.

In summary, a number of non-anatomical factors potentially contributing to obstructive sleep apnoea pathogenesis were explored in this thesis. Substantial improvements in OSA severity from stage 1 to 4 non-REM sleep were found in a large group of subjects with and without OSA. These improvements were at least of a similar order to well known supine versus lateral posture and REM versus non-REM effects. The major implication of this finding is that the majority of patients with OSA, ranging from mild to severe disease, appear to be able to compensate for an anatomically smaller upper airway, at least some of the time, particularly during slow wave sleep, on the assumption that upper airway anatomy is unlikely to

be altered significantly by sleep stage alone. This suggests that non-anatomical factors play a key role in at least modulating OSA severity and potentially underpin key pathogenic mechanisms in OSA. Increased ventilation and sustained improvements in upper airway function on a background mild hypercapnia/increased ventilatory drive (Chapter 4) supports that ventilatory neuromuscular activation, at least when present *before* significant flow limitation develops, does improve airflow mechanics in OSA patients. However, minimal improvements in airflow and ventilation over time despite large increases in ventilatory drive developing in the presence of flow limitation (Chapter 5), suggest that once flow limitation commences, neuromuscular ventilatory compensation mechanisms alone have a very limited capacity to improve ventilatory output without arousal, at least in severe OSA patients. This may not be case in patients without OSA who appear to have a better ability to compensate. Furthermore, in the absence of any significant differences in passive airway function, or ventilatory compensation responses to flow limitation, combined with consistently delayed and a reduced propensity for arousal in slow wave compared to stage 2 sleep, arousal factors appear most likely to explain OSA improvements in slow wave sleep. Consequently, further studies are needed to clarify the role of arousal responses in explaining OSA improvements in deep sleep and their potential role in the pathogenesis of cyclical breathing disturbances in OSA. Likewise, a detailed exploration of apnoea / hypopnoea duration and level of ventilation in a group of OSA subjects comparing stage 2 and SWS epochs may shed further light on the apparent ‘improvement’ of OSA during slow wave sleep.

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