THE CONTROL OF RESPIRATION AND UPPER AIRWAY MUSCLE ACTIVITY IN HEALTHY YOUNG MEN AND WOMEN

by

Amy Jordan

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Department of Physiology
University of Adelaide
South Australia, 5005.

Sleep Disorders Unit
Repatriation General Hospital
South Australia, 5041.

TABLE OF CONTENTS

TABLE OF FIGURES

ABSTRACT

Sleep apnea syndromes are characterised by repetitive cessation of breathing during sleep. They are more common in men than in women and currently the reason for this is poorly understood. The central control of respiration and upper airway dilator muscle function have been implicated in the pathogenesis of these disorders. In this thesis, several aspects of the control of ventilation and an upper airway dilator muscle (the genioglossus) have been compared between healthy men and women, in an attempt to identify a gender difference that may contribute to the high male prevalence of sleep apnea.

In the first study presented in this thesis, the phenomenon of respiratory afterdischarge of the genioglossus and diaphragm muscles were compared between men and women during wakefulness. Respiratory afterdischarge is the slow decline in respiratory drive following removal of a brief respiratory stimulus. A short respiratory afterdischarge (rapid decline to baseline) represents an "under-damped" system in which further oscillations in respiratory output are more likely. In this study the respiratory afterdischarge of the diaphragm and genioglossus muscles were found to be similar between genders.

In the second study, the ventilatory and upper airway muscle responses to repetitive isocapnic hypoxia were compared between men and women. In animals, repetitive hypoxia induces long-term facilitation (a progressive increase in ventilation during and following repeated stimulation), which is thought to help

stabilise respiratory patterns. Long-term facilitation has not been consistently found in humans nor compared between genders. In this study, repetitive hypoxia did not elicit long-term facilitation of ventilation or upper airway muscle activity in either gender.

In the third study the ventilatory response to arousal from NREM sleep was compared between healthy men and women. The degree of hyperventilation following arousal from sleep has been implicated to contribute to subsequent respiratory instability. The ventilatory response to arousal from sleep was found to be higher in men than in women, and men subsequently developed a larger transient ventilatory undershoot.

In summary, several aspects of respiratory and upper airway dilator muscle control have been investigated and compared in healthy men and women. Respiratory afterdischarge and the response to repetitive hypoxia appear similar between genders during wakefulness, however the ventilatory response to brief awakening from sleep was found to be higher in men than women. It is possible that this difference contributes to the high male prevalence of sleep apnea syndromes.

PUBLICATIONS

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Amy Jordan

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GLOSSARY OF ABREVIATIONS

AHI	Apnea hypopnea index (events·hr^{-1} sleep)
AI	Arousal index (arousals·hr^{-1} sleep)
BMI	Body mass index (kg·m^{-2})
BSA	Body surface area (m^2)
CHF	Congestive heart failure
CPAP	Continuous positive airway pressure (cmH_2O)
CSA	Central sleep apnea
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG_{DI}	Diaphragm electromyogram
EMG_{GG}	Genioglossus electromyogram
$EMG_{GG(200)}$	Genioglossus electromyogram at 200 ml·s^{-1} flow
EMG_{SUB}	Submental electromyogram
EOG	Electrooculogram
ESS	Epworth sleepiness scale
F_B	Breathing frequency (breath·min^{-1})
FEV_1	Forced expiratory volume in 1 second (% predicted)
FRC	Functional residual capacity (l)
FVC	Forced vital capacity (% predicted)
LTF	Long-term facilitation
MVA	Motor vehicle accident
NREM	Non rapid eye movement sleep
OSA	Obstructive sleep apnea
$P_{0.1}$	Occlusion pressure (cmH_2O)
P_{CHO}	Choanal pressure (cmH_2O)

P_{CRIT}	Critical closing pressure (cmH_2O)
P_{EPI}	Epiglottic pressure (cmH_2O)
$PETCO_2$	End-tidal partial pressure of carbon dioxide (Torr)
PIF	Peak inspiratory flow ($l \cdot min^{-1}$)
P_{MASK}	Mask pressure (cmH_2O)
R_{200}	Upper airway resistance at 200 $ml \cdot s^{-1}$ flow ($cmH_2O \cdot l^{-1} \cdot s$)
RAD	Respiratory afterdischarge
REM	Rapid eye movement sleep
R_{NA}	Nasal (choanae - mask) resistance ($cmH_2O \cdot l^{-1} \cdot s$)
R_{PH}	Pharyngeal (epiglottis - choanae) resistance ($cmH_2O \cdot l^{-1} \cdot s$)
R_{UA}	Upper airway (epiglottis - mask) resistance ($cmH_2O \cdot l^{-1} \cdot s$)
RV	Residual volume (l)
SaO_2	Arterial oxygen saturation (%)
SDB	Sleep disordered breathing
SEM	Standard error of the mean
SOL	Sleep onset latency (minutes)
T_E	Expiratory time (s)
T_I	Inspiratory time (s)
TLC	Total lung capacity (l)
TST	Total sleep time (minutes)
T_{TOT}	Total breath time (s)
UA-CSA	Upper airway cross sectional area (cm^2)
V_T	Inspiratory tidal volume (l)
$\dot{V}_I$	Inspiratory minute ventilation ($l \cdot min^{-1}$)

CHAPTER 1. GENERAL INTRODUCTION

1.1 General background

Sleep-related breathing disorders are increasingly recognized as an important cause of morbidity. They include a number of distinct syndromes defined by agreed clinical and polysomnographic criteria (6). Common to each is the presence of episodic disturbances of ventilation during sleep with consequent sleep disruption and abnormal gas exchange. The sleep apnea syndromes are usually divided into obstructive and central types, however, within a single patient, both central and obstructive apneas and hypopneas may be observed. Both the common obstructive sleep apnea syndrome (OSA) and central sleep apnea (CSA) show a striking male predominance (125, 159), the reasons for which are at present poorly understood. Increased knowledge of the pathophysiological mechanisms underpinning these gender differences will likely lead to new insights into the basic biology of sleep disordered breathing, which may ultimately have important therapeutic spin-offs. The aim of this doctoral study was to examine possible physiological mechanisms for this difference in healthy men and women, in the hope to identify an underlying gender difference that may contribute to the high male prevalence of sleep apnea syndromes. This chapter examines the literature with regard to differences and similarities between men and women with both obstructive and central sleep apnea, and reviews the current understanding for the high prevalence of these disorders in men.

1.2 Obstructive sleep apnea

Obstructive sleep apnea is characterised by episodic collapse or narrowing of the upper airway during sleep. During apnea or hypopnea, progressive hypoxia and hypercapnia leads to incrementally larger breathing efforts that culminate in arousal, re-opening of the upper airway and brief hyperventilation followed by return to sleep. There are several known risk factors for OSA including increased body mass, craniofacial skeletal dysmorphology and increased age (see (35) for review). Male gender is also a well-recognized risk factor.

1.2.1 Prevalence: community versus clinic populations

The prevalence of OSA in the general population has been estimated in several large studies (17, 18, 40, 74, 100, 116, 159). The populations studied, number of subjects, measurement methods and definitions of OSA have varied between studies and so not surprisingly the estimated prevalence of OSA has also differed. However, common to all reports is a higher prevalence of OSA in men compared with women, with male to female ratios of approximately 2 or 3:1 (17, 74, 100, 116, 159). In the first large, polysomnographic study of sleep disordered breathing (n=602), OSA (as defined by an apnea-hypopnea index (AHI) of ≥ 5 with concurrent hypersomnolence) was estimated to occur in approximately 4% of men and 2% of women (159). Similar findings have been reported amongst relatives and neighbors of patients with sleep disorders (116) and Spanish residents (40). Post-menopausal women have been found to have a significantly greater risk of OSA than either pre-menopausal women or post-menopausal women taking hormone replacement

therapy (17). Thus female sex hormones may protect against OSA and contribute to the observed gender difference in OSA prevalence. Against this is the finding that the experimental administration of female hormones does not, at least in the short term, reduce the severity of OSA (31, 32, 114).

Interestingly, the ratio of men to women with OSA in clinical studies (33, 51, 116) appears to be considerably higher than in the community (116, 159) (approximately 5 to 8:1 versus 2 to 3:1). This does not seem to have changed significantly in the last decade as judged by the gender ratio (4 to 10:1) of patients entered into recent clinical trials (15, 86). However, one recent report of a large sleep clinic cohort reported a gender ratio comparable to that reported in epidemiological studies (97). The reason for the apparent gender discrepancy between community and clinic populations is not clear. However, it has potentially important public health implications, particularly if large numbers of women with OSA are unaware of their condition or for some other reason are not presenting for treatment.

1.2.2 Polysomnographic findings

There are two large retrospective clinical studies that show women with OSA have a lower AHI during non rapid eye movement (NREM) sleep but the same AHI during rapid eye movement (REM) sleep (97, 142). The gender difference in AHI during NREM appears to be confined to men and women under the age of 60 years (142). It is possible that women, because of advantageous upper airway anatomy or mechanics, have less propensity to upper airway obstruction in sleep, but experience more muscle hypotonia in REM, greater central respiratory instability

during REM or more diaphragm fractionations (20-100ms pauses in diaphragm activity which have been postulated to contribute to the diminished ventilatory response to occlusion in REM (127)). I am not aware of any studies investigating these possibilities. Alternatively, women may have similar upper airway dimensions or mechanics and similar REM neurophysiology and propensity for obstruction compared with men, but have a respiratory control mechanism that protects them from obstructive events in NREM sleep. Female hormones could well be involved since older men and women (>60 years) do not show the NREM AHI difference (142). Increased pharyngeal dilator muscle tone or a more stable respiratory control system have each been implicated in the lower prevalence of OSA in pre-menopausal women (see Pathogenic mechanisms, page 9).

The duration of apneas and hypopneas are reported to be 3-5s shorter in women than men and correspondingly women are reported to experience less desaturation (77, 142). A more rapid change in blood gases during apnea would appear unlikely to account for the shorter apnea duration in women, as desaturation was less in women. If hypoxic or hypercapnic chemosensitivity was higher in women than men, women may increase breathing efforts during obstructive events more rapidly and arouse earlier. However, hypoxic ventilatory responses are not convincingly different in either healthy men and women (3, 117, 145, 146) or OSA patients (126), and respiratory effort ($P_{0.1}$ responses) appears to augment at a similar rate in men and women during progressive hypercapnia (140). The arousal threshold may be reduced in women causing them to arouse more quickly following an obstruction. Finally, it is possible that men, because of more severe OSA, have

greater sleep pressure and therefore require a larger stimulus to arouse from sleep and terminate apnea. I am not aware of any study comparing arousal threshold to respiratory stimuli in men and women either with or without OSA.

1.2.3 Symptoms and neurobehavioral changes

In epidemiological surveys, women with OSA appear to report the classical symptoms associated with OSA in men, namely loud or disruptive snoring, snorting or gasping and witnessed breathing pauses (101, 116, 158). However, in the study of Redline et al. (116), the proportion of women with an AHI >15 who reported these symptoms was approximately half that found in men with the same level of sleep disordered breathing. A similar (non-significant) trend was observed in the study of Young et al. (158), with approximately 40% of women versus 20% of men with AHI >15 reporting none of the classical symptoms. While women who suffer from OSA appear to perceive similar symptoms to men with OSA, they more frequently report a number of other possibly unrelated sleep symptoms. In clinic based studies, women with OSA report more difficulty initiating sleep (4), morning headache (4) and fatigue than men with OSA (4, 29). Importantly, women who present to sleep clinics and turn out not to have OSA, also report more morning headache and difficulty initiating sleep than men without OSA (4). In the Wisconsin Sleep Cohort Study women reported more excessive sleepiness (159) and more morning headache in all apnea severity groups (158). Women were more likely to be dissatisfied with life, which was unrelated to OSA severity (158). In the more recent Sleep Heart Health Study women were found to report "difficulty initiating and maintaining sleep" more than men, even though fewer women had sleep

disordered breathing (14). A linear relationship was also reported between AHI and the score on the Epworth sleepiness scale (ESS) and this was not affected by gender (50).

Excessive sleepiness is thought to contribute to the elevated risk of motor vehicle accidents (MVA) amongst patients with OSA. The risk for MVA has been assessed in licensed drivers enrolled in the Wisconsin Sleep Cohort Study (156). This study reported that during a 5 year period, men with sleep disordered breathing were 3 times more likely to have a MVA than men without sleep disordered breathing (SDB) after controlling for average number of miles driven, usual alcohol consumption, education, age and body mass index (BMI). Interestingly, no such association was found in women. However, an association between having multiple accidents and sleep disordered breathing was present in both genders. Several reasons for the apparent reduction in accident risk for female OSA patients were postulated. Firstly, women may not suffer the same degree of driving impairment for the same level of sleep disordered breathing. However, there is no evidence for less sleepiness or neurocognitive impairment in females with OSA (50, 73). Secondly women may perceive sleepiness more accurately than men and therefore take preventative action (stop driving, rest etc). Finally, the authors proposed that men may continue driving despite equivalent perception of sleepiness. The last possibility is supported by the observation that men have an increased frequency of risky driving behaviors compared to women (66).

1.2.4 Cardiovascular disease

In the Wisconsin Sleep Cohort Study (160) sleep disordered breathing was found to be an independent risk factor for hypertension but there was no significant interaction effect of AHI and gender. A similar association of OSA and hypertension has been reported from the Sleep Heart Health Study although in this instance the association was stronger in men than in women (96). A third population based study of 1,741 men and women (16) has also reported sleep disordered breathing to be a significant independent risk factor for hypertension in both men and women. The Sleep Heart Health Study has demonstrated increased risk of stroke and ischaemic heart disease associated with OSA but it appears that gender does not influence this risk (123). It therefore seems that although women are less likely to suffer from OSA than men, the cardiovascular risk associated with sleep disordered breathing is similar between genders.

1.2.5 Survival

Given the association of OSA with cardiovascular disease, it is pertinent to ask whether there is a relationship between survival and AHI and whether this is influenced by gender. In 1996, Ancoli-Israel and colleagues reported survival in a community sample of elderly individuals (65-95) who had sleep disordered breathing (7). The follow up ranged from 7.9 - 10 years and while age and cardiovascular risk factors were found to be independently associated with mortality, AHI and gender were not independent predictors of mortality. More recently, survival has been reported in men and women from a sleep clinic

population studied with full polysomnography. The vital status of 354 patients who attended the University of Wisconsin Sleep Disorders Clinic was determined 5 years after diagnosis by searching state mortality records (157). The survival of men and women without OSA was equal, whereas of the patients diagnosed with OSA, more women died than men in the follow up period. The mean age at death was significantly lower in women so older age does not explain this finding. Women had a slightly but not significantly higher AHI (53 versus 40 events per hour). A weakness of this study was the inability to fully account for co-morbidities and other confounders. Further research assessing survival of men and women in population based studies is required before conclusions can be made regarding the survival of men and women with OSA.

1.2.6 Summary of gender differences in obstructive sleep apnea

OSA is more common in men and it appears that many women with OSA do not attend sleep clinics despite displaying similar symptoms to men. It is possible that the high reporting of excessive sleepiness, headache and fatigue by women without OSA could lead to primary care and specialist physicians mis-diagnosing OSA in women. The neurocognitive and cardiovascular consequences of OSA appear similar between men and women except that the risk for a MVA is higher in men. However this may be an exaggeration of the increased risk taking behavior of men.

There are several polysomnographic differences between genders in sleep clinics. Women demonstrate a greater change in severity from NREM to REM sleep and

have shorter respiratory events. There are no comparable NREM-REM comparisons in epidemiological studies. The significance of the polysomnographic findings with regard to the pathogenesis and cardiovascular and neurobehavioral consequences of OSA is unknown.

1.3 Pathogenic mechanisms

1.3.1 Obesity

Since obesity is a major risk factor for OSA, it is possible that the gender difference in OSA prevalence may be, at least in part, related to gender differences in body mass. The 1997 US National Health Interview Survey reported that although more men (62%) are overweight (BMI >25 $kg \cdot m^{-2}$) than women (46.6%), the prevalence of obesity (BMI >30 $kg \cdot m^{-2}$) is actually similar between the sexes (18.8% in men, 19.3% in women) (94). These results are almost identical to the 1995 National Nutrition Survey in Australia which found 66.3% of men and 45.7% of women were overweight while 17.6% of men and 16.1% of women were obese (8). The severity of OSA increases with body mass but the slope of this relationship, adjusted for other factors, is unclear and almost certainly differs between men and women (see below). However, more men than women in the overweight range (BMI 25-30) could contribute to the increased prevalence of OSA in men. It is important to remember that in clinic based studies women are generally more obese for a given severity of OSA (51, 77, 97, 142). Thus the gender difference in body weight does not fully explain the gender difference in OSA prevalence.

Obese men with OSA have significantly higher levels of visceral fat than equally obese male controls (141). The distribution of body fat may therefore be important in determining risk for OSA and may contribute to the high male prevalence because women have lower waist to hip ratios than men. This is supported by regression analysis performed on the Wisconsin Sleep Cohort Study population (155) to determine the odds ratio for men (compared to women) having sleep disordered breathing (AHI >5 events·hr^{-1}) with different body habitus parameters included in the regression model. With only age and gender in the model, men were 2.7 times more likely than women to have SDB. When BMI was included, the male risk increased to 5.8 times that of women. However, when waist-hip ratio or neck girth were included in the model instead of BMI, the risk for OSA was no longer different between men and women. These data suggest that the finding of lower AHI in women compared with equally obese male OSA patients may be related to different patterns of fat deposition. A recent report from the Sleep Heart Health Study also suggests an independent effect of neck girth on the odds for having an AHI ≥15, however the effect of waist to hip ratio was weaker and neither factors eliminated the influence of sex on OSA prevalence (161).

Central rather than peripheral obesity may be deleterious to breathing during sleep in several ways. Resting lung volume is reduced with weight gain and high waist to hip ratios are associated with greater reductions in forced vital capacity (52). Respiratory stability may be decreased with reduced lung volume, because a given change in ventilation will cause a greater change in blood gases due to the reduced buffering ability of the lung (69). Greater fluctuations in arterial blood gases will in

turn lead to more ventilatory oscillation. Therefore obese male OSA patients may, by virtue of greater central fat distribution than equally obese female patients, have more ventilatory instability and more severe OSA. To my knowledge this has not been formally tested.

An alternative explanation is that increased central fat deposition may push the diaphragm rostrally thereby reducing caudal traction on the upper airway. The importance of caudal traction on upper airway stability has been demonstrated in cats (135) and dogs (139) however the role in humans is uncertain. There is an increased incidence of snoring during pregnancy (80), a situation in which women develop increased abdominal mass. Preliminary data suggest that upper airway compliance (as measured by the change in airway area from seated to supine postures) is increased during pregnancy when compared to non pregnant control subjects (119), an effect that may be caused by reduced caudal traction on the airway.

It is therefore possible that women of equal BMI to men may be protected from OSA because of lower central obesity such that they have a more stable respiratory control system, more caudal traction on the airway or a combination of these two effects. Importantly, the recent report of regression analysis of men and women in the Sleep Heart Health Study showed gender remained as an independent predictor of OSA after correction for BMI, waist to hip ratio and neck girth (161). This suggests that while distribution of fat may play a role in the male

predominance, it is not likely to be the sole factor responsible for the gender difference in OSA prevalence.

1.3.2 Upper airway anatomy and physiology

1.3.2.1 Anatomy

To my knowledge only two studies have compared the upper airway anatomy in men and women with OSA (51, 93). Guilleminault and co-workers obtained lateral cephalometric roentgenograms in 27 women and 110 men who presented to their sleep laboratory and were diagnosed with OSA (51). The men and women did not differ in terms of age or AHI however the women had significantly higher body mass indices than men. The only cephalometric variable that differed between genders was the length of the soft palate which was greater in men than in women. Of particular importance was the finding that the posterior airway space was not different between men and women. More recently Mohsenin (93) reported the upper airway cross sectional area (UA-CSA) measured by acoustic reflection was significantly smaller in women when compared to more severely affected OSA men, even after correction for differences in height.

There have been several studies comparing the upper airway anatomy in healthy men and women (23, 25, 85, 134). Male subjects have been reported to have larger UA-CSA than females measured by the acoustic reflection technique while awake and seated (23, 25, 85, 134). However, the upper airway size is dependant on lung volume and it appears that the reduction in airway area from functional

residual capacity (FRC) or total lung capacity (TLC) to residual volume (RV) is greater in men than in women (23, 25). This difference did not persist after correction for the differences in body surface area between men and women (25). The UA-CSA is reduced on assuming the supine position in young subjects (85, 134). This effect is reported to be greater in men than in women in one study (85) but not in another (134). These studies therefore suggest that the UA-CSA is greater in men than women and that this probably reflects a larger body size in men.

The UA-CSA is only one factor determining the likelihood of upper airway collapse. Airway length and shape may also be involved and there are preliminary data suggesting the upper airway is longer in men than in women (82). Many other factors also influence collapsibility including the intrinsic pharyngeal wall compliance, the pressure in the tissues surrounding the airway and the negative pressure gradient across the airway. If any of these factors differ between genders, they may contribute to the observed change in UA-CSA with a change in body position or lung volume and may render the male airway more prone to collapse.

1.3.2.2 Muscle activity

The activity of upper airway dilator muscles is thought to contribute to airway stability. The genioglossus is the largest upper airway dilator muscle and has therefore attracted most attention with regard to sleep disordered breathing. The genioglossus muscle is innervated by the hypoglossal nerve and has respiratory related activity. This is believed to occur partly due to a reflex response to the

negative pressure generated during inspiration and partly due to central activation from respiratory premotorneurons (54, 62, 78). During sleep the genioglossal negative pressure reflex response is reduced (55, 143). This is thought to render the airway less able to respond to negative pressure generated by inspiration and therefore to contribute to pharyngeal collapse during sleep (55, 143).

The genioglossus is found to be more active during wakefulness in OSA patients than in controls, suggesting a compensatory reflex hyperactivity secondary to compromised upper airway anatomy (90). This increased activity is lost in most patients at sleep onset, a time when upper airway obstruction first occurs (89). This contributes to the notion that the genioglossus may play an important role in the maintenance of upper airway patency in OSA, and that reduced muscle activity may predispose to airway collapse.

The resting activity of the genioglossus muscle has been reported to be higher in healthy women than men during wakefulness (111) and in women, higher in the luteal than follicular menstrual phase. (112). In post-menopausal women, 2 weeks of hormone replacement (progesterone and oestrogen) has been shown to increase the tonic and inspiratory phasic activity of the genioglossus (112). Female sex hormones were therefore implicated in the difference in resting muscle activity between men and women. However several more recent studies have found no gender difference in the genioglossus muscle activity of healthy individuals (110, 147). It is therefore uncertain if a gender difference in the resting level of genioglossus muscle activity exists in healthy subjects.

1.3.2.3 Resistance and collapsibility

The combined influence of upper airway size, muscle activity and intrinsic properties of the airway wall and surrounding tissues on upper airway function can be assessed with measurement of pharyngeal resistance and the critical closing pressure (P_{CRIT} - the nasal pressure below which the pharynx collapses). During wakefulness the pharyngeal resistance has been reported to be higher in men in one study (148) and not different between genders in another (111). Studies of upper airway resistance during sleep have reported equally variable results. During sleep onset, upper airway resistance has been reported to fluctuate markedly but not differently between genders (68). The same group subsequently found that during stable NREM sleep resistance was higher in men than in women (136). Thurnheer and associates (134) have also compared upper airway resistance in young and old men to age and BMI matched women during both wakefulness and sleep. In both genders there was a significant increase in upper airway resistance at high inspiratory flow during stable NREM and REM sleep. The wake to sleep changes were generally comparable in men and women although women appeared to have more dynamic upper airway closure (i.e. increased resistance at high flow) than men. This finding opposes that reported by Trinder et al. (136). Rowley and colleagues recently reported that airway resistance was not different during stage 2 sleep between 33 men and 27 women (120). Thus there is considerable discordance between studies of airway resistance in healthy men and women, both awake and asleep. These differences are difficult to explain based on the methodologies used and may reflect the inherent variability of measurements of

upper airway resistance or subtle differences in body mass between subjects in the various studies.

Sforza et al. (122) have measured P_{CRIT} in stage 2 sleep in patients with OSA. The men and women studied did not differ in terms of age or AHI, however women had significantly higher mean BMI and smaller neck girth than men. Men were found to have higher P_{CRIT} than the women, indicating that the upper airway of male OSA patients is more collapsible than that of women with equal severity OSA, despite the men having lower mean BMI.

A recent study by Pillar et al. in healthy men and women supports this finding (110). External inspiratory resistive loading applied during NREM sleep resulted in a greater increase in pharyngeal resistance and reduction of tidal volume in men than in women. Although central drive was not measured, the authors considered that the reduced tidal volume was related to upper airway collapse rather than reduced drive as the relationship between ventilation and total load (external resistive load plus pharyngeal resistance) did not differ between genders. Their results, which suggest a more dynamically collapsible airway in men compared with women is at variance with the report by Thurnheer (134). Also, Rowley and colleagues report that P_{CRIT} did not differ between 8 men and 8 women (with equivalent body mass indices) in stage 2 sleep (120). In summary, it is impossible to know from these conflicting results whether there is a difference in upper airway resistance or collapsibility in healthy men and women.

1.3.3 Respiratory control

The central control of respiration has been implicated in the pathogenesis of OSA (60, 154). OSA patients have been reported to have a less stable respiratory controller than less severe patients (154) and healthy controls (60). Differences in ventilatory responses to chemical stimuli have been postulated to contribute to increased respiratory instability in sleep (70). These responses have therefore been compared between men and women with and without OSA in several studies with mixed findings. The hypoxic ventilatory response has been reported to be lower in awake women (145, 146), higher in awake women (3) and not different between genders both awake (117) and asleep (145). The hypercapnic ventilatory response is generally reported to be lower in women awake (3, 146), however it has also been reported to be similar between men and women awake and asleep (39). Sin et al. (126) compared the hypercapnic ventilatory response between men and women, with and without OSA and found that women had a lower hypercapnic ventilatory response than men, but not following correction for FEV_1. Recently, van Klaveren and Demendts reported that although the hypoxic and hypercapnic ventilatory responses were higher in healthy men than women, the $P_{0.1}$ responses were not different between genders (140) suggesting central drive may be similar between genders.

During sleep, the level of CO_2 is critically important in determining the level of ventilation. If the arterial CO_2 falls below a critical threshold (apneic threshold) ventilation ceases until the CO_2 rises back above the threshold for ventilation. Zhou and colleagues (164) have compared the susceptibility of men and women to

develop central apnea with mechanical ventilation during sleep. These authors found that women required a greater reduction in arterial CO_2 to cause central apnea than men. If a larger reduction in CO_2 is also required for women to develop a hypopnea, women may develop less respiratory instability and be protected from OSA (69).

Respiratory controlling neurones demonstrate plasticity and memory such that a given respiratory stimulus may alter respiration well after the stimulus ceases (44, 113). Several of these mechanisms have been extensively studied in animals but there are fewer studies in humans. Respiratory memories can be inhibitory or facilitatory (44, 113) and may influence both respiratory and upper airway stability (45). Respiratory afterdischarge (RAD) and long-term facilitation (LTF) are two facilitatory memories that may protect against cyclical breathing patterns and be important in the pathogenesis of OSA.

Respiratory afterdischarge is the gradual decline in respiration following the cessation of a brief respiratory stimulus that typically lasts for 15-60s in humans (13, 48, 131). RAD is thought to reflect short-term potentiation of brainstem respiratory controlling neurones and is elicited following a variety of respiratory stimuli including carotid stimulation (direct electrical stimulation (43) or with hypoxia (13, 48)), calf stimulation in animals (41, 42) and voluntary hyperventilation in humans (131). An abnormally short rate of decline in ventilation following removal of a respiratory stimulus could predispose an individual to cyclical breathing, particularly in sleep where it may contribute to repetitive apneas (153). In

agreement with this hypothesis, patients with OSA are found to have RAD that is about half the length of that in normal men (49). Female sex hormones have been suggested to alter RAD (67, 131), however studies comparing the duration of RAD in the luteal compared to follicular menstrual phase have reported mixed findings (67, 131). Also, we have recently shown healthy young men and women have similar duration of RAD following brief isocapnic hypoxia during wakefulness (67).

Long-term facilitation (LTF) is another respiratory memory that has been suggested to contribute to the pathogenesis of sleep related breathing disorders (2, 9). Long-term facilitation is the progressive rise in ventilation above the eupnic level that occurs during and following repeated carotid body stimulation (45, 91). LTF is a centrally mediated, serotonin dependent process (10) that can last from several minutes to hours depending on species and protocol used (45, 113). By preventing periods of low respiratory drive, LTF is thought to stabilise respiratory patterns and possibly upper airway patency. LTF has been demonstrated in numerous animal models and found to be affected by gender and the estrus cycle in rats (162) but it has proven more difficult to elicit in humans (2, 9, 87). Whether LTF is similarly influenced by sex hormones or is different between genders in humans in currently unknown.

As previously mentioned, the upper airway dilator muscles also receive inputs from the respiratory controller and have inspiratory related activity (62, 78). It is therefore not surprising that they have been demonstrated to exhibit respiratory memory in animals (10, 46, 65). However, the activities of respiratory pump and upper airway

muscles do not always correspond. They have been shown to become uncoupled following alcohol (19, 75), brain hypoxia (128) and benzodiazepene administration (20). It is therefore possible that respiratory neural plasticity or memory may differ between upper airway dilator muscles and respiratory pump muscles. If during or immediately after increased pump muscle activity, the activity of upper airway dilator muscles is preferentially reduced, then the airway would be at least theoretically, prone to collapse. Correspondingly, if the respiratory memory in upper airway dilator muscles differs between genders, then airway stability may also differ.

1.3.4 Sleep and arousal from sleep

Sleep is of critical importance to the pathogenesis of all sleep apnea syndromes. There are multiple physiological changes that occur at sleep onset that are likely to contribute to breathing disturbance. These include the reduction in activity of postural and some upper airway muscles (132), which may contribute to the observed reduction in airway size and increase in pharyngeal collapsibility and upper airway resistance (61, 136). Lung volume may be reduced (59) particularly in individuals with a central pattern of obesity and this in turn may increase respiratory instability (see Obesity, page 9). The central respiratory drive is also reduced during sleep as the "wakefulness stimulus" to breathe is lost (107) and chemoresponses suppressed (38, 39, 144, 145). At arousal from sleep, many of these changes are rapidly reversed with the sudden restoration of airway patency and increased chemoresponsiveness. Physiologic changes following arousal from sleep also appear to be influenced by a unique "waking reflex" (56, 137). Arousal

from sleep may transiently increase ventilation above the stable waking level and induce mild hypocapnia. Thus arousal from sleep may destabilise respiration and be important in sleep apnea syndromes (70, 151). I am unaware of any prior studies that compared the ventilatory response to arousal between men and women. However, if the response is different between genders then respiratory stability in sleep may also differ.

1.4 Central sleep apnea

Central sleep apnea (CSA), like OSA is also characterised by repetitive apneas interspersed with periods of hyperventilation and arousal from sleep. However, in contrast to obstructive upper airway events, central apneas are related to reduced central respiratory drive. CSA is less common than its obstructive counterpart, however Javaheri et al. have reported that 45% of men with congestive heart failure (CHF) have CSA (64). More recently, Sin et al. (125) compared polysomnographic findings in 382 men and 68 women with CHF (125). These authors reported a higher incidence of both central and obstructive sleep apnea in men than in women despite age, BMI, indices of heart failure and medications being similar. Whether idiopathic CSA is also more common in men than in women is unknown given the sparsity of research on this relatively rare condition. However of the reports of consecutive patients with idiopathic CSA there has been only one female identified (150, 151) providing some weak evidence that idiopathic CSA may also be more prevalent in men than in women. Also, in two large community studies Bixler and colleagues showed the prevalence of CSA was 0.4% in males but no women were identified with CSA (17, 18).

The primary problem in both idiopathic and CHF related CSA appears to be hyperventilation such that CO_2 levels are driven below the apneic threshold in sleep (149, 150). While patients with CHF do also have increased circulatory delays (which have been suggested to increase respiratory instability (71)), Naughton et al. (95) have recently shown that CHF patients without CSA had similar lung to ear circulation times to CHF patients with CSA. Additional support for the role of Pa_{CO_2} comes from reports that both forms of CSA may be improved or eliminated by raising the Pa_{CO2} with either an added dead-space or supplemental CO_2 during sleep (149). It would appear likely that the increased apneic threshold for $PaCO_2$ demonstrated in women (164) may help protect women from CSA. If the ventilatory response to arousal differs between men and women then the susceptibility to central apnea may also differ.

The upper airway has also been implicated in the pathogenesis of CSA because stimulation of upper airway reflexes can result in central apnea in experimental animals and pre-term infants (34, 53). In some patients central respiratory events are predominantly seen in the supine position (22), a result that may be caused by a reflex response to airway narrowing in this position. However it is also possible that functional residual capacity may change with body position and impair respiratory control (see Obesity, page 9) such that ventilatory stability is reduced. Support for a role of the upper airway in CSA comes from the finding that snorers with idiopathic CSA have significantly greater changes in upper airway area associated with changes in lung volume than snorers with no apnea (21).

Central sleep apnea, like OSA, appears less common in women than men, suggesting there may be gender differences in respiratory control that protect women from both sleep apnea syndromes. On the other hand the upper airway may be involved in CSA and the low prevalence of both disorders in women may be related to a more stable upper airway in women.

1.5 Summary and aims of thesis

In summary, OSA and CSA are more common in men than in women. This may reflect differences in airway anatomy or physiology, however previous research has failed to consistently identify such a difference. The site of fat deposition may be important in the gender difference, however this has as yet not been systematically tested. The difference in severity of OSA from NREM to REM sleep has been demonstrated in two sleep clinic based studies to differ between the genders. This appears likely to reflect a difference in the central control of respiration or upper airway dilator muscles as other anatomical based differences remain unchanged between stages. The aim of this doctoral study was therefore to examine several aspects of respiratory and upper airway muscle control in men and women, to try and identify gender differences that may contribute to the difference in expression and prevalence of OSA between men and women.

CHAPTER 2. GENERAL METHODS

2.1 Subjects and study conditions

Overall 43 men and 45 women participated in the studies presented in this thesis. All subjects were informed of the risks associated with the experiments and informed written consent was obtained from all subjects. The studies were approved by the Research and Ethics Committee of the Repatriation General Hospital, Daw Park, South Australia and the Human Research and Ethics Committee of the University of Adelaide. The subjects were healthy non-smokers with normal lung function (spirometry and body plethysmography) who did not take any regular medication, including the oral contraceptive pill. Female subjects had regular menstrual cycles not longer than 35 days. The physical characteristics of the subjects are presented for each study in the relevant chapter.

All experiments were performed in a temperature controlled (23 ± 1°C), sound insulated bedroom in the Sleep Disorders Unit, Repatriation General Hospital, South Australia. All studies were performed with the subjects lying on a hospital bed with one pillow. The posture assumed in each study is presented in the relevant chapter.

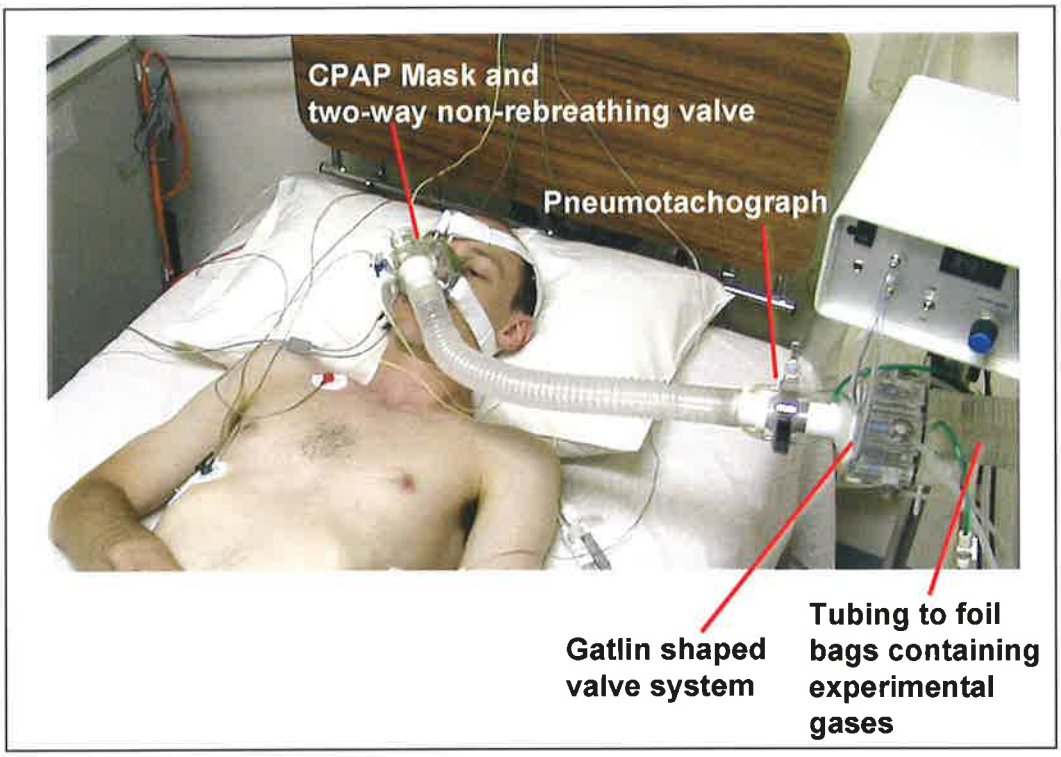
2.2 Equipment and measurements

2.2.1 Respiratory measurements

A nasal mask (Gel mask, Respironics, PA) with two-way non-rebreathing valve attached (series 2600, Hans Rudolph, Kansas City, MO), allowed measurement of inspiratory minute ventilation ($\dot{V}_I$) via a pneumotachograph (PT36, Erich Jaeger, Germany) attached to the inspiratory side of the breathing valve. The flow signal from the pneumotachograph was electronically integrated to give tidal volume (V_T). The peak inspiratory flow (PIF), inspiratory (T_I), expiratory (T_E) and total (T_{TOT}) breath times were determined from the flow signal and breathing frequency (F_B = 60/Ttot) and minute ventilation ($\dot{V}_I = V_T \times F_B$) calculated on a breath-by-breath basis. Mask pressure (P_{MASK}) and end-tidal partial pressure of CO_2 (P_{ETCO_2}) were measured continuously through ports in the mask (P_{MASK} - model 78342A, Hewlett Packard, Andover, MI; P_{ETCO_2} - POET II model 602-3, Criticare systems Waukesha, WI).

For experiments 1 and 2 (Chapters 3 and 4), inspiratory gases were delivered through a Gatlin-shaped valve system (series 2440C, Hans Rudolph, Kansas City, MO) which consisted of 1 output port attached to the pneumotachograph and 4 inputs, 3 of which were connected to foil bags (300 litres, Scholle Industries, Adelaide, Australia) containing the inspiratory gas mixes (see Chapters 3 and 4 for details). The fourth port was open to room air. Only one input port was open at any time and all changes between ports were conducted during expiration. This experimental setup is depicted in Figure 1.

Figure 1 Experimental setup for studies 1 and 2 (Chapters 3 and 4)



Experimental setup showing the breathing circuit. Subjects wore a nasal CPAP mask with two-way non-rebreathing valve attached, the inspiratory side of which was connected to the pneumotachograph and Gatlin shaped valve system for delivery of experimental gases.

2.2.2 Upper airway measurements

Epiglottic pressure (P_{EPI}) was measured with a pressure transducer tipped catheter (MPC-500, Millar, Houston, TX) in experiments 1 and 2 (Chapters 3 and 4), and Millar catheters or disposable air perfused catheters in the final experiment (Chapter 5). Air perfused catheters were custom made from 40cm lengths of polyethylene tubing (2.1 mm OD, Microtube Extensions, North Rocks NSW). Approximately 10-15 radially arranged ~1-mm holes were drilled in the distal 1-cm of each catheter. A bead of acrylic glue was placed at the tip of the cut end of the catheter to minimise airway irritation. Both types of catheters were placed in the same manner. After both nostrils were decongested (0.05% oxymetazoline HCl) and the nostril through which the catheter was passed anaesthetised (2% lignocaine), the epiglottic catheter was advanced 1cm below the tongue base under direct visualisation. Catheters were secured at the nose with tape. Upper airway resistance (P_{EPI} to P_{MASK}) was calculated ($\Delta P/Flow$) at peak inspiratory (R_{UA}) and 200 $ml \cdot sec^{-1}$ flow (R_{200}).

2.2.3 Electromyographic recordings

The electromyogram of the genioglossus muscle (EMG_{GG}) was recorded in the standard manner (90) with 2 intramuscular Teflon insulated stainless steel wire electrodes (316SS5T wire, Medwire, Mt Vernon, NY) inserted 4 mm either side of the frenulum to a depth of 1½ -2 cm after surface anesthesia (4% lignocaine). Surface electrodes were placed in the right 6^{th}, 7^{th} and 8^{th} intercostal spaces adjacent to the costal margin for measurement of diaphragm muscle activity. The diaphragmatic electromyogram (EMG_{DI}) was recorded from the pair of electrodes

with the highest inspiratory phasic activity. These positions were chosen to minimise intercostal and abdominal muscle artifact on the diaphragm signal (76). The electrocardiogram (ECG) was also recorded and the ECG R-wave used as a trigger to blank ECG artifact from the EMG_{DI} signal (CWE, Ardmore, PA). Both genioglossal and blanked diaphragmatic EMGs were band-pass filtered (0.3-1kHz) and recorded at 1kHz. The raw signals were rectified and moving time averaged with a time constant of 100 msec. For each breath the expiratory tonic (Tonic) and peak inspiratory phasic (Phasic) EMG activities were determined. The genioglossal activity at 200 $ml \cdot sec^{-1}$ of flow ($EMG_{GG(200)}$) was also calculated in experiment 1 (Chapter 3).

The maximal electromyographic activity of the genioglossus muscle was determined by performing three of each of the following maneuvers: swallows, deep sniffs and maximal tongue protrusions against the top teeth. The highest activity recorded during these maneuvers was taken to be the maximal activity of the genioglossus. During each maximal tongue protrusion, the subject was given strong verbal encouragement until a plateau in activity was reached to ensure maximum activity was recorded. The EMG_{GG} activity was then expressed as a percentage of maximal activity by scaling the moving time averaged signal between electrical zero and the maximum activity level. This well established technique (90) allowed the EMG_{GG} signals to be averaged within and between subjects and compared between genders.

2.2.4 Electroencephalographic and electrooculographic recordings

The electroencephalogram (EEG, position C3-A2) and right electrooculogram (EOG-R) were recorded in all studies using surface electrodes and a commercial sleep laboratory amplifier (Compumedics S series preamplifier, Abbotsford, Victoria, Australia) to confirm wakefulness (experiments 1 and 2) or determine sleep stage (experiment 3).

2.2.5 Arterial oxygen saturation recording

Arterial oxygen saturation (SaO_2) was measured in all studies (POET II model 602-1 Criticare systems). This allowed documentation of the degree of hypoxemia in experiments 1 and 2, and assisted in confirming that the subjects studied in experiment 3 had no evidence of sleep disordered breathing.

2.3 WINDAQ data acquisition system

All data were acquired on an IBM laptop computer using an analogue to digital converter (DATAQ Instruments, Akron, OH) at a sampling rate of 200Hz for all signals other than EMGs (1 kHz). Custom designed software developed using Excel Visual Basic (Excel97 SR-2, Microsoft Corporation, USA) was used to calculate each variable on a breath-by-breath basis for subsequent analysis.

2.4 Statistical analyses

Anthropometric and resting data were compared between genders with two sample Student's t-tests (Excel97 SR-2, Microsoft Corporation, USA) assuming unequal variance. Measurements made at repeated intervals were analysed using analysis of variance (ANOVA, SPSS Release 6.0 –10.0.5, SPSS Inc, Chicago, IL) for repeated measures including the Greenhouse-Geisser correction for multisample asphericity (81). One assumption of ANOVA is that the data displays multisample sphericity, or that the variance at each repetition (time point) is similar. In the studies described in this thesis, the variance increased with the mean violating the multisample sphericity assumption. ANOVA main and interaction effects were considered the principle test of a given hypothesis. However, where appropriate, Tukey's post-hoc tests were used to further investigate significant ANOVA effects.

Group data are expressed as means ± standard error of mean (SEM) and $p<0.05$ was considered significant. Where changes approached significance the p value is reported separately.

CHAPTER 3. GENIOGLOSSUS MUSCLE ACTIVITY AT REST AND IN RESPONSE TO BRIEF HYPOXIA IN HEALTHY MEN AND WOMEN

3.1 Introduction

Obstructive sleep apnea (OSA) is more common in men than in women for reasons that are not clearly understood. While many investigations have focussed on identifying upper airway anatomical differences between men and women (25, 85), more recently a difference in respiratory neural control of upper airway dilator muscles has been implicated in this gender difference (111). The respiratory activity of the genioglossus muscle is partly due to a reflex response to the negative airway pressure during inspiration and partly to central activation independent of the reflex (spontaneous activity from respiratory pre-motor neurones) (62). Either of these elements may differ between the genders and may contribute to the high male prevalence of OSA.

The resting inspiratory activity of the genioglossus muscle has been reported to be higher in women than in men (111). This could be, at least in part, a result of hormonal differences as the genioglossal activity was later reported to be higher in the luteal menstrual phase than the follicular phase (112). However, more recent studies by the same investigators have failed to replicate the finding of a gender difference in resting genioglossal activity during wakefulness (147) or sleep (110).

There is therefore uncertainty as to whether a gender difference in resting genioglossal activity actually exists.

Following removal of a respiratory stimulus there is a respiratory afterdischarge (RAD) in minute ventilation, which can be observed as a slow decline in ventilation to the resting level (48). The duration of respiratory afterdischarge has been proposed to be important in determining ventilatory stability (153), with a short RAD representing an under-damped respiratory controller that is predisposed to unstable patterns. Consistent with this theory is the finding that the duration of RAD is shorter in OSA patients than healthy controls (49) suggesting a possible respiratory control abnormality in these patients. We recently found RAD, as measured by the rate of decline in ventilation after brief hypoxia, to be the same in men and women, and the same between the luteal and follicular menstrual cycle phases (67). The current study was conducted to examine whether there was respiratory afterdischarge of the genioglossus muscle, and if so, whether it parallels that seen in respiratory pump muscles or is different between the genders. A slow decline in respiratory phasic hypoglossal nerve activity has been described in cats following carotid sinus or superior laryngeal nerve stimulation (65), and in humans the activity of the diaphragm and genioglossus muscles have been shown to change proportionately during hypoxia (106) and hypercapnia (105). These findings suggest that phrenic and hypoglossal motor neurone control are coupled during periods of physiological stimulation. However it was not known whether on abrupt termination of a respiratory stimulus, there is RAD in hypoglossal motorneurones and if so, whether this is coupled to the RAD of respiratory pump muscles. There

are a variety of situations where the activities of the hypoglossal and phrenic motor neurone pools become uncoupled. These include following alcohol ingestion (75), benzodiazepene administration (79), anaesthesia and cyanide brain hypoxia (128). The two motor neurone pools are also seen to behave differently in patients with obstructive sleep apnea, because although both muscles increase to a similar degree during an apnea, the genioglossus increases out of proportion to the diaphragm on termination of the apnea (104). The current study was therefore conducted to determine if the genioglossus and diaphragm muscle activities follow each other and ventilation after sudden termination of hypoxia, and whether the muscle groups behave similarly between genders. If gender differences in RAD were observed in the electrical behavior of these two muscles, it may have implications for the mechanical behavior of the upper airway following episodes of brief hypoxia such as occur in OSA.

In awake normal subjects the activity of the genioglossus muscle has recently been reported to correlate with pharyngeal pressure during application of inspiratory resistive loads (83). It is postulated that in awake subjects a negative pressure neural reflex (63, 78) is a major contributing factor to genioglossal activity during tidal and stimulated breathing (83). Whether the negative pressure reflex or the relationship of pharyngeal pressure to upper airway dilator activity during tidal breathing is different between genders has not been investigated previously.

I therefore aimed to compare three aspects of upper airway dilator function in healthy young men and women: 1) resting inspiratory genioglossus muscle

(EMG_{GG}) activity, 2) the respiratory EMG_{GG} afterdischarge following a brief hypoxic stimulus, and 3) the relationship between the EMG_{GG} and pharyngeal airway pressure during normoxia and isocapnic hypoxia.

3.2 Methods

3.2.1 General

16 men and 14 women were recruited to the study. Women were tested in the luteal menstrual phase (20-24 days following the first day of menstruation). Plasma progesterone levels were measured (ACS:180 Progesterone Assay, Chrion Diagnostics, Chrion Healthcare, Victoria, Australia) to confirm menstrual phase and that ovulation had occurred. The data of 1 subject was subsequently removed due to anovulation (plasma progesterone < 7 $nmol \cdot L^{-1}$ in the luteal phase). Inadequate data were obtained in 4 men and 1 woman (see Data analysis) leaving 12 men and 12 women for analysis.

3.2.2 Experimental gases

The 3 inspiratory gases contained in the foil bags attached to the input ports of the Gatlin valve system (see GENERAL METHODS, page 24) were 100% O_2, 100% N_2, 9% O_2 in N_2. The fourth port was open to room air. Only one input port was open at a time and all changes between ports were conducted during expiration. 100% CO_2 was bled into the inspirate immediately up-stream of the pneumotachograph as required to maintain isocapnia.

3.2.3 Protocol

Subjects attended the laboratory for a preliminary visit during which time respiratory function testing was performed (spirometry and whole body plethysmography) and subjects familiarised with experimental equipment. The subjects then returned on a second occasion in the morning after a light breakfast without caffeine. They were instrumented (as described in GENERAL METHODS, page 24) and lay supine. Maximal electromyographic activities were determined after which subjects were given earphones through which they listened to the radio. They were instructed to relax, keep their eyes open, stay awake and to breathe only through their nose. They were informed that during hypoxia they might experience slight dizziness or breathlessness, but that these responses were normal and so to remain relaxed. After 5 minutes of baseline room air breathing, the subjects were exposed to 45-75 seconds of isocapnic hypoxia (9% O_2), which was rapidly reversed with 1 breath of 100% O_2. Each hypoxic period was initiated with 1-6 breaths of 100% N_2 to cause a rapid fall in SaO_2 and the number of breaths and duration of hypoxia adjusted such that the ventilatory increase by the end of the hypoxic period was approximately 160% of the resting ventilation level. Each subject received 12-20 hypoxic exposures separated by at least 7 minutes of room air breathing to avoid the ventilatory depression associated with prolonged hypoxia. Maximal EMG maneuvers were repeated at least 5 minutes after the last hypoxic period.

3.2.4 Data analysis

To enable respiratory afterdischarge to be measured it was necessary to select trials in which the baseline breath-by-breath ventilation was relatively stable and free from artifact and in which there was a clear hypoxic respiratory neural stimulus (evident in ventilation, and both the diaphragmatic and genioglossus muscle activities). Therefore individual trials were only included in the analysis if the following criteria were met: 1) baseline ventilation was stable (the co-efficient of variation in resting minute ventilation in the 30 seconds before a trial was less than 25%); 2) Minute ventilation at the end of hypoxia was increased ≥ 20% above the resting level; 3) The EMG_{DI} and EMG_{GG} both increased ≥ 10% above the resting level by the end of hypoxia; 4) Isocapnia was maintained (the end-tidal CO_2 both during and following hypoxia was within 2 Torr of the mean of the 30s pre-hypoxia level) ; 5) There were no sighs in the last 20 seconds of the hypoxic period or in the 25 seconds following termination of hypoxia (sighs were characterised by a change in V_T of > 75% between two adjacent breaths when accompanied by a change in T_E of > 40% between the same two breaths) and 6) There were no swallows in the last 20 seconds of the hypoxic period or in the 25 seconds immediately following the hypoxia (swallows were visually determined from the epiglottic pressure and genioglossal EMG traces). If swallows or sighs occurred elsewhere within a trial, then the breath during which it occurred was removed, but the trial remained in the analysis. One female and four male subjects had no trials that met these criteria and were excluded from further analysis. To allow breath-by-breath measurements to be averaged within and between subjects the remaining trials were interpolated at 4-second intervals starting 10s after the hyperoxic breath, to allow for inspiratory

circuit (4s) and peripheral circulatory (6s) delays (71). The last 20 seconds of the hypoxic period was also interpolated at 4-second intervals to ascertain the degree of hypoxic stimulation. Resting variables were determined for the 20 seconds immediately prior to each trial. EMG_{GG} versus P_{EPI} comparisons were made for breaths during and after hypoxia to give a range of pressures and genioglossal activities. The slopes and correlation coefficients and the theoretical genioglossal activity at zero pharyngeal pressure (y-intercept of the pressure versus genioglossal activity plot) were compared between genders. The latter should theoretically reflect the inspiratory pre-motor neural activity of the muscle. That is, inspiratory phasic activity arising from the respiratory pattern generator uninfluenced by the negative pressure reflex.

3.2.5 Statistical procedures

Resting data and data following hypoxic stimulation were compared as described in GENERAL METHODS, page 24. All measured variables ($\dot{V}_I$, V_T, T_{TOT}, phasic and tonic EMG_{GG} and EMG_{DI}, $EMG_{GG(200)}$, PET_{CO2}, SaO_2, P_{EPI}, $P_{EPI(200)}$, P_{MASK}, PIF and $R_{(200)}$) were compared between men and women for the last 20 seconds of hypoxia and for 24 seconds after termination of hypoxia with two-way analyses of variance (ANOVA). In addition, Student's t-tests were used to compare the slope, intercept and correlation coefficients (R) for epiglottic pressure-genioglossal muscle activity relationships.

3.3 Results

3.3.1 Trial exclusion

There were 117 hypoxia trials that met the inclusion criteria (57 trials in 12 men, 60 in 12 women). There were approximately equal numbers of trials excluded in men and women for each exclusion criteria except the "minute ventilation increase" criterion in which 32 trials were excluded in men and only 16 in women.

3.3.2 Resting characteristics

There were no differences in age, body mass index or lung function between the 12 men and 12 women in this study (Table 1). Resting ventilation did not differ between the men and women in the luteal menstrual phase, however the $P_{ET_{CO_2}}$ was significantly lower in the women, consistent with the respiratory stimulant effect of progesterone (Table 2). The mean plasma progesterone level in the 12 women studied was 36.12 ± 4.16 $nmol \cdot L^{-1}$ (range 15.0 - 62.8 $nmol \cdot L^{-1}$) confirming recent ovulation in all females. PIF, P_{EPI}, $R_{(200)}$ and EMG_{GG} (Phasic, Tonic and $EMG_{GG(200)}$) were not different between the genders at rest.

3.3.3 Hypoxic stimulation and afterdischarge

There were no time or gender by time interaction effects in $P_{ET_{CO_2}}$ during or after hypoxia indicating satisfactory maintenance of isocapnia (Figure 2). The mean SaO_2 at rest (98.1 ± 0.2 % women, 97.9 ± 0.3 % men) and in the last 20s of hypoxia (86.0 ± 2.0 % women, 87.1 ± 0.9 men) were not significantly different

between genders. There were no significant gender or gender by time interaction effects in $\dot{V}_I$, P_{EPI} (Figure 3), phasic (Figure 4) or tonic (Figure 5) activity of the EMG_{GG} or EMG_{DI} during or after hypoxia. There were also no significant gender or gender by time interactions in $R_{(200)}$, P_{MASK} , PIF, V_T or T_{TOT}.

3.3.4 Epiglottic pressure – Genioglossus activity relationship

There were no gender differences in the correlation coefficient, slope or intercept of the relationship between genioglossus muscle activity and epiglottic pressure when group mean values were compared. In some subjects the relationship between epiglottic pressure and genioglossus muscle activity did not reach statistical significance, however when only the subjects with significant relationships were compared there were still no differences in correlation coefficient, slope or intercept between genders. Individual responses are shown in Table 3.

Table 1 Anthropometric data

	Men (n=12)	Women (n=12)	p value
Age (years)	27.8 ± 1.7	28.4 ± 1.9	0.817
BMI ($kg \cdot m^{-2}$)	24.1 ± 0.6	23.8 ± 1.0	0.748
FEV_1 (% predicted)	105.8 ± 2.1	106.2 ± 3.2	0.928
FVC (% predicted)	103.0 ± 2.8	100.3 ± 2.6	0.481
Progesterone ($nmol \cdot L^{-1}$)	-	36.2 ± 4.2	-

Age, body mass index (BMI), forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) in the men (n=12) and women (n=12) studied. Progesterone levels in females are also presented. All values are means ± SEM, $p<0.05$ significant.

Table 2 Resting characteristics in men and women

	Men (n=12)	Women (n=12)	p value
$\dot{V}_I$ ($l \cdot min^{-1}$)	7.40 ± 0.29	7.07 ± 0.23	0.386
P_{ETCO_2} (Torr)	40.7 ± 0.84	36.6 ± 1.02	0.005
V_T (l)	0.53 ± 0.04	0.44 ± 0.02	0.091
F_B ($breaths \cdot min^{-1}$)	14.73 ± 0.88	16.06 ± 0.68	0.166
PIF ($l \cdot min^{-1}$)	24.9 ± 0.7	26.8 ± 0.9	0.141
P_{EPI} (cmH_2O)	-2.13 ± 0.18	-2.30 ± 0.11	0.446
$P_{EPI(200)}$ (cmH_2O)	-1.20 ± 0.12	-1.27 ± 0.01	0.559
$R_{(200)}$ ($cmH_2O \cdot l^{-1} \cdot s$)	2.05 ± 0.24	1.78 ± 0.56	0.663
Phasic EMG_{GG} (% maximum)	3.16 ± 0.59	3.75 ± 0.79	0.557
$EMG_{GG(200)}$ (% maximum)	2.83 ± 0.66	2.98 ± 0.62	0.864

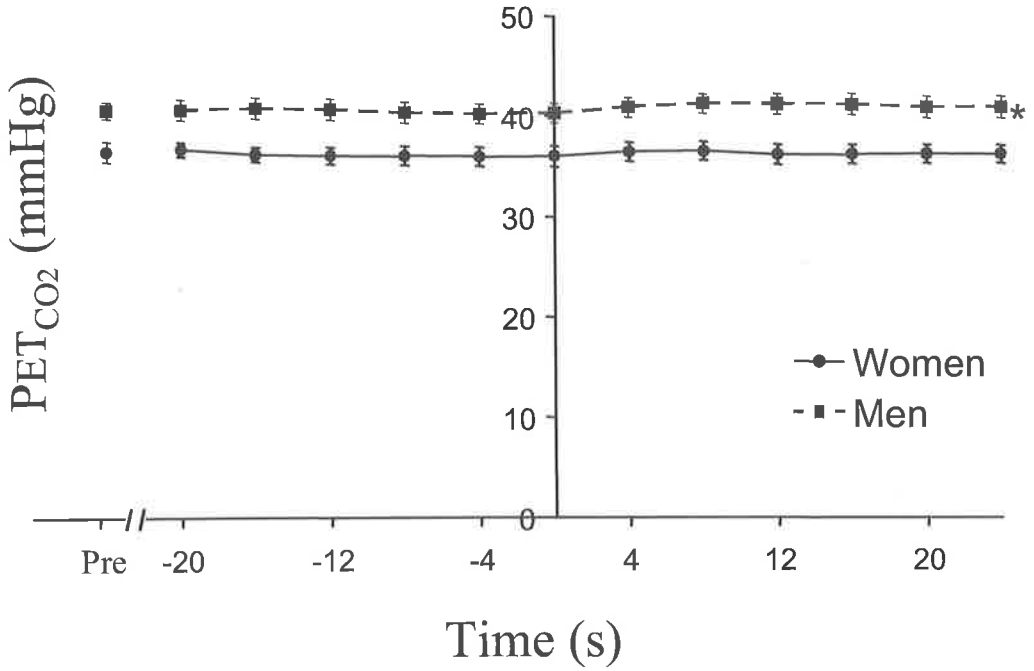
Minute ventilation ($\dot{V}_I$), end-tidal partial pressure of CO_2 (P_{ETCO_2}), tidal volume (V_T), breathing frequency (F_B), peak epiglottic pressure (P_{EPI}), inspiratory phasic genioglossal (Phasic EMG_{GG}) activity and epiglottic pressure, resistance and genioglossal activity at 200 $ml \cdot sec^{-1}$ flow ($P_{EPI(200)}$, $R_{(200)}$ and $EMG_{GG(200)}$ respectively) in the men (n=12) and women (n=12) studied. All values are means ± SEM, $p<0.05$ considered significant.

Table 3 The relationship between inspiratory phasic genioglossus activity and peak negative epiglottic pressure

Women					Men				
Subject	**R**	**p value**	**Intercept**	**Slope**	**Subject**	**R**	**p value**	**Intercept**	**Slope**
KK	-0.536	0.022	4.304	-0.078	**JW**	-0.820	0.000	1.659	-0.538
TO	-0.203	0.418	10.703	-0.428	**FO**	-0.848	0.000	1.747	-0.420
YD	-0.592	0.010	3.852	-0.372	**JJ**	0.046	0.857	5.429	0.029
JO	-0.845	0.000	-0.094	-1.010	**GD**	-0.182	0.471	2.260	-0.032
PL	-0.296	0.233	0.764	-0.575	**JV**	-0.118	0.653	2.855	-0.351
AJ	-0.912	0.000	1.221	-0.341	**TR**	-0.637	0.004	4.133	-1.205
LC	-0.390	0.109	4.890	-0.345	**PC**	-0.621	0.006	3.820	-0.303
CT	0.714	0.001	3.451	0.424	**SM**	-0.966	0.000	0.749	-0.688
ZM	-0.825	0.000	1.943	-0.128	**AB**	-0.556	0.017	0.127	-0.454
MC	-0.412	0.089	-0.986	-1.884	**BW**	-0.904	0.000	-1.180	-0.922
DW	0.502	0.034	2.308	0.212	**LK**	-0.962	0.000	1.962	-2.587
AD	-0.680	0.002	-2.702	-3.748	**SA**	-0.277	0.266	0.637	-0.043
Mean	-0.373		2.471	-0.689	**Mean**	-0.570		2.016	-0.626
SEM	0.15		0.992	0.33	**SEM**	0.10		0.534	0.21

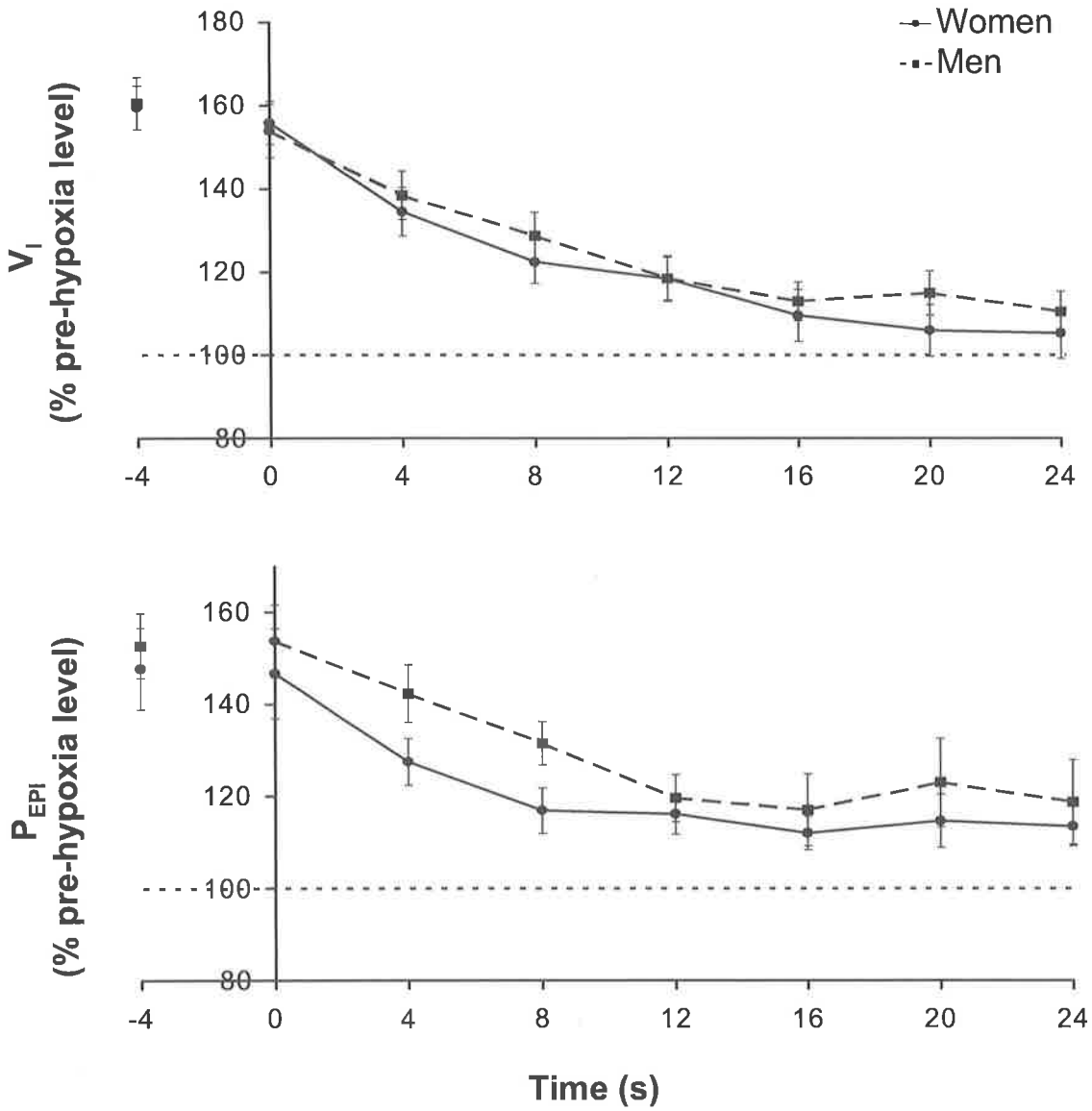
Correlation coefficients (R), significance (p-value), slopes and intercepts of epiglottic pressure versus inspiratory phasic genioglossal electromyogram activity in each subject. There were no significant differences between genders for any variable measured, $p<0.05$ considered significant.

Figure 2 **P_{ETCO_2} before, during and following brief hypoxia**



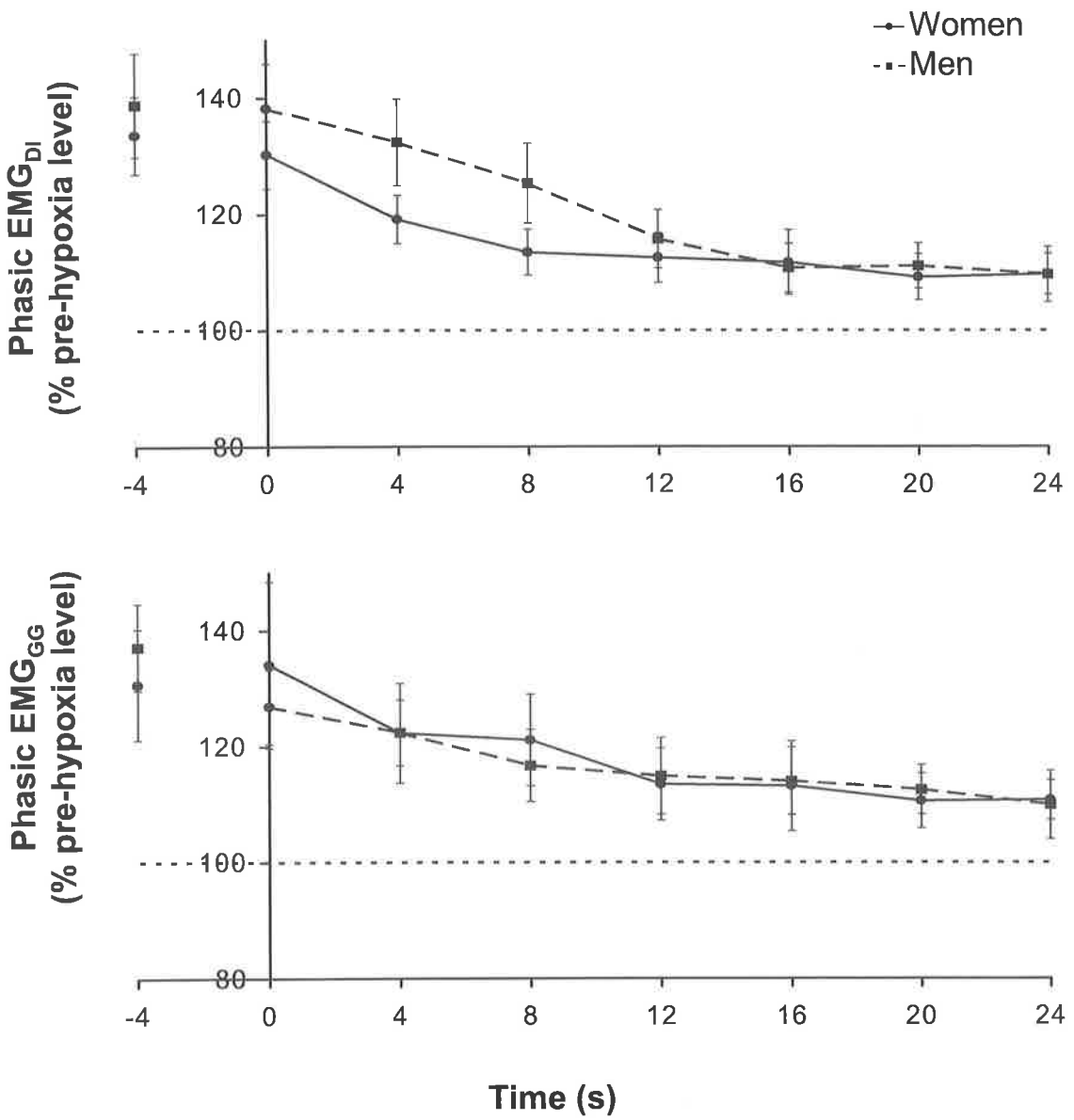
End-tidal partial pressure of CO_2 (P_{ETCO_2}) before (Pre), during (-20 to -4s) and after removal (0-24s) of brief hypoxia in men (n=12) and women in the luteal menstrual phase (n=12). There were no significant time or gender by time interaction effects indicating strict maintenance of isocapnia. * Indicates that there was a significant effect of gender on P_{ETCO_2} consistent with the resting differences (p=0.002, men higher than women). Values are means ± SEM.

Figure 3 **Ventilation and epiglottic pressure during and following removal of brief hypoxia**



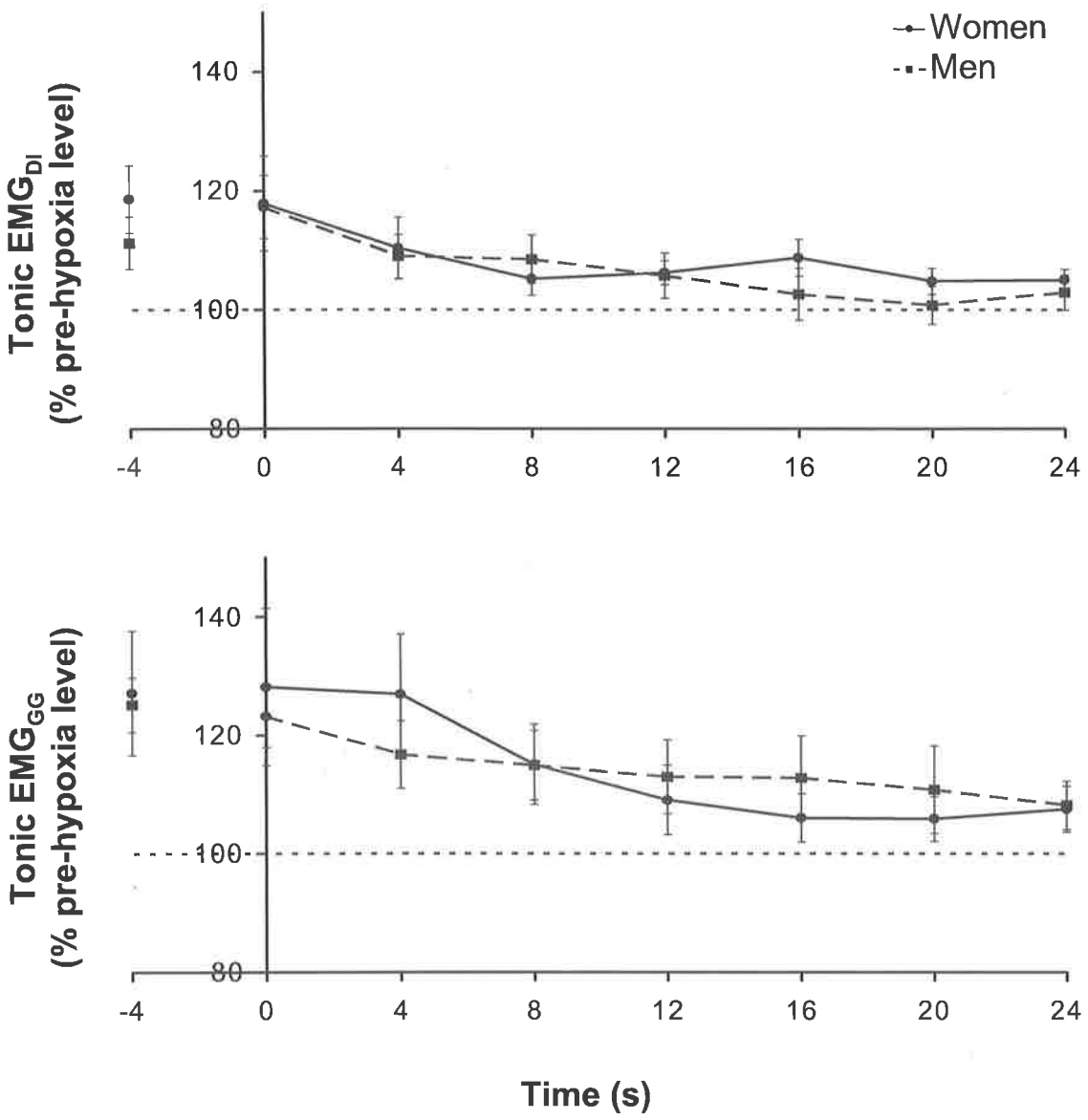
The decline in minute ventilation ($\dot{V}_I$) and peak epiglottic pressure (P_{EPI}) after brief isocapnic hypoxia in men (n=12) and women (n=12). Data at –4s are the average of the last 20s of hypoxia. All data are expressed as a percent of the pre-hypoxia level. There were no significant gender, or gender by time interaction effects in either variable. Values are means ± SEM.

Figure 4 **Inspiratory phasic diaphragmatic and genioglossal muscle activity during and following removal of brief hypoxia**



The decline in inspiratory phasic (Phasic) genioglossus (EMG_{GG}) and diaphragmatic (EMG_{DI}) muscle activities after brief isocapnic hypoxia in men (n=12) and women (n=12). Data at –4s are the average of the last 20s of hypoxia. All data are expressed as a percent of the pre-hypoxia level. There were no significant gender, or gender by time interactions for either variable. Values are means ± SEM.

Figure 5 **Expiratory tonic genioglossal and diaphragmatic muscle activity during and following removal of brief hypoxia**



The decline in expiratory tonic (Tonic) genioglossus (EMG_{GG}) and diaphragmatic (EMG_{DI}) muscle activities after brief isocapnic hypoxia in men (n=12) and women (n=12). Data at –4s are the average of the last 20s of hypoxia. All data are expressed as a percent of the pre-hypoxia level. There were no significant gender, or gender by time interactions for either variable. Values are means ± SEM.

3.4 Discussion

In this study the electrical activity of an inspiratory pump muscle (diaphragm) was compared to an upper airway dilator muscle (genioglossus) at rest, during and following brief hypoxic episodes. The hypoxic exposures were deliberately kept brief (45-75s) and given at infrequent intervals (7 minutes between episodes) to avoid the suppressant effect on the hypoglossal motor neurone pool which has been demonstrated previously with either prolonged (98) or more condensed intermittent hypoxic exposures (87). I therefore believe, that these data provide important information on the intrinsic neural activity and behavior of hypoglossal and phrenic motor neurone pools at rest and in response to a brief respiratory stimulus. The main findings of this study were that the resting respiratory related activity of the genioglossus muscle was not different between women in the luteal menstrual phase and men, and that both the genioglossus and diaphragm muscles declined proportionately during respiratory afterdischarge following removal of brief hypoxia. The genioglossus muscle activity was correlated with the negative epiglottic pressure during inspiration in the majority of men and women studied and the mean slope of this relationship was not different between the genders.

3.4.1 Resting genioglossal activity

Previous reports of resting genioglossal activity in healthy men and women are inconsistent (110, 111, 147). Contrary to the results of the present study, Popovic and White (111) reported that the resting level of genioglossal activity was 11% higher in women than in men. The 22 subjects studied by Popovic and White were

slightly older (34 yrs men, 32 yrs women) and had similar BMIs (24.4 men, 22.2 women) to those in the current study, however in their study the BMI was significantly higher in the men than the women. It would seem unlikely that the elevated BMI in men would explain the difference in genioglossal activity found, because a higher BMI would be expected, if anything, to be associated with a reduced upper airway size and therefore a higher resting level of genioglossal activity (108). Popovic and White found the women had a smaller mean BMI yet had higher resting genioglossal activity.

White and colleagues have more recently reported that the resting level of genioglossal activity is not statistically different between men and women during wakefulness (147) and sleep (110). The study conducted during wakefulness (147) used healthy men and women (9 of each sex) who were of similar age (mean 30yrs) to both the current study and their previous study (111). No details of BMI were given. The study of sleeping subjects (110) was conducted in BMI matched men and women (8 of each sex) who were of similar age (mean 27yrs) to the previous studies. Thus, while the latter finding may represent a depressant effect of sleep on genioglossus activity that is greater in women than in men, it seems more likely that no gender difference in resting genioglossal activity exists in healthy young men and women awake or asleep.

3.4.2 Genioglossal and diaphragmatic activity following brief hypoxia

The duration of respiratory afterdischarge is thought to be important in stabilising respiratory patterns (153). If RAD is short, ventilation may become unstable and the subject more susceptible to an undershoot in respiratory drive following any brief respiratory stimulus (such as a brief arousal from sleep). Further, the upper airway is more prone to collapse at times of low respiratory drive (11, 102). We have recently demonstrated that the RAD duration is not different between healthy young men and women (67) suggesting it is unlikely that a fundamental gender difference in RAD exists. However, whether RAD occurs in the genioglossus and parallels that of the diaphragm RAD in humans has not been previously studied, nor has the RAD been compared in the two muscles groups between genders. The phrenic and hypoglossal motor neurone pools have been shown to behave similarly in some circumstances (at rest, during hypoxia (106) and hypercapnia (105)) but differently in other situations such as following alcohol (75), diazepam (79), cyanide hypoxia and anaesthesia (128). In male cats the alcohol induced reduction of hypoglossal output was reduced by pretreatment with a progesterone analogue (129), suggesting that progesterone may influence hypoglossal activity and that there may be a gender difference in genioglossal control. In this study, the decline in genioglossus activity was found on abrupt termination of a brief hypoxic stimulus parallels the decline in diaphragm muscle activity and ventilation, and was not different between genders. This suggests that the phrenic and hypoglossal motor neurone pools are closely coupled during respiratory afterdischarge following hypoxia and are not different between young men and women during wakefulness.

Whether this coupling of RAD activity in the genioglossus and diaphragm is the result of similar RAD in respiratory pre-motor neurones projecting to hypoglossal and phrenic motor neurone pools, or whether the genioglossal activity is coupled via the negative pressure reflex cannot be determined by this experiment. Caution must be exercised when extrapolating the findings to the mechanical behavior of the upper airway in men and women following a brief respiratory stimulus. Little is known about the electromechanical coupling of upper airway dilator muscles in humans and no data exists comparing this in men and women. It is possible for example, that the same degree of electrical activation of upper airway dilator muscles in the genders may result in different degrees of stiffening or dilatation of the airway.

It is not known whether the close relationship observed in this experiment between upper airway and respiratory pump muscle activity in men and women persists in sleep. In stable NREM sleep upper airway resistance has been reported in one study to be increased more in healthy men than in women (136) an effect also seen during the application of an external resistive load (110). A possible explanation for these findings is that in NREM sleep the respiratory pump and upper airway muscle activities are less tightly coupled in men compared to women. Finally, our data should not be interpreted to indicate that hypoxia may not, following more prolonged exposure, lead to gender differences in central neural control of phrenic or hypoglossal motor neurones. It is possible, for example, that more intense, repetitive hypoxia could lead to more depression of respiratory activity in the genioglossus (87) or more periodic breathing (58, 102) in men than in women.

3.4.3 The relationship between epiglottic pressure and genioglossus muscle activity

Consistent with the finding that there are no gender differences in the genioglossal activity at rest or following increased respiratory drive, the slopes, intercepts and correlation coefficients of the genioglossus muscle versus epiglottic pressure relationship were also not different between men and women. It is important to note the correlation coefficients in this and an earlier study (83), although significant were relatively weak (mean R=-0.47) suggesting that other factors importantly influence genioglossal activity. Behavioral influences or postural reflexes may be important. Furthermore, while it seems that the stimulus for negative pressure activation of the genioglossus is probably mucosal pressure receptor or stretch receptor activation due to airway distortion, an alternative view is that the muscle activity may affect airway compliance and size, and therefore the airway pressure. In this way, the most negative pressures would be expected when genioglossal activity is lowest. It is therefore possible and I feel probable, that the epiglottic pressure is both a stimulus for, and a result of the upper airway EMG activity. This may explain the relatively weak correlation between the variables.

3.4.4 Methodological considerations

Given the negative results of the current study, power calculations were performed to determine the minimum difference in genioglossal activity that could be detected given the number of subjects used and standard deviations measured in this study.

It was determined that a gender difference in resting genioglossal activity of 2.7% of maximal activity could be detected with a study power of 80%. This corresponds to a doubling of resting genioglossus activity. In the study of Popovic and White (111), a difference of 10% of maximal genioglossal activity was reported between genders and this also corresponded to a 2-fold difference. It would therefore appear unlikely that the results reflect type II statistical error.

There are other methodological considerations with regard to this study that should be considered. The EMG activity of any muscle is not necessarily representative of the muscle force or movement. Consequently, although no gender differences in the EMG activity of the genioglossus were found at rest or in response to a brief hypoxic stimulus, this does not exclude differences between genders in tongue movement during inspiration and therefore differences in airway size or collapsibility. The genioglossus muscle is also only one of many airway dilator muscles and the activity of the genioglossus may not be representative of all dilator muscles. Surface diaphragm recordings may include EMG activity from intercostal or abdominal muscles, however I believe the recordings largely reflect diaphragm activity as the recording electrodes were placed in the manner described by Lansing and Savelle (76).

The method used to target minute ventilation was to vary the number of N_2 breaths and the duration of hypoxia. It is possible therefore that the hypoxic stimulus may have been greater in one sex than the other. However, the SaO_2 was not significantly different between men and women during hypoxia making this

possibility seem unlikely. The strict trial exclusion criteria resulted in many trials being discarded from further analysis, leaving the possibility that there was a preferential removal of trials in one gender. In fact the number of trials discarded due to each of the criteria was very similar between genders, except for the minute ventilation criteria in which twice as many trials were excluded in men than in women. In these trials, the mean SaO_2 was 82% in women while 89% in men. This indicates that the reason for the inadequate increase in minute ventilation in these men was probably due to the inability to reduce SaO_2 significantly. These stringent trial exclusion criteria were essential to allow for unbiased comparisons between genders in terms of the relative increase and time course of decay in diaphragmatic and genioglossal EMG activity and I feel is unlikely to bias results in either gender. The fact that the study was conducted during wakefulness limits the relevance of the findings to OSA, however if a difference existed between genders while awake, it is likely that this would persist in sleep.

3.4.5 Summary

In summary, measurements of diaphragm and genioglossus muscle activity were compared between young healthy men and women at rest, during and after a brief hypoxic stimulus while awake. The pharyngeal pressure and genioglossal activity relationship were also compared between the genders. No difference in the genioglossal activity was identified at rest or during hypoxia. There was also no gender difference in the rate of genioglossal or diaphragmatic afterdischarge following abrupt removal of hypoxia and the relationship between pharyngeal pressure and genioglossal activity was similar between the two sexes. This study is

the first to demonstrate that the hypoglossal and phrenic motor neurone pools behave similarly following removal of a brief hypoxic stimulus in healthy men and women. These results do not necessarily exclude gender specific changes in the output of the hypoglossal or phrenic motor neurone pools during sleep, or with increased age or body weight.

CHAPTER 4. LONG-TERM FACILITATION OF VENTILATION IS NOT PRESENT DURING WAKEFULNESS IN HEALTHY MEN OR WOMEN

4.1 Introduction

Obstructive sleep apnea (OSA) is more common in men than in women (159). The reason for this gender difference is currently unknown, however it is potentially related to differences in central respiratory control, upper airway neuromuscular control, or upper airway anatomy. Several studies have compared the airway anatomy in healthy men and women and reported that the cross sectional area of the pharynx is larger in men than in women (23, 25, 84). However only one study has corrected for the difference in body size between genders, and this study did not find a gender difference in pharyngeal cross sectional area (25). The resting activity of the upper airway dilator muscles has been implicated in the pathogenesis and high male prevalence of OSA, however the activity of the largest airway dilator, the genioglossus, has been reported to be similar between men and women (110, 147) in all but one study (111). The central control of respiration may also play a role in the pathogenesis of OSA because the airway is more prone to collapse during periods of low respiratory drive (11, 102). Theoretically, it would seem that if periods of low drive were prevented and cyclical respiratory events minimised, then the upper airway would be stabilised and airway collapse less likely.

Long-term facilitation (LTF) is the progressive increase in ventilation that persists for minutes to hours following cessation of a repetitive hypoxic stimulus. LTF is a centrally mediated, serotonin dependent process (10) that has been shown to exist in various awake animals (1, 10, 26, 47, 92, 138). LTF not only prevents periods of low drive but could, at least theoretically, minimise respiratory cycling and therefore stabilise the upper airway. There is disagreement in the literature regarding the existence of LTF in humans (2, 9, 87, 88, 130). These studies have been predominantly conducted in men and thus far there have been no gender comparisons of LTF in humans. Progesterone is a known respiratory stimulant which not only increases resting ventilation (36), but also increases the hypoxic ventilatory response (133). Recently LTF has been reported to be greater in diestrus than estrus in female rats (162). It is therefore possible that LTF may be influenced by female sex hormones and may be different between the genders. If LTF were more readily elicited or more pronounced in women than in men, then it could provide partial protection from cyclical respiratory events in women and may help protect them from further sleep disordered breathing events.

Long-term facilitation is seen in both the hypoglossal and phrenic motor neurones in animals (10). It would therefore appear likely that the genioglossus muscle activity would increase during and following repetitive hypoxia. However, the genioglossus has been reported to be depressed during repetitive hypoxia in healthy male subjects, while diaphragm activity and ventilation were not different from the resting level (no LTF) (87). However there is evidence to suggest that repetitive hypoxia may augment upper airway dilator activity. Aboubakr and

colleagues (2) reported that repetitive hypoxia during sleep in patients with partial upper airway obstruction results in a progressive reduction of upper airway resistance without changing minute ventilation (2). It is therefore possible that the genioglossus muscle may be facilitated or depressed during repetitive hypoxia. The response of the genioglossus may differ between genders because other respiratory depressants (alcohol and benzodiazepines) that cause selective depression of genioglossus or hypoglossal nerve activity (20, 75) do not depress the genioglossus as much in women as men (75) and the depression is reduced in male cats when pre-treated with progesterone (129).

I therefore conducted the current study to determine: 1) whether long-term facilitation of ventilation occurs in healthy men and/or women, and 2) whether depression or facilitation of genioglossus and diaphragm muscles occur during repetitive hypoxia, and if this is the same in both genders.

4.2 Methods

4.2.1 General

12 men and 13 women gave informed written consent and participated in the study. Female subjects were tested in the luteal menstrual phase (days 20-24 following the 1st day of menstruation) as confirmed by plasma progesterone levels (ACS:180 Progesterone Assay, Chrion Diagnostics, Chrion Healthcare, Victoria, Australia). Three females were subsequently found to be anovulatory (plasma progesterone < 7 nmol·L^{-1} in the luteal phase) and their results were excluded from the analysis.

4.2.2 Experimental gases

The inspiratory gases used in this study were 100% N_2, 50% O_2 in N_2 and 9% O_2 in N_2. 100% CO_2 was manually bled in to the inspirate immediately up stream of the pneumotachograph as required to maintain isocapnia.

4.2.3 Protocol

Subjects attended the laboratory for a preliminary visit in which respiratory function testing was performed and subjects were familiarised with laboratory equipment. The subjects then attended the laboratory on another morning after a light breakfast without caffeine. They were instrumented (as described in GENERAL METHODS, page 24). In addition to this equipment, subjects had a second pressure tipped catheter (MPC-500, Millar, Houston, TX) inserted through the same nostril as the epiglottic catheter to measure the pressure at the level of the choanae (P_{CHO}). The choanal catheter was advanced until it touched the posterior nasopharyngeal wall and was then retracted 0.5cm. The peak inspiratory supraglottic (epiglottis to nares), nasal (choanae to nares) and pharyngeal (epiglottis to choanae) airway resistances were calculated (R_{UA}, R_{NA} and R_{PH} respectively).

When all equipment was attached, subjects lay supine with one pillow and when comfortable performed maximal EMG maneuvers (as described in GENERAL METHODS, page 24). Subjects were then given earphones through which they

listened to the radio and were instructed to relax, keep their eyes open, stay awake and to breathe only through their nose. They were informed that during hypoxia they might experience slight dizziness or breathlessness but that these responses were normal and so to remain relaxed. After 5 minutes of baseline room air breathing, subjects were exposed to ten 2-minute episodes of hypoxia separated by 2-minutes of room air. The hypoxic gas was blended with room air, as necessary via a three way tap, to maintain arterial oxygen saturation at 80%. Each hypoxic period was initiated with 3 breaths of 100% N_2 to cause a rapid fall in SaO_2 and was terminated with 1 breath of 50% O_2. Isocapnia was maintained during and following repetitive hypoxia by manually bleeding CO_2 into the inspirate. Subjects breathed room air for a further 25 minutes after the 10^{th} hypoxic period before the maximal EMG maneuvers were repeated.

4.2.4 Data and statistical analysis

Breaths which were contaminated with movement artifact, swallows, sighs or sleep were removed from analysis. All variables were averaged at 30-second intervals to condense the 70 minutes of breath-by-breath data. Resting variables were determined from the average of the 5 minutes immediately prior to the start of the repetitive hypoxia. The maintenance of isocapnia was assessed with ANOVA for repeated measures on P_{ETCO_2} data for the entire protocol. All measured variables ($\dot{V}_I$, V_T, T_I, T_E, T_{TOT}, phasic and tonic EMG_{GG} and EMG_{DI}, SaO_2, P_{EPI}, P_{MASK}, peak inspiratory flow (PIF), R_{UA}, R_{NA} and R_{PH}) were compared between men and women with two-way ANOVA for repeated measures. Separate ANOVA were used to 1) detect long-term facilitation or depression (data during the second minute of

normoxia between hypoxic exposures and during recovery following the 10th hypoxic exposure analysed) and 2) detect "roll-off" during repetitive hypoxia (data during the second minute of each hypoxic exposure analysed). Long-term facilitation was also calculated in the manner described by Babcock et al. (9). This involved determining whether the minute ventilation was greater than 10% above the resting level at 5 and 20 minutes after the 10th hypoxic exposure.

4.3 Results

Adequate ventilatory and diaphragm EMG data were obtained in all 22 subjects. One of either the epiglottic or choanal pressure signals were inadequate in 5 subjects (large drift in 3 subjects and catheter failure in 2 subjects) so all pressure and resistance data were excluded in these subjects. The genioglossal signal was poor in 2 subjects (intermittent signal in one subject and complete signal loss in the other subject mid protocol). Analysis of the genioglossus and diaphragm muscle activity was therefore limited to 20 subjects.

4.3.1 Resting data

The 12 men and 10 women studied did not differ in terms of age, body mass index or lung function (Table 4). While resting $\dot{V}_I$ was not different between genders, there were significant differences in breathing pattern, V_T and P_{ETCO_2} at rest (Table 5). The low P_{ETCO_2} in women with equivalent $\dot{V}_I$ likely reflects the elevated plasma progesterone level in the 10 women studied, 22.4 ± 1.1 nmol·L^{-1}. The resting level

of genioglossal activity did not differ between genders and the airway pressures and resistance were also not different between men and women (Table 5).

4.3.2 Repetitive isocapnic hypoxia

The SaO_2 at rest, during or following repetitive isocapnic hypoxia did not differ between genders (Figure 6). There were no time, or gender by time interaction effects for P_{ETCO_2} during and following hypoxia indicating adequate maintenance of isocapnia (Figure 6).

The increase in minute ventilation during the hypoxic periods did not differ between men and women and there was no gender by time interaction effect. Similarly the normoxic ventilation between hypoxic exposures and during recovery was not different between genders (Figure 7). There was no significant change over time in minute ventilation during normoxic periods of the protocol indicating that no depression or facilitation of ventilation occurred in either gender. There were also no gender differences in V_T (Figure 7), T_I, T_E (Figure 8) and T_{TOT} during the hypoxic periods, or in the normoxic periods during or following repetitive hypoxia indicating these variables also showed no facilitation or depression. When the minute ventilation data were analysed by the method described by Babcock et al. [(9) see Data and statistical analysis, page 59] to determine whether individual subjects demonstrated long-term facilitation, only one male and one female subject were identified that fit their criteria for long-term facilitation of ventilation.

The upper airway resistance, genioglossus and diaphragm muscle responses were more variable than the minute ventilation data, however the same patterns existed. There were no gender differences or gender by time interaction effects in any of these variables during the hypoxic periods or in the 2-minute normoxic periods between hypoxic exposures. However, the inspiratory phasic genioglossus muscle activity was lower in women than in men at 5 and 20 minutes after the 10^{th} hypoxic exposure (Figure 9). This occurred despite unchanged ventilation, diaphragm muscle activity and upper airway resistance (Figure 10) during this recovery time.

Table 4 Anthropometric Data

	Men (n=12)	Women (n=10)	p value
Age (yrs)	25.3 ± 0.7	25.0 ± 0.8	0.926
BMI ($kg \cdot m^{-2}$)	23.6 ± 0.9	22.3 ± 0.4	0.245
FEV_1 (%predicted)	112 ± 4	106 ± 1	0.380
FVC (%predicted)	107 ± 4	96 ± 1	0.091

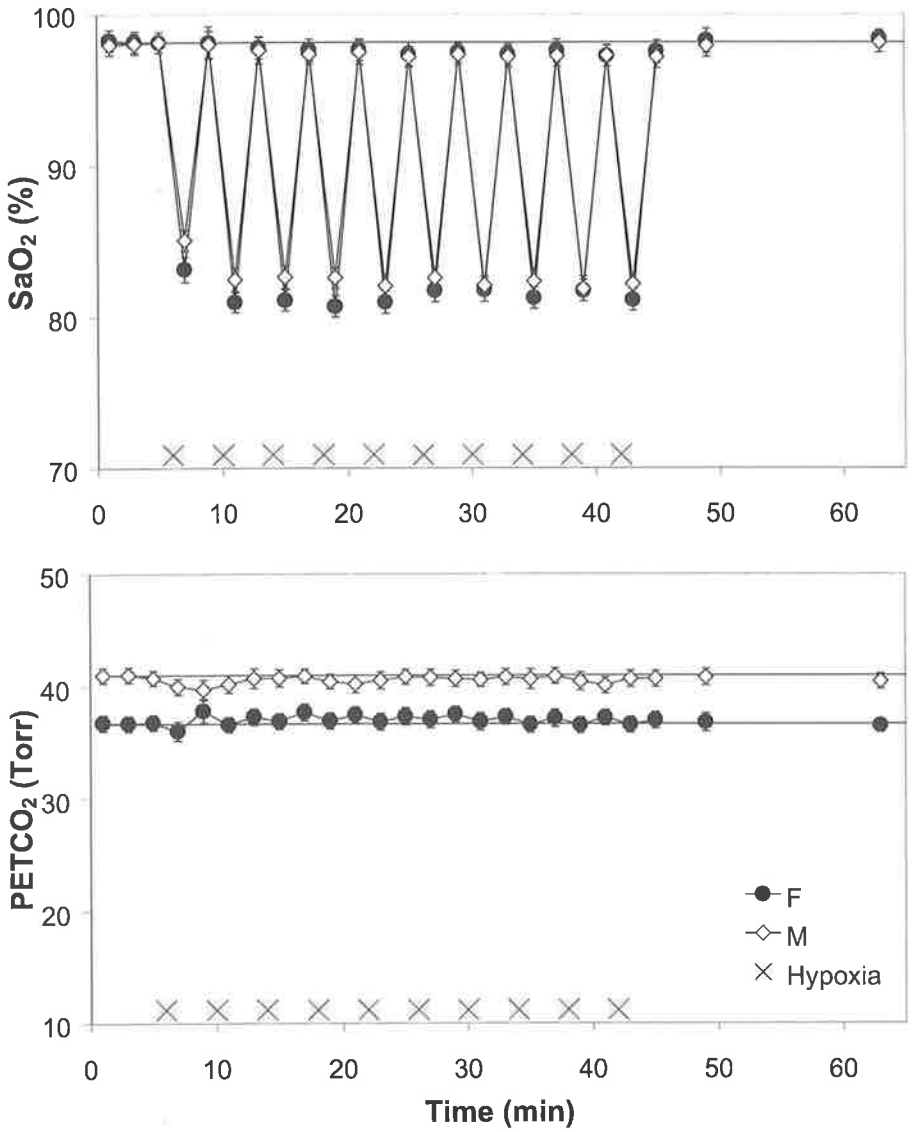
Age, Body Mass Index (BMI), and lung function (FEV_1 and FVC) in the men and women studied. Data are means ± SEM, $p<0.05$ considered significant.

Table 5 Resting breathing characteristics in men and women.

	Men (n=12)	Women (n=10)	p value
$\dot{V}_I$ ($l \cdot min^{-1}$)	7.67 ± 0.32	7.59 ± 0.49	0.893
P_{ETCO_2} (Torr)	40.92 ± 0.67	36.77 ± 0.68	<0.001
V_T (l)	0.56 ± 0.04	0.43 ± 0.03	0.021
F_B ($breaths \cdot min^{-1}$)	14.34 ± 1.03	17.76 ± 1.06	0.031
T_I (s)	2.04 ± 0.15	1.63 ± 0.06	0.025
T_E (s)	2.41 ± 0.21	1.85 ± 0.14	0.038
Phasic EMG_{GG} (% maximum)#	4.91 ± 1.61	7.34 ± 2.63	0.443
Tonic EMG_{GG} (% maximum)#	1.68 ± 0.51	2.91 ± 1.01	0.300
P_{EPI} (cmH_2O)*	-2.65 ± 0.33	-2.75 ± 0.35	0.837
P_{CHO} (cmH_2O)*	-2.44 ± 0.33	-2.40 ± 0.39	0.932
R_{UA} ($cmH_2O \cdot l^{-1} \cdot s$)*	2.67 ± 0.69	3.00 ± 0.63	0.732

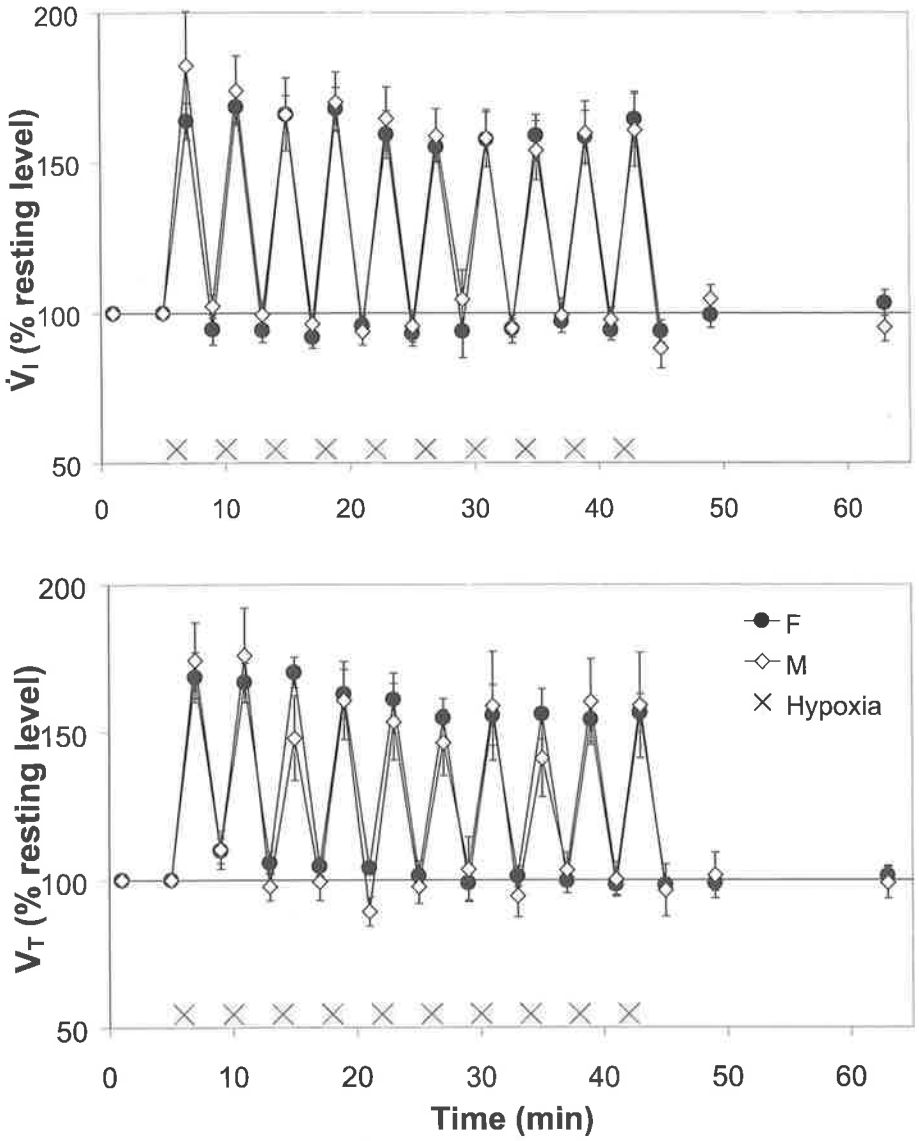
Resting minute ventilation ($\dot{V}_I$), end-tidal partial pressure of carbon dioxide (P_{ETCO_2}), tidal volume (V_T), Breathing frequency (F_B), inspiratory (T_I) and expiratory times (T_E), inspiratory phasic (Phasic EMG_{GG}) and end expiratory tonic (Tonic EMG_{GG}) genioglossus activity, epiglottic (P_{EPI}) and choanal (P_{CHO}) pressures and upper airway resistance (R_{UA}). Data are means ± SEM. #n=10 men, *n=7 men.

Figure 6 **Changes in arterial oxygen saturation and $PETCO_2$ levels during and following repetitive hypoxia**



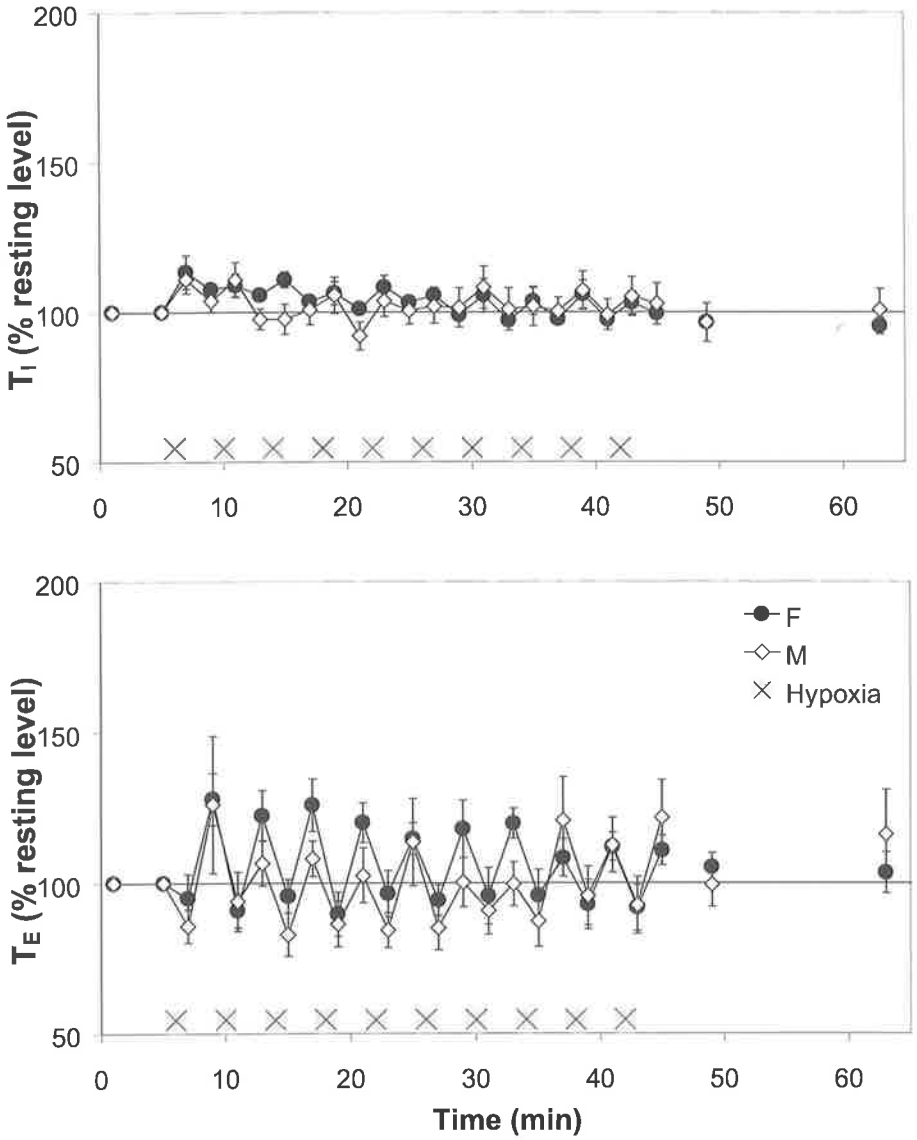
Arterial oxygen saturation (SaO_2) and end-tidal partial pressure of CO_2 ($PETCO_2$) at rest, during and 5 and 20 minutes following repetitive hypoxia in men (n=12) and women (n=10). Data are means ± SEM. There was a significant effect of gender on $PETCO_2$. There was no significant change in $PETCO_2$ over time in either gender. There were no other significant gender or gender by time interaction effects.

Figure 7 **Minute ventilation and tidal volume during and following repetitive hypoxia**



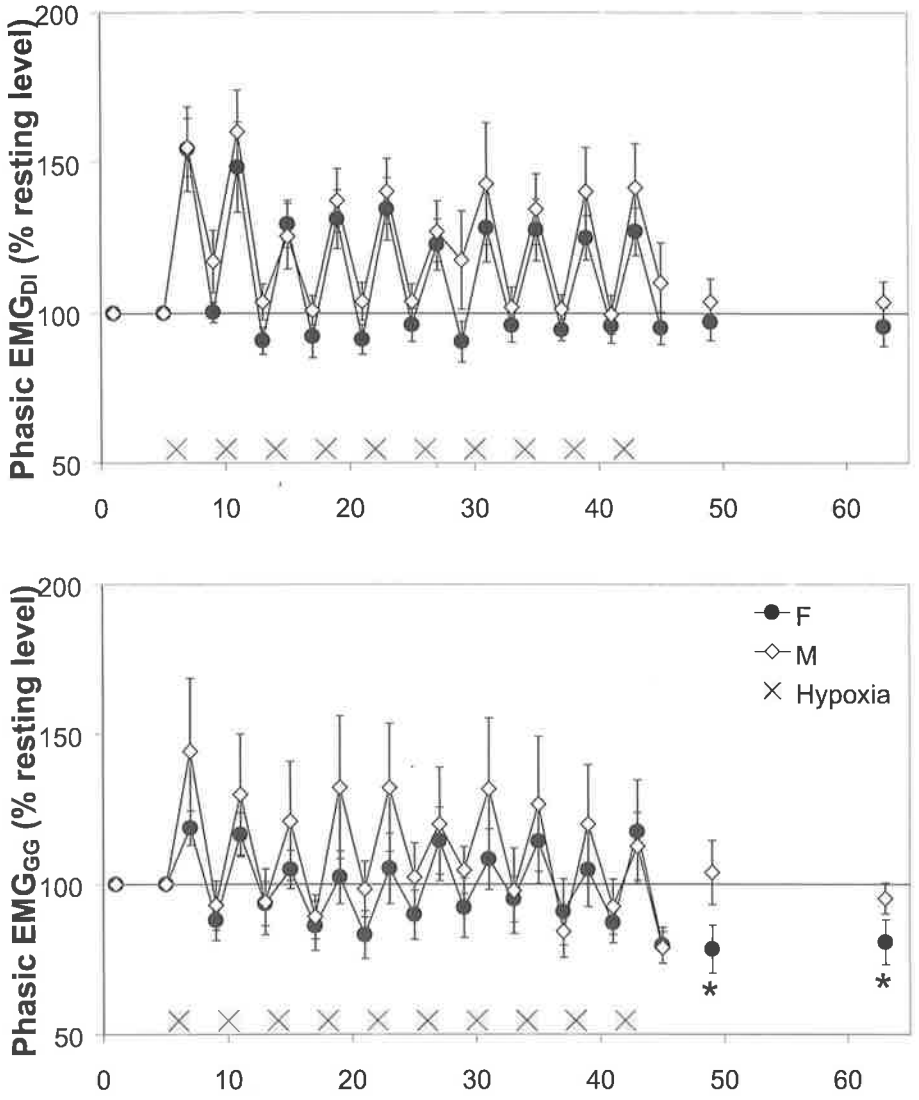
Minute ventilation ($\dot{V}_I$) and tidal volume (V_T) at rest, during and 5 and 20 minutes following repetitive hypoxia in men (n=12) and women (n=10). Data are means ± SEM. There were no significant gender or gender by time interaction effects.

Figure 8 **Inspiratory and expiratory times during and following repetitive hypoxia**



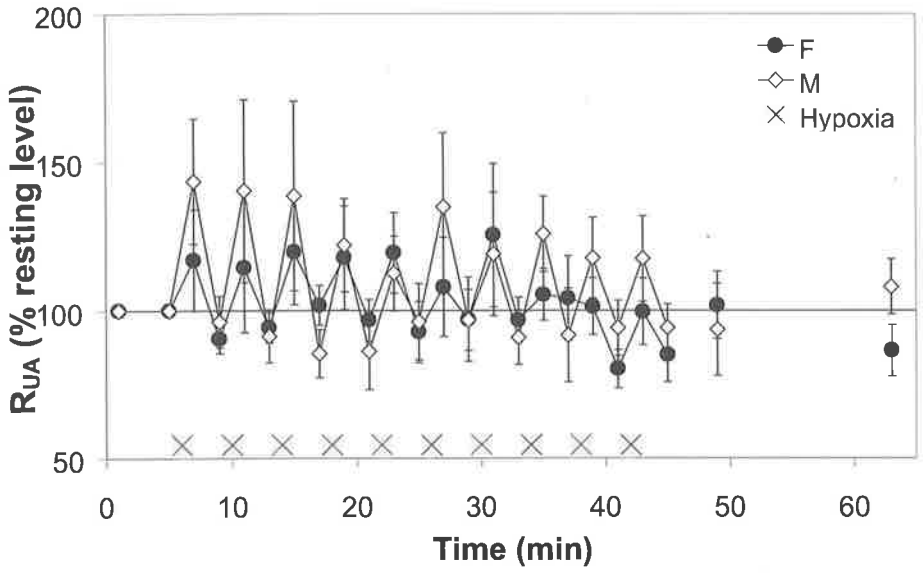
Inspiratory (T_I) and Expiratory (T_E) times at rest, during and 5 and 20 minutes following repetitive hypoxia in men (n=12) and women (n=10). Data are means ± SEM. There were no significant gender or gender by time interaction effects.

Figure 9 **Inspiratory phasic diaphragm and genioglossus muscle activity during and following repetitive hypoxia**



Inspiratory phasic diaphragm (Phasic EMG_{DI}) and genioglossus (Phasic EMG_{GG}) muscle activities at rest, during and 5 and 20 minutes following repetitive hypoxia in men (n=10) and women (n=10). Data are means ± SEM. * Significantly lower in women than the corresponding time point in men. There were no significant gender or gender by time interaction effects.

Figure 10 Upper airway resistance during and following repetitive hypoxia



Upper airway resistance (R_{UA}) at rest, during and 5 and 20 minutes following repetitive hypoxia in men (n=7) and women (n=10). Data are means ± SEM. There were no significant gender or gender by time effects.

4.4 Discussion

In this study, the ventilatory and respiratory muscle responses to repetitive eucapnic hypoxia have been compared between healthy young men and women during wakefulness. No long-term facilitation of ventilation, diaphragm or genioglossus muscle activity was identified during or following repetitive hypoxia. The genioglossus muscle activity was depressed in women during recovery from repetitive hypoxia, however this was not apparent in men.

4.4.1 Resting gender differences

Although resting minute ventilation was not different between genders, there were significant differences in the pattern of breathing in the young men and women studied. The resting tidal volume was lower in women than in men, however breathing frequency was higher in women due to lower inspiratory and expiratory times (Table 5). The end-tidal partial pressure of CO_2 was lower in women than in men consistent with the respiratory stimulating effects of progesterone. The relevance, if any, of these differences in resting breathing pattern to the current study and to the high male prevalence of sleep disordered breathing syndromes is uncertain. There were no gender differences in the resting genioglossus muscle activity or upper airway resistance.

4.4.2 Long-term facilitation of ventilation.

Long-term facilitation of ventilation is the prolonged increase in ventilation following repetitive stimulation of the carotid bodies that has been proposed to stabilise breathing patterns. Although long-term facilitation has been extensively studied in various animal models, there have been relatively few studies in humans. In the early 1990's there were two abstract reports from the same laboratory, one showing long-term facilitation in 14 healthy volunteers (130), the second showing LTF in 14 OSA patients but not in 5 healthy controls (88). Since these initial reports McEvoy et al. (87) studied 11 male volunteers during wakefulness and failed to find evidence of LTF. These three studies all used the same "2-minute on, 2-minute off" hypoxia protocol causing desaturation to approximately 80-85%. More recently Babcock et al. (9) have used a "3-minute on, 5-minute off" hypoxia protocol (FiO_2 = 8%) during sleep in healthy subjects, and reported no long-term facilitation of ventilation in the group, although some individuals appeared to demonstrate LTF. These authors commented that the presence or absence of LTF in an individual subject appeared to be related to whether the subjects snored and had inspiratory flow limitation (LTF existed in snorers). More recently the same laboratory has conducted a similar study in OSA patients with optimal or sub-optimal continuous positive airway pressure (CPAP, sub-optimal to allow inspiratory flow limitation) levels during sleep. Using the same "3-minute, on 5-minute off" protocol, the authors were unable to demonstrate LTF in either CPAP condition. In the present study long-term facilitation was not found to occur in men during wakefulness and this has been extended to awake women. Given the results of this and previous studies I feel it likely that LTF does not exist, or is rare in healthy humans when

exposed to repetitive hypoxia of a few minutes duration and at physiologically significant levels. These negative findings contrast the many awake animal studies (1, 10, 26, 47, 92, 138) and may be related to species differences.

4.4.3 Respiratory muscle activity and upper airway resistance

The genioglossus and diaphragm muscles both show inspiratory phasic activity that increases during respiratory stimulation and decreases with generalised respiratory depression. These relative changes in muscle activity are often proportional suggesting that the hypoglossal and phrenic motor neurone outputs are coupled. However, there are situations when the activity of the two muscles differ. These include following alcohol (75) and benzodiazepene (79) ingestion in humans, and following anaesthesia and cyanide brain hypoxia in animals (128). Repetitive eucapnic hypoxia has previously been reported to also cause preferential depression of genioglossal activity in healthy men during wakefulness without altering diaphragmatic activity (87). In the current study of normal individuals during wakefulness there was a statistically significant reduction in genioglossus muscle activity after the 10^{th} hypoxic exposure in women but not in men. Given the variable nature of genioglossus EMG signals, power calculations were performed to determine the minimum detectable difference of genioglossus activity in both men and women. These calculations revealed that with the coefficient of variation of genioglossal activity measured at rest in the 10 women studied, a change in genioglossal activity below 87% of the resting level could be detected with 80% power (hence the power was adequate). In men however, the coefficient of variation in genioglossal activity was almost twice that of women (20.9 in men, 12.8

in women) such that only changes below 79% of baseline could be detected with 80% power. It is therefore possible that the non-significant result in men was the result of Type II error. However, it must be noted that the genioglossal data in men did not appear to show a trend toward depression during the 5 and 20 minute recovery times despite being below baseline for the last three hypoxic episodes.

Repetitive (87) and sustained (98) isocapnic hypoxia have been shown to disproportionately suppress genioglossal compared with diaphragm activity. Why the current findings differ from the earlier studies using repetitive hypoxia (87) is uncertain. The current protocol was almost identical to this previous study with the exception that the hypoxic gas used in my study was a 9% O_2 in N_2 gas compared with an 11% O_2 gas in the earlier study. Despite this, the level of hypoxemia was similar between studies (85% SaO_2 in the earlier study and 82% in the current study) probably reflecting the fact that the earlier study was conducted at moderate altitude. In the earlier study each hypoxic period was terminated with one breath of 100% O_2 whereas in the current study each hypoxic period was terminated with one breath of 50% O_2. I feel it unlikely that these differences would give rise to the different findings between studies. If anything, the subjects in the current protocol would be likely to experience more central depression because of the lower saturation levels achieved. There are some differences in the subject characteristics between studies. Notably, the 11 subjects used in the earlier study had a mean age of 36 yrs compared to 25 yrs in the current study. This may contribute to the inability of the current study to detect a reduction in genioglossal activity in men if this phenomenon is, at least in part, age related as it is in rats

(162, 163). Therefore, it is possible that the inability to demonstrate depression of the respiratory phasic genioglossal activity in the men in the current study results from either the high variability of the genioglossus in these men and/or the age of the experimental subjects.

Previous studies in humans during NREM sleep (2, 9) have indicated that upper airways resistance decreases following repetitive hypoxia perhaps implying facilitation of upper airway dilator muscles, although muscle activity was not measured in either study. Data from this and a previous study during wakefulness (87) do not support relative augmentation of genioglossal activity with repetitive hypoxia in healthy human subjects.

4.4.4 Methodological considerations

There are several important methodological issues to consider when interpreting these results. Firstly, the level of inspired oxygen was adjusted in the current study to target an arterial saturation of 80% in both men and women. If the hypoxic ventilatory response was different between men and women then one gender may have received a lower oxygen concentration in the inspirate than the other. However, SaO_2 and ventilation were not different between genders indicating that the stimuli for LTF was probably not different between genders.

The second consideration regards the recording of genioglossal and diaphragmatic EMGs. The electrical activity of any muscle may not represent the functional changes in muscle force or movement. If the electromechanical coupling of the

genioglossus and diaphragm muscle differed between genders then any EMG comparison would be of limited significance regarding muscle force generation or perhaps upper airway caliber. However, I am unaware of any data showing a gender difference in electromechanical coupling of upper airway dilator muscles. The surface recording of diaphragm muscle activity can be confounded by activation of abdominal muscles during respiration, however diaphragm EMG electrodes were placed in positions to minimise abdominal muscle contamination.

Finally, this study was conducted during wakefulness and so the findings may not persist in sleep. This limits the significance of the findings with regard to sleep disordered breathing. However, if a gender difference was identified during wakefulness, it would appear likely to persist in sleep and therefore be important in the pathogenesis of sleep disordered breathing.

4.4.5 Summary

In summary, no evidence of long-term facilitation of ventilation, genioglossal or diaphragm muscle activity was found in healthy men or women during wakefulness. The present finding in awake men and women are consistent with previous reports in awake males (87), sleeping normal subjects (9) and OSA patients (2) and suggest that long-term facilitation of ventilation is either absent or at least difficult to elicit in humans. Genioglossus muscle activity was depressed in women during recovery from repetitive hypoxia, as it has previously been shown in men (87). The results do not support the hypothesis that different ventilatory or respiratory muscle

responses to repetitive hypoxia could explain the gender difference in OSA and other related sleep breathing disorders.

CHAPTER 5. THE VENTILATORY RESPONSE TO BRIEF AROUSAL FROM SLEEP IS GREATER IN MEN THAN IN WOMEN

5.1 Introduction

Obstructive sleep apnea (OSA) and central sleep apnea (CSA) associated with congestive heart failure are more common in men than in women (125, 159). The cause for this gender difference in prevalence is not known. Respiratory control instability has been proposed as a mechanism for sleep disordered breathing. It is possible therefore that some central respiratory control components differ between genders and help explain the difference in prevalence. Arousal from sleep is believed to have a destablising influence on the control of breathing (12, 70, 151). In support of this theory, Xie and colleagues (151) found in CSA patients, 37 of 48 episodes of periodic breathing arising from stable sleep were preceded by arousal, brief hyperventilation and a reduction in transcutaneous CO_2.

The increase in ventilation following arousal from sleep is thought to arise from increased chemoresponsiveness on return to the waking state, a relatively high $PaCO_2$ that develops in sleep prior to arousal (see (109) for review), and a sudden reduction in upper airway resistance (70). Given that there is some evidence that the hypercapnic ventilatory response is higher in men than in women (3, 140, 146), men may be expected to have a larger ventilatory response on awakening for a given sleep related increase in $PaCO_2$. However, Khoo and colleagues have

suggested that the ventilatory response to arousal is largely determined by upper airway resistance changes, with central drive only playing a small part in the response. Evidence for this suggestion comes from a study of ventilatory changes following tone-induced arousals from stage 2 sleep in 9 healthy men (70). In this study, mean inspiratory flow and tidal volume were increased above the pre-arousal level for seven breaths following the start of a 5s tone. In three subjects upper airway resistance was measured and found to decrease immediately and remain reduced for approximately 30s following the tone, whereas $P_{0.1}$ (measured in the other 6 individuals) was increased only on the second and third breaths following the tone. The authors therefore suggest that the increased mean inspiratory flow on the 1^{st} and 4^{th} through 7^{th} breaths is likely to be due to reduced upper airway resistance. During NREM sleep, upper airway resistance has been reported to be higher in men than in women in one study (136) but not different between genders in another (120). If upper airway resistance were higher in men than in women during sleep, the ventilatory response to arousal may also be elevated in men, secondary to a release of resistance.

Horner and colleagues have measured ventilatory responses to auditory arousal from NREM sleep in tracheostomised dogs and compared this to ventilatory changes during wakefulness following 1) the application of a tone used to arouse the dogs during sleep and 2) addition of CO_2 to match the sleeping level (56). During wakefulness, tone presentation did not affect ventilation. Also the change in ventilation caused by increasing CO_2 to the sleeping level was smaller than the increase in ventilation following tone-induced arousal. Since their dogs breathed

through an endotracheal tube, changes in upper airway resistance were not likely to have contributed to the result. These findings provide evidence for an independent stimulus to ventilation following arousal from sleep, or a "waking reflex". This concept is further supported by the finding that healthy human volunteers increase diaphragm muscle activity following spontaneous arousal from sleep, when sleep related changes in ventilation and P_{ETCO_2} are prevented by passive ventilation (137). Furthermore, the magnitude of the diaphragm response following arousal was the same during mechanical ventilation as during spontaneous ventilation. Khoo and colleagues have also determined that the ventilatory response to arousal from sleep persists in OSA patients while treated with nasal CPAP despite upper airway resistance changing minimally (72). This provides additional evidence for a reflex stimulation of respiration following brief arousal from sleep. If the waking reflex is similar to a startle reflex, it may be higher in men than in women, as Reyes del Paso and Vila (118) reported a greater increase in breathing amplitude (pneumatic transducer) in response to a brief startling tone (100dB) in men compared to women. This study was designed to test the hypothesis that the ventilatory response to arousal from sleep is greater in men than women due to one or more of the above-mentioned factors.

If the ventilatory response to arousal importantly contributes to respiratory instability, a period of hypopnea would be expected to follow hyperpnea. Hypopnea could occur due to low central respiratory drive following hyperventilation, loss of the waking stimulus to breathe and increased upper airway resistance following resumption of sleep. The upper airway has been proposed to be prone to collapse

during periods of low respiratory drive (103). Therefore, arousal with secondary brief hyperpnea followed by hypopnea could be an important component of the pathophysiological sequence in OSA. However, Badr and colleagues have been unable to demonstrate hypopnea (with Respitrace) following tone-induced arousal from sleep (12). Khoo and colleagues reported that 3 normal subjects occasionally developed periodic breathing following tone-induced arousal (70). Furthermore, modeling analysis predicted that arousal is capable of inducing respiratory instability. Khoo et al. also reported that the degree of hyperpnea and the rate of decline of ventilation following arousal are important determinants of the subsequent reduction in ventilation (70). It may therefore be expected that if men show an elevated ventilatory response to arousal, they may also have a greater subsequent hypopnea and develop greater respiratory and upper airway instability.

Most of the studies mentioned have measured responses to tone-induced arousal from sleep. These responses may differ from spontaneous arousals. Previous studies have generally been conducted in stage 2 NREM or stages 2-4 NREM sleep combined, with subjects in the supine position. However, stage of sleep and position may influence the ventilatory response to arousal. The relevance of previous findings to the normal sleeping situation may therefore be limited. The current study was conducted to investigate the influence of gender, sleep stage and body position on the ventilatory and upper airway responses to brief arousal from sleep. In addition, ventilatory responses following tone-induced arousal were compared to spontaneous arousal in healthy young men and women.

5.2 Methods

15 men and 18 women gave informed written consent and participated in the study. No subject reported any auditory, cardiovascular or sleeping problems and no subjects reported regular (>1 night/week) snoring. Female subjects were tested in the follicular menstrual phase (days 5-14 following the first day of menses).

5.2.1 Measurements

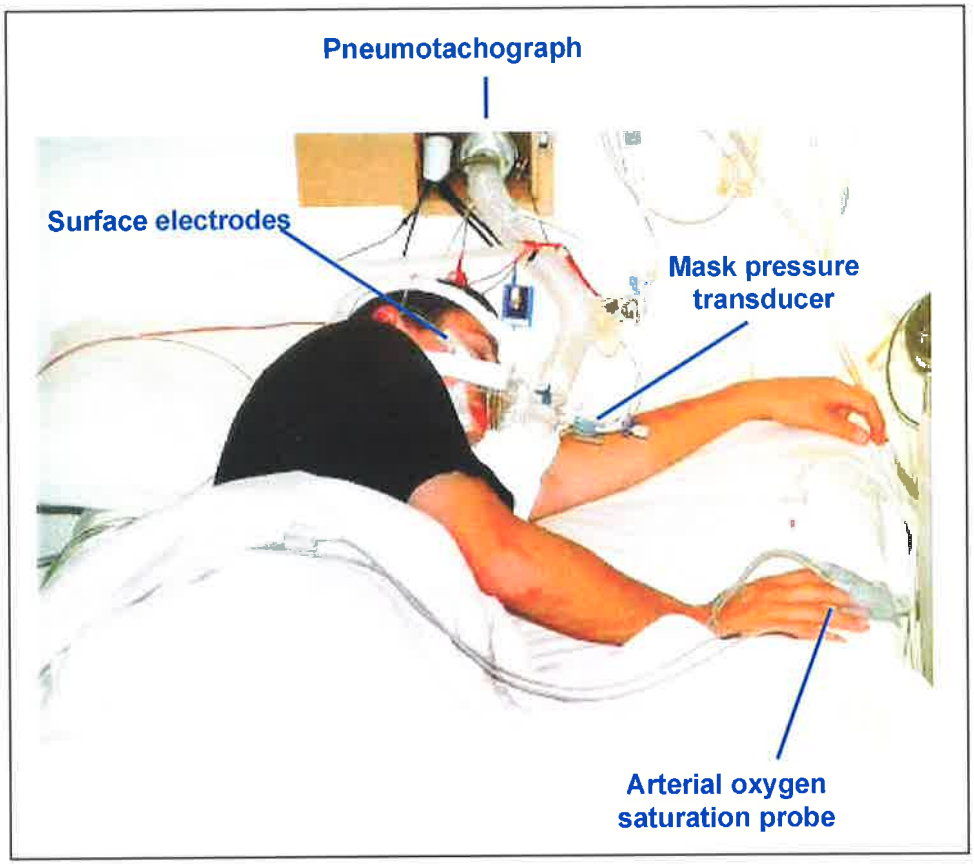
A nasal mask (Gel mask, Respironics, PA) was fixed to the subjects' face with double-sided tape and a head strap. The mouth was taped closed and subjects were instructed on how to remove the tape if required. A perforated tube was threaded around the outside of the nasal mask and was connected to a CO_2 analyser (POET II model 602-1, Criticare Systems Waukesha, WI) to serve as a leak detector. The expirate was sampled to determine the end-tidal partial pressure of CO_2 (P_{ETCO_2}, POET II model 602-3, Criticare systems Waukesha, WI) via tubing incorporating a 30-cm length of Nafion (Permapure, NJ) tubing to prevent condensation from blocking the sample line. The experimental setup is shown in Figure 11.

Epiglottic pressure (P_{EPI}) was measured in the first 8 subjects with a Millar pressure tipped catheter (MPC-500, Millar, Houston, TX), however build up of secretions on this catheter caused the signal to be intermittent across the night and unusable in 2 subjects (both male). Air perfused catheters (see. GENERAL METHODS, page 24) were used for the remainder of subjects to avoid this problem.

In addition to the equipment described above and in the GENERAL METHODS (page 24), subjects were also instrumented with two channel electroencephalography (EEG, C3-A2 and C4-A1), left and right electrooculograms (EOG-L and EOG-R respectively) and submental electromyogram (EMG_{SUB}) for sleep staging and arousal scoring. Immediately prior to the start of recording, subjects were fitted with ear-insert headphones (E-A-Tone, Cabot Saftey Corporation/Auditory Systems Division, Indianapolis, USA) through which 11 computer generated auditory tones could be presented (1 kHz, 0.5s duration, 5dB increments range 55-105dB; Cool Edit, Syntrillium Software Corporation, AZ, USA).

Data were acquired on two separate recording systems. The first system (Compumedics S series, Abbotsford, Victoria, Australia) was used for recording of EEGs, left and right EOG, EMG_{SUB}, ECG, SaO_2, sound and an event channel. The sampling rates varied between 250Hz for event, sound and EEG to 50Hz for EOGs. Sleep staging and arousal scoring were performed on this system. The second system is described in the GENERAL METHODS, (page 24). The signals recorded on this system were: inspiratory flow, inspired volume, CO_2, one EEG, EOG-R, ECG, P_{EPI}, P_{MASK}, sound, leak, event and raw, rectified and averaged EMG_{GG} and EMG_{DI}. These data were used for breath-by-breath calculations of ventilation, upper airway resistance and muscle activity. An event mark was simultaneously placed on both recording systems coincident with the onset of each auditory tone allowing both data acquisition systems to be accurately linked in time.

Figure 11 The experimental setup for study 3 (Chapter 5)



Subjects wore a nasal CPAP mask with two-way non-rebreathing valve attached. The pneumotachograph was attached to the inspiratory side of the breathing valve and inspiratory tubing was directed above the subjects' head so that position could be changed with relative ease.

5.2.2 Protocol

Subjects presented to the laboratory in the evening approximately 2 hours before their normal bedtime (bedtime range 9:30-12:30pm) having abstained from caffeine for at least 8 hrs. They were instrumented and lay on their left side with one pillow. Maximal electromyographic activity of the genioglossus muscle was determined (see GENERAL METHODS, page 24). Following this, earphones were inserted and two sample tones were played (60dB and 95dB). Subjects were then requested to lie on their left side, keep their eyes open and stay awake for 10 minutes before allowing themselves to fall asleep. The light was left on for the first 10 minutes to facilitate this and baseline wakefulness data were collected at this time. Subjects were instructed to sleep as much as possible on their left hand side, however when they became uncomfortable they could sleep on their back. Sleep was restricted to these two positions. The primary objective was to compare ventilatory responses to arousal with subjects in the lateral position to minimise any effects of sleep-induced increases in UA resistance on the arousal response.

Auditory tones were administered at intervals during sleep according to the following protocol. Following 5 minutes of stable stage 2 sleep (or higher) a 55dB tone was played. If this failed to arouse the subject the next highest volume tone was played a minimum of 2 minutes later. If subjects had a spontaneous arousal the next tone was played at least 2 minutes after the re-establishment of stable sleep. After a full awakening from sleep, 5 minutes of stable stage 2 sleep (or higher) was required before another tone was played. All tones were played during expiration since ventilatory responses to arousal have been shown to be more

reproducible when given in expiration rather than inspiration (70). Tone presentation continued in all sleep stages and body positions until sufficient data were collected, the subject reached the end of their sleep period or otherwise requested the study be terminated. On terminating data collection, subjects were awoken fully and maximal tongue maneuvers were repeated. Several practice trials of each maneuver were used to ensure the subjects were fully alert and correctly performed the maneuvers. The highest activity recorded during the maneuvers (morning or evening) was taken to be the maximal activity of the genioglossus.

5.2.3 Data analysis

5.2.3.4 Sleep staging and arousal scoring

A single trained sleep observer, blinded to all event, sound and respiratory signals, scored the sleep stages and arousals in 30s epochs in the standard manner (5, 115). Following analysis of the whole study, arousals that fit the following criteria were identified: 1) the sleep stage in the epoch prior to the arousal was scored as stage 2 or higher, 2) The arousal did not represent a full awakening (arousal duration <15s and the epochs during and immediately following arousal not scored as wake), 3) there was only one arousal identified in the epoch of the arousal, 4) there were no arousals or auditory tones in the epoch preceding or the epoch following this epoch. In addition, if the arousal occurred in the same epoch as a tone, it was only considered suitable for analysis if the arousal occurred within 5s of the onset of the tone and did not precede it. In this case the arousal was assumed to be a tone-induced arousal. The times at which suitable tone-induced and

spontaneous arousals occurred were noted for breath-by-breath analysis of respiratory and EMG data. The position and sleep stage corresponding to the time of the arousal was also noted.

5.2.3.5 Ventilatory, resistance and EMG analysis

Breath-by-breath data were determined for the whole recording period. Data during the period of resting wakefulness were averaged and compared to data during the 30s prior to all suitable arousals in a given sleep stage on the left side. Data for 30s prior to and 60s after an arousal were interpolated at 4s intervals from the start of the arousal (time zero). The raw data was visually checked before and after every arousal in all subjects and excluded from analysis if snoring, inspiratory flow limitation and/or mask leak was detected. If rapid drift of the P_{EPI} catheter occurred R_{UA} and P_{EPI} data were excluded for that arousal, however, ventilation data were retained. If the arousal was followed by a swallow genioglossal, P_{EPI} and R_{UA} data were excluded. This usually occurred only after the first few arousals at the beginning of the night. Diaphragm data were excluded if ECG blanking was no longer adequate due to a change in body position. Genioglossal data was excluded for the whole night in one subject in whom a wire electrode became dislodged during the night. Interpolated data from remaining trials were expressed as a percent of the pre-arousal level and averaged within a type of arousal (spontaneous or tone-induced) for a given sleep stage and body position for 60s following arousal. There were too few arousals that fit these criteria during REM sleep to allow analysis in this sleep stage.

5.2.4 Statistical analysis

Resting data during wakefulness and sleep were compared between genders and sleep stages with ANOVA for repeated measures. ANOVA for repeated measures were also used to compare the percent changes in all variables for 60s following an arousal between men and women, sleep stages, body positions and type of arousal.

5.3 Results

5.3.1 Anthropometric characteristics and sleep architecture

Four female subjects had markedly abnormal sleep. The average sleep onset latency in these subjects was 186.6 ± 112.5 minutes, sleep efficiency was 22.9 ± 7.7%, arousal index was 47.4 ± 8.1 arousals·hr^{-1} and none of these subjects had any REM sleep. There were no arousals that fit the criteria for analysis in these subjects, so all of their data were excluded. One female and one male subject snored for a large portion of the night and there were no suitable spontaneous or tone-induced arousals without snoring in these subjects, hence these subjects were also excluded from further analysis. Nine other subjects (4 men) were found to snore at some time during the night and data during periods of snoring or flow limitation were discarded.

13 men and 13 women had adequate sleep and arousal data. The men and women did not differ in terms of age, body mass index or respiratory function (FEV_1 or FVC), however the women studied had significantly smaller mean body surface

area (Table 6). The sleep architecture did not differ between men and women (Table 6) and an average of 63 tones were played to each subject (range 37-99, not significantly different between genders).

5.3.2 Resting data.

The resting characteristics during wakefulness and stable NREM sleep are displayed in Table 7. $\dot{V}_I$, V_T and peak inspiratory flow (PIF) were significantly higher and P_{EPI} lower in men than in women in wakefulness and sleep. During NREM sleep, $\dot{V}_I$ was reduced and P_{ETCO_2} levels increased from the waking level. Breathing frequency was reduced in NREM sleep due to lengthening of both T_I and T_E, while V_T did not differ from the waking level. Upper airway resistance also rose from wakefulness to stage 2 sleep, as PIF decreased despite unchanged P_{EPI}. A similar trend was observed from wakefulness to stages 3/4 but did not reach statistical significance. There were no significant changes in phasic or tonic EMG_{GG} from wakefulness to NREM sleep. The sleep related changes in all variables were similar in both genders and no gender by stage interaction effects were observed.

5.3.3 Ventilatory response to spontaneous arousal in the left lateral position

25 subjects (13 men) had suitable (see Sleep staging and arousal scoring, page 85) spontaneous arousals from NREM sleep while in the left lateral position (average 15.6 ± 1.9 arousals per subject). The average duration of arousal was not different between men and women (6.8 ± 0.3 and 6.3 ± 0.4s respectively). The

reduction in ventilation and rise in P_{ETCO_2} and R_{UA} from wakefulness to sleep in the 30s prior to arousal did not differ between genders. Following arousal from sleep, $\dot{V}_I$ rose briefly, before rapidly declining below the pre-arousal level (Figure 12). This was primarily related to changes in V_T (Figure 13), however significant changes over time were also found in F_B (Figure 13), T_I and T_E. Corresponding to the increase in $\dot{V}_I$ there was an increase in phasic EMG_{DI} (Figure 14) and reductions in P_{ETCO_2} (Figure 12) and R_{UA} (Figure 14). Both phasic and tonic EMG_{GG} (Figure 15), and tonic EMG_{DI} failed to showed statistically significant changes over time following arousal from sleep.

The changes in $\dot{V}_I$ were more marked in men than in women (significant gender and gender by time interaction effects, Figure 12). V_T (Figure 13) and P_{ETCO_2} ($p=0.053$, Figure 12) also showed more pronounced responses in men than women while no other variables differed between genders over time. The absolute values for $\dot{V}_I$, P_{ETCO_2} and R_{UA} are compared between men and women in wakefulness, pre-arousal and at 4s (peak $\dot{V}_I$) and 20s (trough $\dot{V}_I$) post-arousal (Figure 16). Although men had a greater ventilatory increase after arousal than women, ventilation did not exceed the level during wakefulness in either men or women. Following arousal R_{UA} did not decrease to the wakefulness level.

5.3.4 Ventilatory responses to spontaneous arousal: stage 2 versus stage 3/4 NREM

Seven women and nine men had arousals from both stage 2 and stages 3/4 NREM sleep. The duration of arousal was not different between stages (6.7 ± 0.3s in stage

2, 6.2 ± 0.5s in stages 3/4) or genders (6.6 ± 0.4s in women, 6.4 ± 0.4s in men). The average pre-arousal $\dot{V}_I$ and R_{UA} did not differ between sleep stages and R_{UA} was not different between genders. The pre-arousal P_{ETCO_2} was slightly but significantly higher in stages 3/4 than stage 2 sleep (44.2 ± 0.7 and 43.8 ± 0.6 Torr respectively) but not different between genders (43.9 ± 0.6 Torr in women, 44.1 ± 0.7 Torr in men). There were no significant differences between arousal responses from stage 2 and stages 3/4 NREM sleep for any variable (no stage by time, stage by gender or stage by time by gender interactions). The gender differences observed in the analysis of all NREM data persisted with significant gender by time interaction effects found for $\dot{V}_I$, V_T and P_{ETCO_2}.

5.3.5 Spontaneous versus Tone-induced arousal responses

Ten women and 13 men had suitable spontaneous and tone-induced arousals from NREM sleep while in the left lateral position. The duration of arousal did not differ between genders (6.2 ± 0.3 in women and 7.0 ± 0.2 in men) or type of arousal (6.5 ± 0.3s spontaneous and 6.8 ± 0.3s tone-induced). The average volume of the tone used to induce arousal was 65.4 ± 1.6 dB and the average latency to arousal was 0.95 ± 0.1s. Neither the tone volume nor latency to arousal differed between genders. The time course of changes in $\dot{V}_I$ (Figure 17), PIF, T_I, P_{ETCO_2} and both phasic and tonic EMG_{GG} differed between tone-induced and spontaneous arousal (type by time effect). V_T showed a trend to a similar effect (p=0.066). Gender by time effects persisted for $\dot{V}_I$ (p=0.057), V_T and P_{ETCO_2}.

5.3.6 The effect of body position on resting ventilation and arousal responses

22 subjects (11 men) had spontaneous arousals from sleep that met the inclusion criteria in both the left lateral and supine positions. The duration of arousal did not differ between positions (6.5 ± 0.3s left and 6.3 ± 0.3s supine) or between men and women (6.6 ± 0.6s and 6.2 ± 0.3s respectively). The pre-arousal characteristics for these subjects in both left and supine positions are presented in Table 8. R_{UA} was increased in the supine position and T_I prolonged, however T_E was reduced resulting in F_B remaining unchanged. PIF and V_T also fell in the supine position. $\dot{V}_I$ fell on assuming the supine position in the whole group, however this was related to a reduction in men but not in women (significant gender by position interaction). Correspondingly, P_{ETCO_2} rose slightly in men and fell in women when assuming the supine position.

Following arousal, T_I, PIF and R_{UA} (Figure 19) demonstrated significant position by time interaction effects with similar trends seen in $\dot{V}_I$ (p=0.055, Figure 18), V_T (p=0.056), P_{ETCO_2} (p=0.073 Figure 18) and tonic EMG_{GG} (p=0.09, Figure 19). Gender by time interaction effects were again found for $\dot{V}_I$ (p=0.051), V_T and P_{ETCO_2} (p=0.055) as was found when examining the arousal response from NREM in the left lateral posture alone. Tonic EMG_{GG} also showed a significant gender by time interaction effect (larger responses in men) when both positions were considered.

While the normalisation process used (expressing post-arousal data as a percent of the pre-arousal level) was required to assess the effect of arousal *per se*, it does not allow valid comparison of the effect of position on arousal responses in men compared to women, because change in position from left to supine affected the pre-arousal conditions differently between genders. Repeated measures ANOVA was therefore performed on the absolute changes in ventilation from pre-arousal, to the maximum (4s post-arousal) and minimum (20s post-arousal) levels in men and women in both left and supine positions (Figure 20). This analysis showed that the effect of position on the ventilatory response to arousal was not different between genders (Figure 20). That is, although ventilation was reduced pre-arousal in the supine position versus the left in men and not different between body positions in women, the effect of position on the ventilatory response to arousal was not different between the genders.

Table 6 Anthropometric and sleep architecture data

	Men (n=13)	Women (n=13)	p value
Age (years)	26.2 ± 1.9	24.1 ± 1.8	0.440
BMI ($kg \cdot m^{-2}$)	22.6 ± 0.5	23.2 ± 0.9	0.561
BSA (m^2)	1.99 ± 0.04	1.74 ± 0.05	<0.01
FEV_1 (% predicted)	110.2 ± 3.1	106.0 ± 4.1	0.420
FVC (% predicted)	105.8 ± 3.1	99.3 ± 3.7	0.194
SOL (minutes)	15.5 ± 3.8	32.0 ± 7.3	0.060
TST (minutes)	317.7 ± 21.4	321.3 ± 17.9	0.899
Sleep efficiency (%)	69.5 ± 3.8	69.1 ± 3.6	0.932
AI ($arousals \cdot hr^{-1}$)	28.9 ± 2.4	24.7 ± 2.5	0.243
% NREM	88.4 ± 2.1	87.7 ± 2.1	0.810
% REM	11.6 ± 2.1	13.3 ± 2.0	0.555

Age, body mass index (BMI), body surface area (BSA), lung function (FEV_1 and FVC), sleep onset latency (SOL), total sleep time (TST), sleep efficiency, arousal index (AI) and percentage of time spent in NREM (% NREM) and REM sleep (% REM) in men and women studied. Means ± SEM are presented, $p<0.05$ considered significant.

Table 7 **Respiratory and upper airway characteristics during wakefulness and sleep**

	Men			Women		
	Wake	**Stage 2**	**Stage 3/4**	**Wake**	**Stage 2**	**Stage 3/4**
$\dot{V}_I$ ($l \cdot min^{-1}$) * † ‡	8.48 (0.30)	7.50 (0.19)	7.54 (0.20)	6.65 (0.36)	5.81 (0.28)	5.93 (0.27)
V_T (l) *	0.55 (0.01)	0.54 (0.02)	0.53 (0.01)	0.42 (0.02)	0.41 (0.01)	0.41 (0.01)
F_B ($breaths \cdot min^{-1}$) † ‡	15.4 (0.65)	13.9 (0.39)	14.3 (0.43)	16.3 (1.11)	14.3 (0.70)	14.6 (0.73)
T_I (s) † ‡	1.81 (0.06)	1.90 (0.04)	1.87 (0.05)	1.73 (0.06)	1.87 (0.06)	1.83 (0.06)
T_E (s) † ‡	2.17 (0.11)	2.44 (0.08)	2.37 (0.09)	2.13 (0.23)	2.41 (0.13)	2.38 (0.14)
P_{ETCO_2} (Torr) * † ‡	42.0 (0.5)	43.9 (0.7)	44.2 (0.8)	41.9 (0.8)	43.9 (0.6)	44.0 (0.6)
PIF ($l \cdot min^{-1}$) * † ‡	28.9 (1.1)	25.1 (0.9)	25.4 (1.1)	23.4 (1.4)	20.1 (0.9)	20.8 (0.9)
P_{EPI} (cmH20) *	-3.1 (0.4)	-3.3 (0.3)	-3.4 (0.3)	-2.3 (0.2)	-2.5 (0.2)	-2.3 (0.2)
R_{UA} ($cmH_20 \cdot l^{-1} \cdot s$) †	2.6 (0.5)	4.2 (0.8)	4.3 (0.9)	2.3 (0.3)	3.7 (0.9)	2.9 (0.5)
Phasic EMG_{GG} (% max)	4.5 (1.4)	3.2 (1.1)	3.1 (1.0)	4.5 (0.9)	4.4 (1.2)	4.1 (1.1)
Tonic EMG_{GG} (% max)	1.8 (0.5)	1.3 (0.3)	1.2 (0.2)	2.4 (0.5)	2.1 (0.5)	2.0 (0.5)

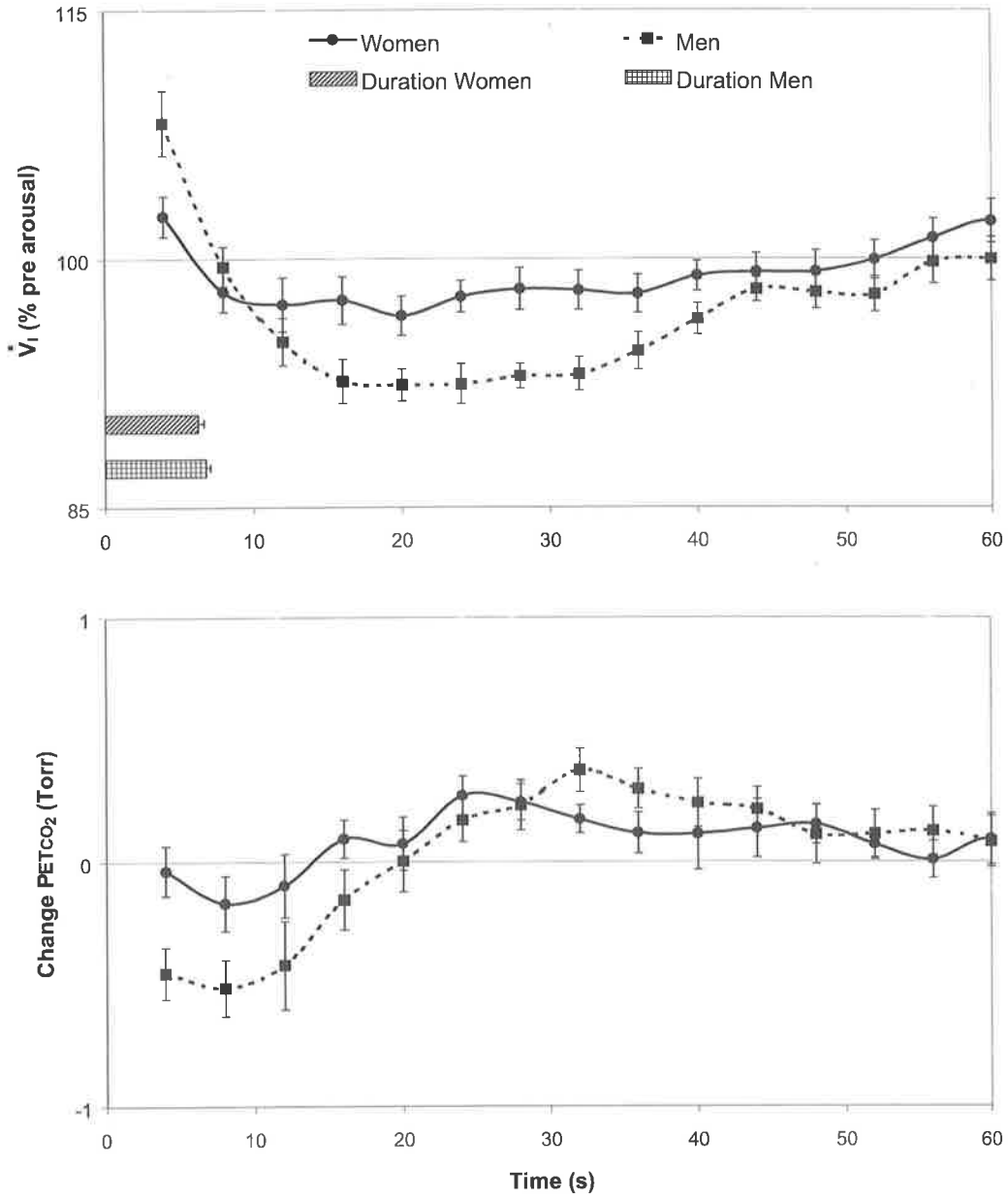
Minute ventilation ($\dot{V}_I$), tidal volume (V_T), breathing frequency (F_B), inspiratory (T_I) and expiratory (T_E) times, end-tidal CO_2 (P_{ETCO_2}), peak inspiratory flow (PIF), minimum epiglottic pressure (P_{EPI}), upper airway resistance (R_{UA}), phasic (Phasic EMG_{GG}) and tonic (Tonic EMG_{GG}) genioglossal activity during wakefulness and sleep. Means (SEM), * gender difference, † wake different to Stage 2 and ‡ wake different to Stages 3/4.

Table 8 **The influence of body position on respiratory variables during sleep.**

	Left		Back	
	Men	**Women**	**Men**	**Women**
$\dot{V}_I$ ($l \cdot min^{-1}$) * † ‡	7.5 ± 0.2	5.9 ± 0.3	7.0 ± 0.2	6.0 ± 0.3
V_T (l) * †	0.54 ± 0.01	0.41 ± 0.01	0.52 ± 0.01	0.40 ± 0.01
F_B ($breaths \cdot min^{-1}$)	14.1 ± 0.5	14.4 ± 0.7	13.8 ± 0.5	14.9 ± 1.0
T_I (s) †	1.90 ± 0.06	1.85 ± 0.06	2.03 ± 0.07	1.90 ± 0.08
T_E (s) †	2.42 ± 0.1	2.40 ± 0.14	2.37 ± 0.08	2.27 ± 0.17
P_{ETCO_2} (Torr) ‡	45.0 ± 0.7	43.8 ± 0.6	45.2 ± 0.7	43.3 ± 0.6
PIF ($l \cdot min^{-1}$) * † ‡	24.5 ± 1.2	20.4 ± 0.7	21.2 ± 1.0	18.9 ± 0.9
P_{EPI} (cmH_20) †	-3.1 ± 0.3	-2.4 ± 0.2	-3.6 ± 0.6	-3.2 ± 0.4
R_{UA} ($cmH_20 \cdot l^{-1} \cdot s$) †	4.1 ± 1.0	3.4 ± 0.6	5.8 ± 1.5	6.6 ± 1.2
Phasic EMG_{GG} (%max) †	3.4 ± 1.3	5.5 ± 1.3	5.5 ± 1.9	6.3 ± 1.7
Tonic EMG_{GG} (%max) †	1.4 ± 0.4	2.7 ± 0.7	2.3 ± 0.7	3.1 ± 0.7

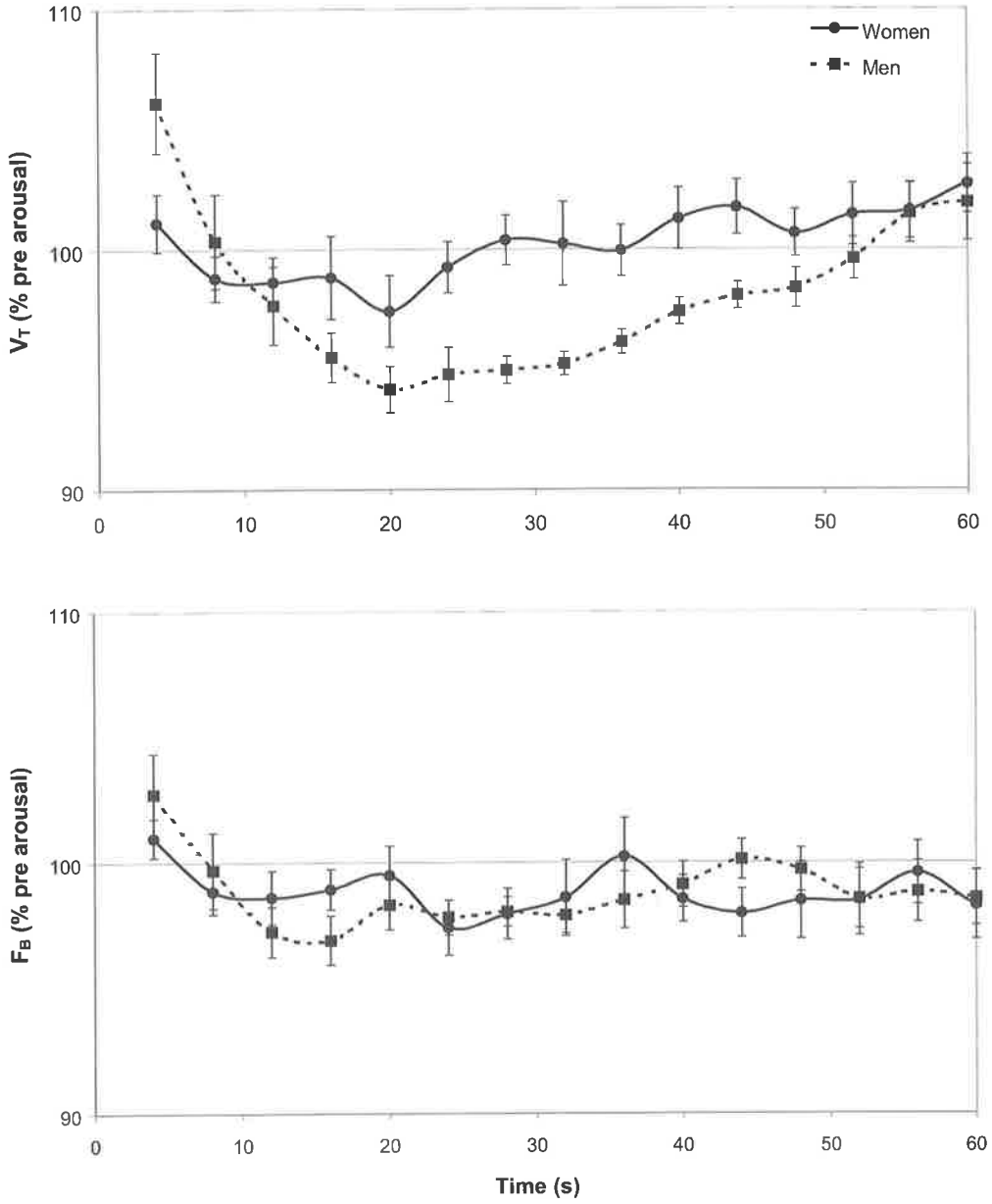
Resting variables in left lateral and supine positions in men and women. See Table 7 for definitions. Means ± presented. * significantly different between genders, † significantly different between body positions, ‡ significant gender by position interaction effect.

Figure 12 Ventilation and $PETCO_2$ following spontaneous arousal from NREM sleep in the lateral position in men and women



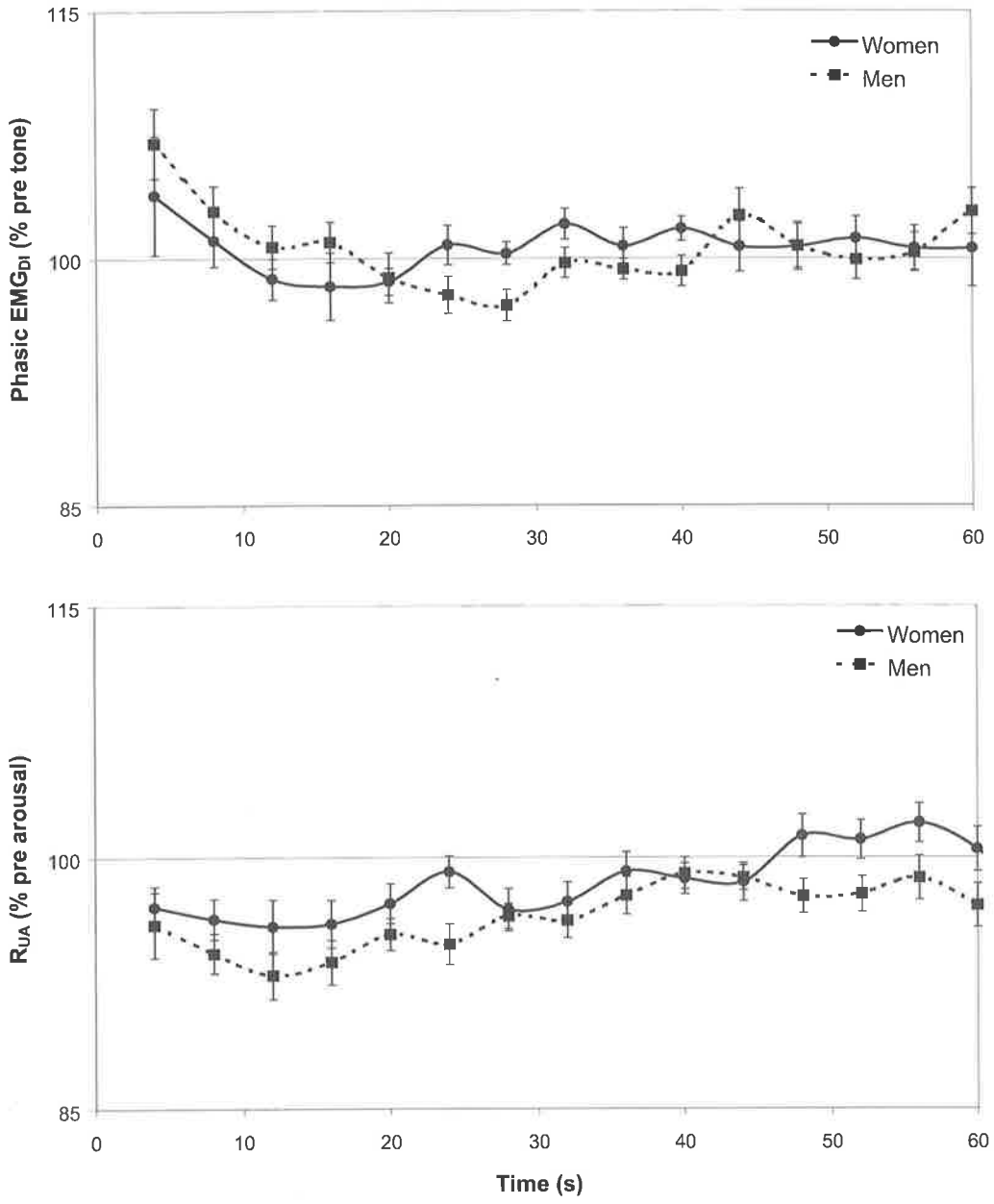
Ventilation ($\dot{V}_I$) and end-tidal CO_2 ($PETCO_2$) interpolated at 4s intervals for 60s after spontaneous arousal from sleep in 13 men and 12 women. The duration of arousal was not different between genders. Significant gender by time interaction effects were found for $\dot{V}_I$. $PETCO_2$ showed a similar trend (p=0.053). Means ± SEM are presented.

Figure 13 Tidal volume and breathing frequency following spontaneous arousal from sleep in the lateral position in men and women



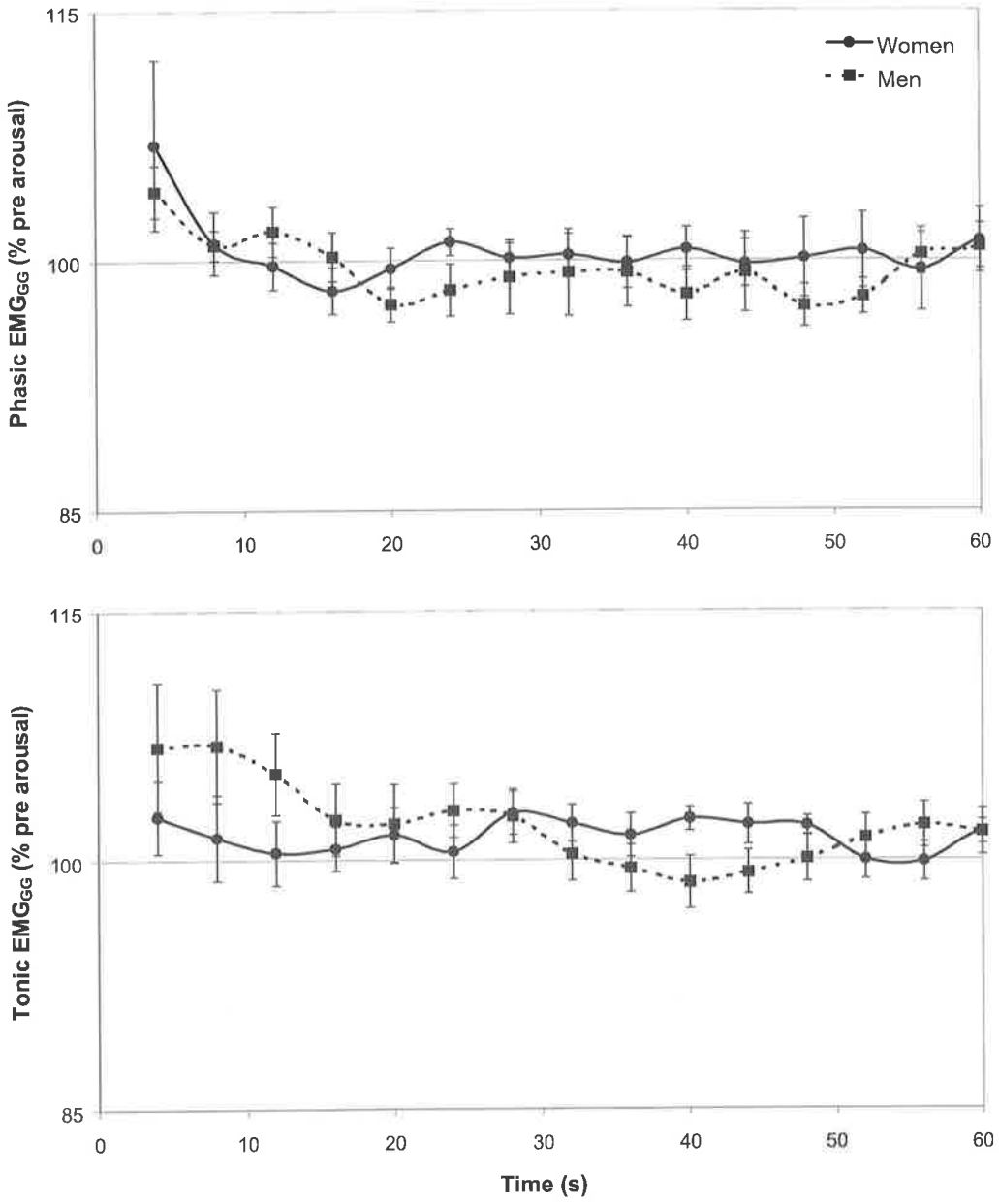
Tidal volume (V_T) and breathing frequency (F_B) interpolated at 4s intervals for 60s after spontaneous arousal from sleep in 13 men and 12 women. Time effects were observed for both variables but only V_T showed a significant gender by time interaction. Means ± SEM are presented.

Figure 14 Inspiratory phasic diaphragm activity and upper airway resistance following spontaneous arousal from NREM sleep in the lateral position in men and women



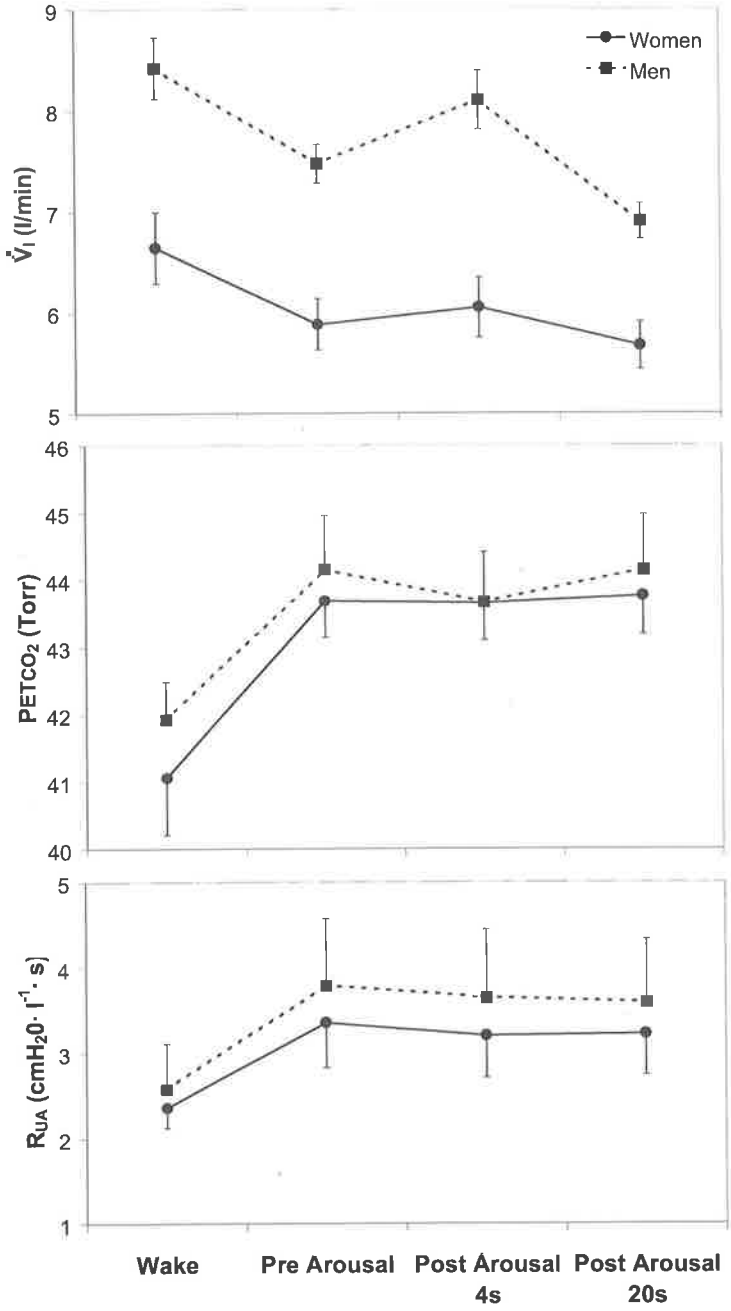
Inspiratory phasic (Phasic EMG_{DI}) diaphragm activity and upper airway resistance (R_{UA}) interpolated at 4s intervals for 60s after spontaneous arousal from NREM sleep in 11 men and 12 women. A significant time effect was found for R_{UA} but no gender or interaction effects were found. Means ± SEM are presented.

Figure 15 **Inspiratory phasic and tonic genioglossus muscle activity following spontaneous arousal from NREM sleep in the lateral position in men and women**



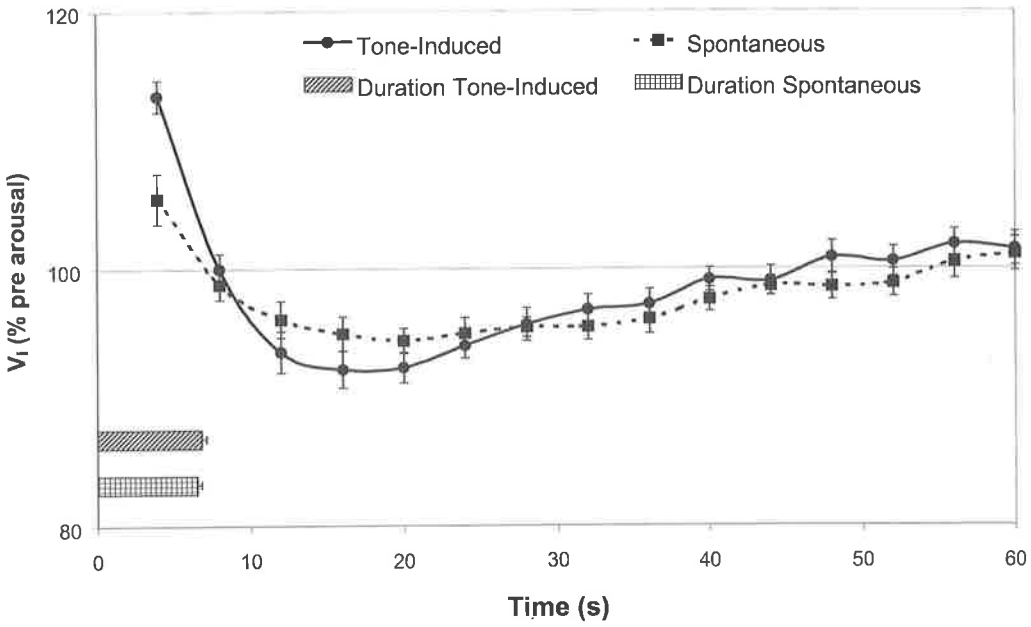
Inspiratory phasic (Phasic EMG_{GG}) and expiratory tonic (Tonic EMG_{GG}) genioglossal activity interpolated at 4s intervals for 60s after spontaneous arousal from NREM sleep in 12 men and 12 women. No significant gender or time effects were found. Means ± SEM are presented.

Figure 16 **Ventilation, P_{ETCO_2} and upper airway resistance in wakefulness, before and after arousal from NREM sleep in the lateral position in men and women**



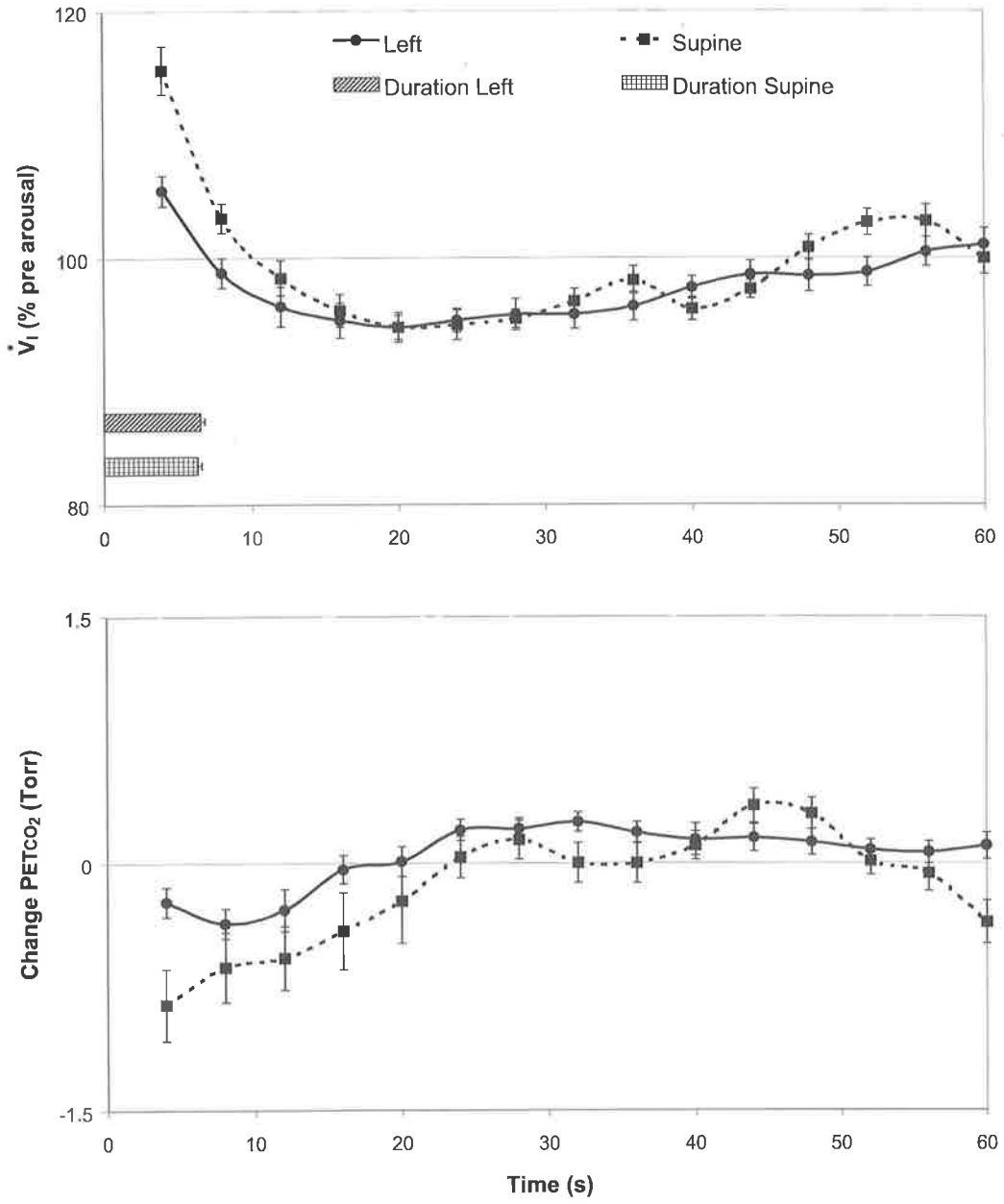
Ventilation ($\dot{V}_I$), $PETCO_2$ and upper airway resistance (R_{UA}) in the left lateral position during wakefulness, stable sleep (pre-arousal) and 4 and 20s post-arousal in men and women. Means ± SEM are presented.

Figure 17 Ventilation following spontaneous and tone-induced arousals from NREM sleep in the left lateral position



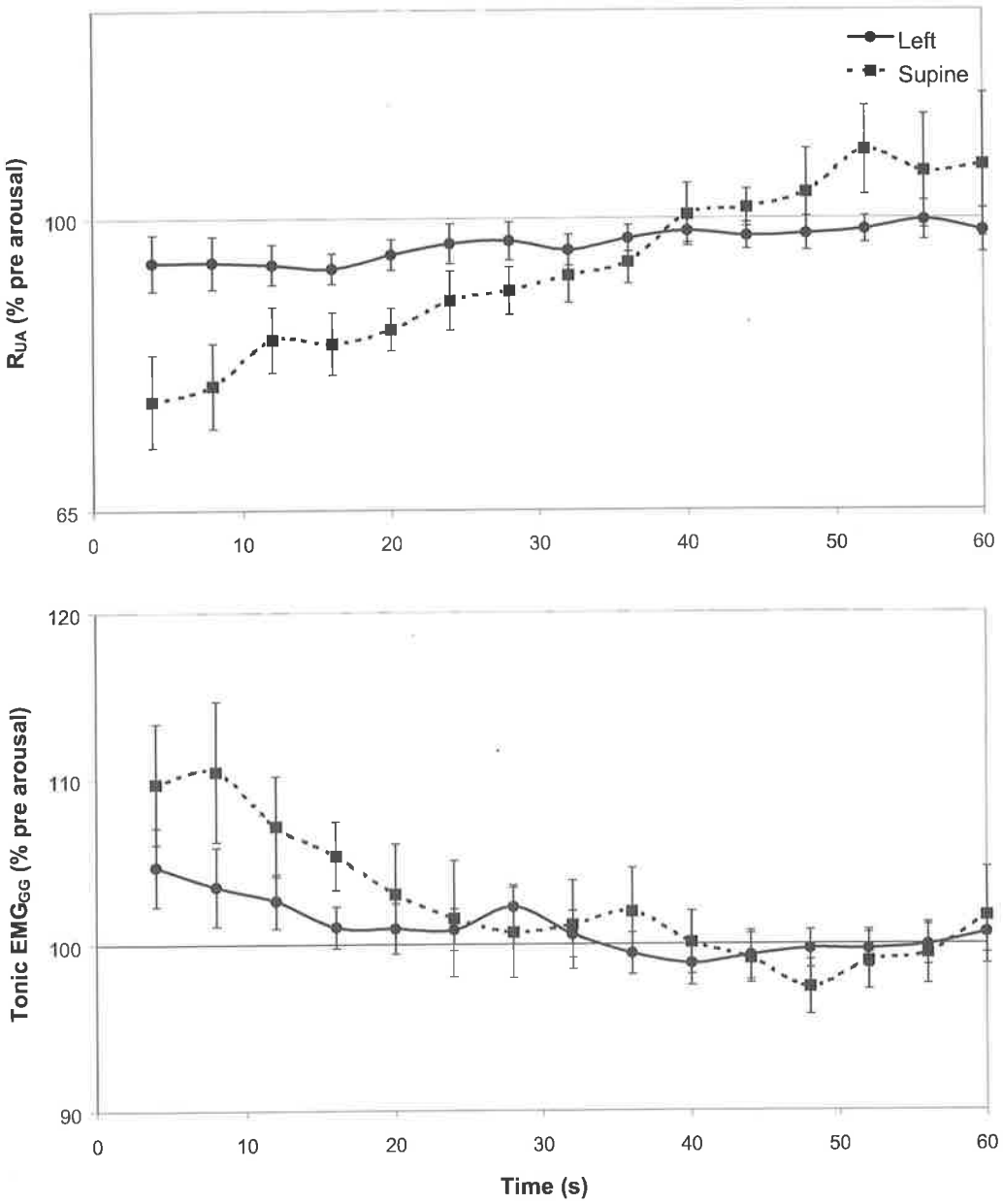
Ventilation ($\dot{V}_I$) interpolated at 4s intervals for 60s after spontaneous and tone-induced arousal from sleep in 23 subjects. The duration of arousal was not different between tone-induced or spontaneous arousal types. Significant type of arousal by time interaction effects were found. Means ± SEM are presented.

Figure 18 The effect of body position on ventilatory and $PETCO_2$ responses following arousal from NREM sleep



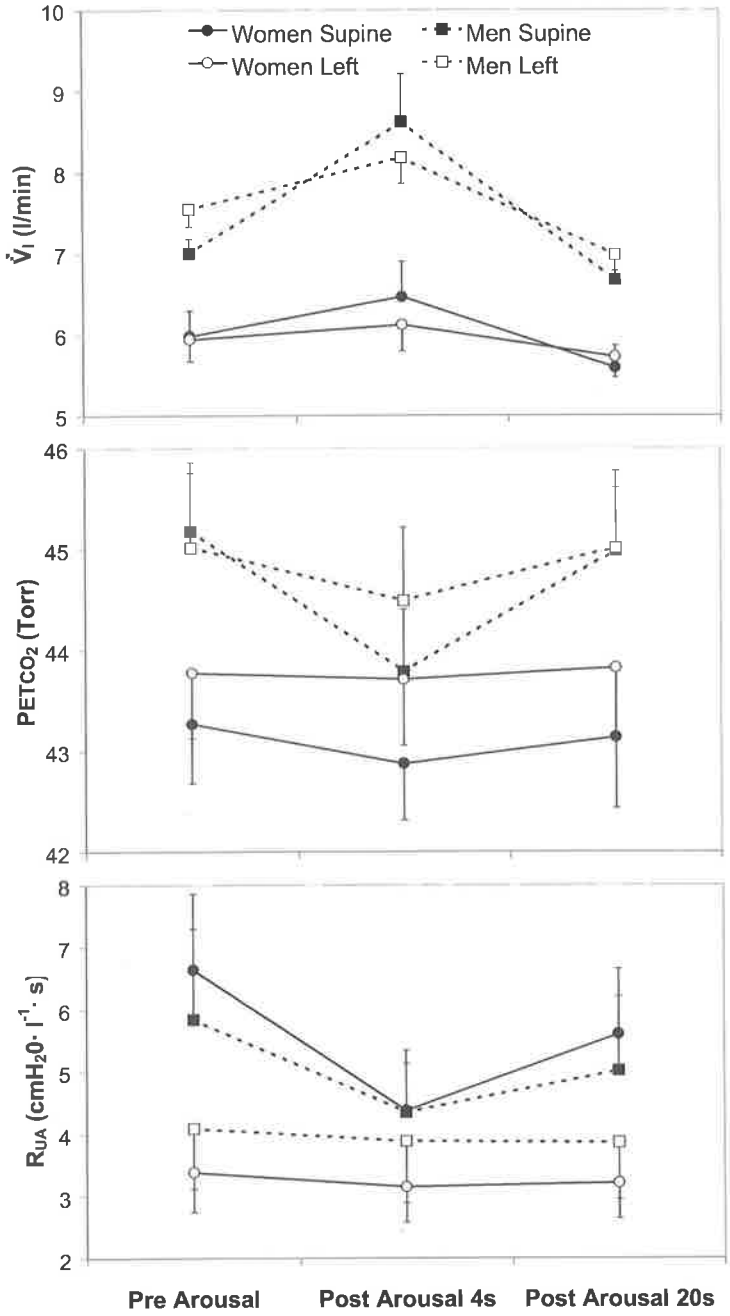
Ventilation ($\dot{V}_I$) and end-tidal partial pressure of CO_2 ($PETCO_2$) interpolated at 4s intervals for 60s after spontaneous arousal from sleep in 22 subjects in the left lateral and supine body positions. The duration of arousal did not differ between the body positions. Both $\dot{V}_I$ and $PETCO_2$ tended to differ between body positions ($p=0.055$ and 0.073 respectively). Means ± SEM are presented.

Figure 19 The effect of body position on upper airway resistance and genioglossal EMG activity following arousal from NREM sleep



Upper airway resistance (R_{UA}, n=18) and expiratory tonic genioglossal EMG (Tonic EMG_{GG}, n=22) activity interpolated at 4s intervals for 60s after spontaneous arousal from sleep in the left lateral and supine positions. Significant position by time interaction effects were found for R_{UA} and a similar trend observed for Tonic EMG_{GG} ($p=0.09$). Means ± SEM are presented.

Figure 20 **Ventilation, P_{ETCO_2} and airway resistance before, during and following arousal from sleep in men and women in the left lateral and supine positions**



Ventilation ($\dot{V}_I$), $PETCO_2$ and upper airway resistance (R_{UA}) in the left lateral and supine positions during stable NREM sleep (pre-arousal) and 4 and 20s post-arousal in men and women. Means ± SEM are presented.

5.4 Discussion

In this study, the ventilatory response to brief spontaneous arousal from NREM sleep has been compared between genders, sleep stages and lateral and supine body positions. In addition, spontaneous arousal responses were compared to tone-induced arousals during NREM sleep. The ventilatory response to arousal was higher in men than in women and men developed greater subsequent hypopnea. This occurred in both the lateral and supine positions and in stage 2 and stages 3/4 NREM sleep. The gender difference was independent of whether the arousal was induced by a tone or occurred spontaneously. These gender, type of arousal and position related differences occurred despite the duration of arousal being equivalent in all comparisons. The magnitude of the initial hyperventilation following arousal from sleep is thought to be related to a combination of the hypercapnic drive (chemoresponsiveness and degree of CO_2 at arousal), the waking reflex (56) and the upper airway resistance changes (70). The subsequent ventilatory pattern on return to sleep appears to be largely determined by the time course of recovery of upper airway resistance but is also influenced by central drive such that the magnitude of the initial hyperventilation and degree of subsequent hypocapnia, also influence recovery from arousal (70). The findings of this study appear consistent with this model, and the gender, position and type of arousal differences can be explained in these terms.

5.4.1 Resting data

Previous comparisons of upper airway resistance between healthy men and women during both wakefulness and sleep have reported mixed findings (68, 111, 120, 134, 136, 148). During wakefulness, resistance has been reported to be higher in men (148) and not different between genders (111). During sleep onset resistance is reported to fluctuate, but similarly between genders (68, 136) and in established sleep resistance is reported to be either higher in men (136) or not different between genders (120, 134). In the current study upper airway resistance was measured in men and women and found to be comparable between genders during both wakefulness and sleep. The differences between this and some previous findings (136, 148) are difficult to explain based on the methodologies used and may reflect slight differences in body mass between genders or the variable nature of upper airway resistance measurements.

Previous reports of resting genioglossus muscle activity in men and women are also somewhat controversial with one report that it is higher in women (111) and others suggesting no difference between genders (110, 147). In the two previous studies described in this thesis (Chapters 3 and 4), no gender differences in resting genioglossal activity were detected while awake. This study again failed to identify a gender difference in genioglossal activity either awake or asleep. The balance of evidence therefore suggests that a gender difference in the resting activity of the genioglossus muscle probably does not exist. The techniques for measurement of genioglossal activity were near identical in all studies and the number and characteristics (BMI, age) of subjects were also similar. The initial report suggesting

women had elevated genioglossal activity (111) may have differed from subsequent studies because it was conducted at moderate altitude (Denver ~ 1,600m) whereas all other studies were conducted at sea level.

5.4.2 The influence of gender on ventilatory response to arousal

The initial increase in ventilation following arousal from NREM sleep was higher in men than in women. This occurred despite the sleep related changes in ventilation, P_{ETCO_2} and upper airway resistance being very similar between genders (Figure 16). Given these similarities, it seems unlikely that the increased ventilatory response to arousal observed in men is related to a difference in the pre-arousal conditions. The increased ventilatory response to arousal from sleep in men may therefore have occurred because of an elevated hypercapnic ventilatory response in men, such that the approximately 3 Torr rise in P_{ETCO_2} during sleep subsequently stimulated ventilation more in men than in women on the return of wakefulness. Alternatively, men may have an increased waking reflex which stimulates ventilation following arousal from sleep more than in women. It was not possible, in this study, to differentiate these two possibilities.

Following the initial hyperpnea and the return to sleep (after 6s), a reduction in ventilation was observed which was more pronounced in men than in women and reached a nadir at approximately 20 seconds after the onset of arousal. During the period of hypoventilation (from 15-35s post-arousal), airway resistance was still lower than the pre-arousal level although it was gradually returning to the sleeping level during this time (Figure 12). The changes in resistance following arousal were

not different between genders. It therefore seems unlikely that the more marked hypoventilation in men was due to elevated upper airway resistance. It is possible that the greater hypocapnia that developed following arousal (approximately 0.5 Torr and 0.1 Torr below pre-arousal level in men and women respectively), when combined with the sleep related fall in chemoresponsiveness, contribute to a reduction of central respiratory drive. However, the small reduction in P_{ETCO_2} may not totally account for the reduction in ventilation found (8% in men, 5% in women) particularly in women who have a lower threshold for apnea (164). The small reduction in ventilation observed following arousal-induced hyperventilation in the current study may also in part be explained by neural inhibition of respiration secondary to the arousal. There is controversy whether neural inhibition of respiration occurs in humans following mechanical ventilation during sleep (37, 152). However if a brief hyperpnea can activate such an inhibition, it could influence the magnitude of ventilation following arousal. It is also possible that the waking reflex itself has a more delayed inhibitory component.

5.4.3 The influence of position on arousal responses

All variables except breathing frequency were different between the left lateral and supine body positions during sleep. Upper airway resistance was increased, inspiratory time prolonged and expiratory time shortened in the supine position suggesting a load compensation response. Despite this, tidal volume was reduced in the supine position as was minute ventilation for the whole group. However, the reduction in minute ventilation occurred only in men suggesting load compensation may not have been as effective in men as in women. This proposal is somewhat

different to that reported in the study of Pillar and colleagues (110) who demonstrated that men decreased tidal volume more than women in response to external resistive loading during sleep. However, these authors suggested that the reduced tidal volume was related to a greater increase in pharyngeal resistance during external loading in men than in women. The relationship between the change in ventilation versus total load applied (pharyngeal + external resistance) was similar between genders suggesting that load compensation was comparable between genders. An alternative explanation for the reduction in ventilation from left to supine postures in men in the current study is that central respiratory drive fell in men on assuming the supine posture.

The ventilatory response to arousal from sleep was larger in the supine than the left position. This may be related to the pre-arousal ventilation being lower supine than left, or the larger decrease in resistance following arousal in the supine position compared to the left (Figure 20). Alternatively, the neural waking reflex may be influenced by posture. The subsequent undershoot in ventilation did not appear to be greater in the supine than the lateral position when expressed as a percent of the pre-arousal level. In the supine position, there was a greater post-arousal reduction in upper airway resistance than occurred in the lateral position, which gradually returned to the pre-arousal level over 30 seconds (Figure 19). Genioglossus muscle activity showed a similar time course of change (Figure 19), however differences between body positions were not statistically significant. It is possible that the more prolonged reduction in airway resistance reflects greater

upper airway stability in the supine position (compared to pre-arousal) which protects against more marked hypoventilation following arousal.

It may appear somewhat surprising that changes in ventilation and P_{ETCO_2} on assuming the supine posture were greater in men than in women, and yet men did not have a more marked position related change in the arousal response when compared to women. One explanation for this result is that the ventilatory response to arousal may be more determined by the waking reflex than the pre-arousal conditions. In this way, the waking reflex may mask the effect of the small differences in pre-arousal conditions found in the current study. This is consistent with the finding that the magnitude of diaphragm activation following arousal from sleep was not different between spontaneous breathing and mechanical ventilation despite P_{ETCO_2} and ventilation being markedly different between conditions (137). Also, Carley and colleagues (27) have reported steady-state hypercapnia does not alter the ventilatory response to tone-induced arousal from sleep, providing additional support for this hypothesis.

5.4.4 Possible relevance to sleep apnea syndromes

The ventilatory response to arousal has been postulated to contribute to the development of periodic breathing in patients with central sleep apnea syndrome (151). In the current study, men were observed to have an increased initial ventilatory response to arousal and greater subsequent hypopnea than in women. This may be an important factor contributing to the male prevalence of CSA, particularly when combined with the observation that men appear to require a

smaller reduction in P_{ETCO_2} than women to develop central apnea (164). In addition, some patients with CSA have been reported to have apneas only in the supine position in sleep (22). The finding in the current study that the ventilatory response to arousal was greater in the supine position may also contribute to this effect.

The relevance of the findings presented in this study to obstructive sleep apnea are unclear. Obstructive sleep apnea patients have large increases in resistance during sleep and might be expected to develop obstructive apnea during hypoventilation following a brief arousal from sleep. However, following arousal from sleep in the supine position (when pre-arousal resistance was elevated compared to left) a larger initial response was observed but the subsequent hypopnea was not more marked when compared that seen on the left. This may reflect the slow return of resistance to the sleeping level. If OSA patients are simply further along this continuum of increased upper airway resistance in sleep, they might be expected to simply show a larger initial ventilatory response without greater undershoot. However, if the time course of change in resistance is shorter in OSA patients then an obstruction may develop during the period of low respiratory drive. Clearly the role of arousal from sleep in the pathogenesis and high male prevalence of sleep apnea syndromes requires further investigation.

5.4.5 Tone-induced versus spontaneous arousal from sleep

Most previous studies examining the ventilatory and airway responses to arousal from sleep have examined responses following tone-induced arousal (12, 27, 28,

56, 70, 72). As mentioned by several of these authors (12, 28, 56, 70), it is possible that the response following tone-induced arousal differs from normal spontaneous arousal from sleep, although this had not been tested previously. In the current study, the initial increase in ventilation following tone-induced arousal was higher than following spontaneous arousal despite the pre-arousal conditions and duration of arousal being similar. This most likely results from an increased waking reflex following arousal from sleep because the hypercapnic ventilatory response would not be expected to differ with the type of arousal. Following return to sleep, subjects developed greater hypopnea following tone-induced than spontaneous arousal. The time course of change in resistance was very similar between arousal types so it appears likely the reduction in ventilation is a result of more pronounced hypocapnia following the initial increase in ventilation, or more neural inhibition.

The ventilatory response to tone-induced arousal reported in the current study appeared smaller and followed a different time course than some (12, 70), but not all (27) previous studies conducted in healthy individuals. Khoo et al. (70) investigated the ventilatory response to arousal from stage 2 sleep in nine healthy men while in the supine position. These authors reported that mean inspiratory flow and tidal volume were elevated for 7 breaths following the presentation of a 5s tone. It is possible that the 5-second auditory stimulus used in their study (compared to 0.5s tone in the current study) contributed to the larger and more prolonged respiratory changes observed. Badr and colleagues (12) measured the change in ventilation, as measured by calibrated Respitrace, in 8 healthy subjects following arousal from NREM sleep. Arousal was induced with a 0.5s auditory tone

presented during inspiration and all subjects were studied supine. These authors reported that ventilation was increased for 2 breaths after the tone, but they did not observe a subsequent hypopnea within 6 breaths of the tone. Subjects did not appear to differ markedly from the current study in terms of age and BMI. It appears likely that the slightly larger increase in ventilation observed by Badr et al. may reflect larger arousals than in the current study, because when Badr et al. analysed only EEG arousals (no submental EMG increase), ventilation was found to be elevated for only 1 breath. It is difficult to explain the lack of undershoot reported in their study. There were significant methodological differences which may have a bearing: In this study tones were presented in expiration, compared with inspiration in the study of Badr et al. In this study ventilation was measured with a mask and pneumotachograph whereas Badr et al. used the less accurate but less intrusive method of inductive plethysmography. Both Khoo et al. and Badr et al. measured ventilatory responses following the onset of the tone, not the onset of arousal as in the current study. However, given that the latency to arousal from sleep following the tone was approximately 1 second in the current and a previous study (27), it would seem unlikely to significantly effect the time course of ventilatory changes.

Another notable difference is that in the current study breath-by-breath measurements were interpolated following arousal whereas previous studies have averaged data by breath number following arousal (12, 27, 28, 70, 137). Interpolation was performed so that men and women could be compared regardless of possible breathing frequency differences. Given that breathing frequency did change following arousal, it appears likely that breath-by-breath

averaging would distort the time course of recovery. However, the interpolation method used in the current study underestimates the magnitude of the first breath. Importantly, to minimise this effect, interpolation was not performed across the arousal. Also, interpolation would tend to an underestimate rather than exaggerate the magnitude of hypopnea. The method of interpolation may therefore contribute to the reduced initial ventilatory response observed in the current study (compared to previous reports), but would not account for the hypopnea found following return to sleep.

Carlson and colleagues measured diaphragm (oesophageal electrode catheter) and genioglossus (intramuscular electrodes) muscle activity following arousal from stage 2 sleep induced by a 0.5s tone (28). All 6 subjects were studied supine and the average duration of arousal was reported to range from 7 and 10s between subjects. These authors reported an increase in the activity of both muscles for 4 breaths following tone-induced arousal. These changes appear very similar to the current study for the phasic and tonic genioglossus muscle activity following arousals in the supine position (Figure 19), however the phasic diaphragm activity measured in the current study did not appear to be increased for this long and more closely followed the decline in ventilation. The difference in diaphragm muscle changes may be related to different measurement techniques as in the current study diaphragm activity was measured with surface electrodes.

5.4.6 Methodological considerations

There are several important methodological considerations with regard to this study. Firstly, the subjects studied were heavily instrumented and it is possible that the arousal response may be altered as a result. The inspiratory circuit added a small (~1 $cmH_2O \cdot l^{-1} \cdot s$) resistance to inspiration and all subjects breathed nasally. Nevertheless, given that all subjects experienced the same conditions, these factors are unlikely to account for the gender, position and type of arousal differences observed.

The epiglottic pressure catheter was prone to drift, presumably because of build up of airway secretions on the catheter. However, the raw data for every arousal identified for breath-by-breath analysis was visually inspected for baseline drift and both epiglottic pressure and upper airway resistance data were removed from trials in which this occurred. This resulted in some subjects having fewer post-arousal measurements of resistance than ventilation. If the arousal duration of trials included for analysis of resistance differed from those included in analysis of ventilation, then the time course of changes in these variables may also appear to differ. The duration of arousal in trials included for analysis of resistance were therefore compared to the duration of trials included in analysis of ventilation and were found to be <0.1s different, suggesting that the results for resistance and ventilation were not likely to be influenced by this problem.

With regard to tone-induced arousals from sleep, it is possible that stage modifies the arousal response to this type of arousal even though it does not appear to

modify the response to spontaneous arousal. There were insufficient tone-induced arousals from stages 3/4 NREM sleep to investigate this possibility. However, if tone-induced arousals were different in stage 2 compared to stages 3/4 sleep, then the fact that tone-induced arousals occurred predominantly from stage 2 sleep may contribute to the observed difference between spontaneous and tone-induced arousal. When type of arousal comparisons were conducted only on arousals from stage 2 sleep, a significant difference for type of arousal was still observed. It therefore seems unlikely that the difference observed between spontaneous and tone-induced arousal from NREM sleep was related to a sampling bias in sleep stage.

If in the case of spontaneous arousals ventilation increased prior to EEG changes, the pre-arousal baseline level of ventilation may be artificially elevated. However if the data immediately prior to arousal (-4s) were excluded the average baseline ventilation would have been only 0.03 $l \cdot min^{-1}$ lower. This would shift the whole spontaneous arousal response curve down by 0.6% and is unlikely to importantly contribute to the results observed.

5.4.7 Summary

In summary, the ventilatory response following brief arousal from NREM sleep is higher in men than in women, and men develop a greater subsequent reduction in ventilation on return to sleep. The ventilatory response to arousal is increased in both genders supine but this postural effect on ventilatory response is not different between genders. The ventilatory response to arousal is greater when induced by

an auditory tone than when occurs spontaneously, and is presumably related to a larger waking reflex component to the arousal following an auditory stimulus. The tendency for men to augment their ventilation more than women immediately after arousal from sleep and to have greater compensatory undershoot in ventilation on resumption of sleep, may contribute to the male predominance of sleep apnea syndromes.

CHAPTER 6. SUMMARY AND CONCLUSIONS

The prevalence of both obstructive and central sleep apnea syndromes is higher in men than in women (17, 40, 74, 100, 116, 159). It has been suggested that this may relate to differences in the anatomy or physiology of the upper airway because patients with sleep apnea are reported to have small (57, 121, 124), compliant (21, 24, 30) upper airways, and OSA patients have abnormal muscle control (90). The stability of the respiratory controller has also been implicated, and patients with obstructive and central sleep apnea have altered respiratory control (60, 150, 154). However, consistent gender differences in airway anatomy, physiology and respiratory control that could explain the high male prevalence of sleep apnea syndromes have not been demonstrated. The studies presented in this thesis were designed to assess several previously untested or controversial aspects of respiratory and upper airway muscle control in healthy men and women.

In male obstructive sleep apneic patients, respiratory afterdischarge of ventilation has been reported to be shorter than in healthy males (49). This effect is believed to reflect an unstable respiratory controller in patients with OSA (153). The duration of respiratory afterdischarge of ventilation has been compared between healthy men and women and found to be similar (67). Whether the upper airway muscles also exhibit respiratory afterdischarge in humans and whether this is similar between men and women was unknown, although respiratory afterdischarge of hypoglossal nerve activity has previously been shown in animals (65). In the first study presented in this thesis, the genioglossus and diaphragm muscle activities

were compared between genders at rest and following brief hypoxia. The resting activity of the genioglossus was not different between men and women and the epiglottic pressure-genioglossus muscle activity relationship were also comparable. The respiratory afterdischarge of both genioglossus and diaphragm muscles were similar in men and women. These findings suggest that a gender difference in genioglossal activity at rest or following removal of a brief respiratory stimulus is unlikely to contribute to the high male prevalence of sleep apnea. However, these experiments were conducted in young subjects during wakefulness. It is possible that gender differences in respiratory afterdischarge in the different motorneurone pools could emerge in sleep or in older subjects.

In the second study presented in this thesis (Chapter 4), repetitive isocapnic hypoxia was given to healthy men and women in an attempt to elicit long-term facilitation of ventilation and genioglossus muscle activity. Long-term facilitation is the progressive rise in respiratory motor output during and following repeated carotid body stimulation that has been shown to occur in a large number of animal species (92, 99, 138). In humans however, long-term facilitation of ventilation has proven more difficult to elicit (2, 9, 87) and although long-term facilitation has been proposed to occur in upper airway dilator muscles (2, 9), healthy male subjects have been shown to have depression, not facilitation of the genioglossus muscle during wakefulness (87). In this thesis, repetitive isocapnic hypoxia was not found to elicit long-term facilitation of ventilation or genioglossus muscle activity in men or women while awake. The lack of long-term facilitation of ventilation is consistent with previous reports in men during wakefulness (87), in men and women during

sleep (9) and OSA patients (2). These studies indicate that long-term facilitation of ventilation is either not present in humans or is difficult to elicit with levels of hypoxia typically experienced by sleep apnea patients. Whether long-term facilitation of upper airway muscles occurs during sleep requires further study.

In the final experiment presented (Chapter 5), the ventilatory and upper airway muscle responses to brief arousal from sleep were compared between healthy men and women. Arousal from sleep has been suggested to contribute to respiratory instability (70), particularly in the central sleep apnea syndrome where an increase in ventilation with arousal appears pivotal to the perpetuation of cyclical respiratory events (151). The ventilatory response to arousal was found to be elevated in men compared to women despite similar wake to sleep changes in baseline ventilation, CO_2 levels and upper airway resistance. Furthermore, men were observed to have a greater reduction in ventilation on return to sleep. These differences persisted whether arousal from sleep occurred spontaneously or following an auditory tone, and whether the subjects were in the left lateral or supine body positions. The role of this gender difference in the pathogenesis of sleep related breathing disorders requires further investigation. Nevertheless, this would appear, at least theoretically, to render men prone to respiratory instability following brief arousal. Genioglossus muscle responses following arousal were in general smaller and more variable than changes in ventilation. In contrast to the ventilatory responses, the genioglossal activity following arousal was generally not different between men and women. However, when arousal responses from both body positions were

combined a significant gender difference in tonic genioglossal activity was observed.

During NREM sleep in the supine position, upper airway resistance was increased by a similar amount in both men and women compared to sleep in the lateral position. However, men were found to have lower ventilation supine than in the lateral position, while there was no apparent difference in ventilation between body positions in women. This may indicate that men compensate for the added resistive load imposed by lying supine less well than women. Alternatively, central respiratory drive may be altered in the supine position in men.

In all three studies the resting activity of the genioglossus muscle was compared between genders. During both wakefulness (all experiments) and sleep (experiment 3, Chapter 5) the genioglossal activity was found to be similar between genders. These findings, when combined with two previous reports showing no gender difference in genioglossal activity (110, 147), indicate that the resting activity of the genioglossus muscle probably does not differ between genders. There is also controversy as to whether the resting upper airway resistance is different between genders (68, 111, 120, 134, 136, 148). In all three studies presented in this thesis, upper airway resistance was found to be similar between men and women. These findings add to the literature suggesting upper airway resistance does not differ between genders.

Several questions have arisen from these studies. Firstly, it would appear important to determine whether long-term facilitation of the genioglossus or other upper airway dilator muscles occurs during sleep. Secondly, the significance of an elevated ventilatory response to arousal from sleep in men, for upper airway and respiratory stability and the pathogenesis of sleep apnea syndromes requires further investigation. Finally it would appear important to determine the mechanism responsible for the increased ventilatory response to arousal in men, if this plays a role in the male predominance of sleep apnea syndromes and whether it is amenable to modification.

REFERENCES

1. Aaron, E. A., and F. L. Powell. Effect of chronic hypoxia on hypoxic ventilatory response in awake rats. *J Appl Physiol* 74: 1635-40, 1993.
2. Aboubakr, S. E., A. Taylor, R. Ford, S. Siddiqi, and M. S. Badr. Long-term facilitation in obstructive sleep apnea patients during NREM sleep. *J Appl Physiol* 91: 2751-7, 2001.
3. Aitken, M. L., J. L. Franklin, D. J. Pierson, and R. B. Schoene. Influence of body size and gender on control of ventilation. *J Appl Physiol* 60: 1894-9, 1986.
4. Ambrogetti, A., L. G. Olson, and N. A. Saunders. Differences in the symptoms of men and women with obstructive sleep apnoea. *Aust N Z J Med* 21: 863-6, 1991.
5. American Sleep Disorders Association Task Force. EEG arousals: scoring rules and examples. *Sleep* 15: 173-84, 1992.
6. American Sleep Disorders Association Task Force. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. *Sleep* 22: 667-89, 1999.
7. Ancoli-Israel, S., D. F. Kripke, M. R. Klauber, R. Fell, C. Stepnowsky, E. Estline, N. Khazeni, and A. Chinn. Morbidity, mortality and sleep-disordered breathing in community dwelling elderly. *Sleep* 19: 277-82, 1996.
8. Australian Instititue of Health and Welfare. National Nutrition Survey. Canberra: Australian Bureau of Statistics and Commonwealth Department of Health and Aged Care, 1995.

9. Babcock, M. A., and M. S. Badr. Long-term facilitation of ventilation in humans during NREM sleep. *Sleep* 21: 709-716, 1998.

10. Bach, K. B., and G. S. Mitchell. Hypoxia-induced long-term facilitation of respiratory activity is serotonin dependent. *Respir Physiol* 104: 251-60, 1996.

11. Badr, M. S. Effect of ventilatory drive on upper airway patency in humans during NREM sleep. *Respir Physiol* 103: 1-10, 1996.

12. Badr, M. S., B. J. Morgan, L. Finn, F. S. Toiber, D. C. Crabtree, D. S. Puleo, and J. B. Skatrud. Ventilatory response to induced auditory arousals during NREM sleep. *Sleep* 20: 707-14, 1997.

13. Badr, M. S., J. B. Skatrud, and J. A. Dempsey. Determinants of poststimulus potentiation in humans during NREM sleep. *J Appl Physiol* 73: 1958-71, 1992.

14. Baldwin, C. M., K. A. Griffith, F. J. Nieto, G. T. O'Connor, J. A. Walsleben, and S. Redline. The association of sleep-disordered breathing and sleep symptoms with quality of life in the Sleep Heart Health Study. *Sleep* 24: 96-105, 2001.

15. Ballester, E., J. R. Badia, L. Hernandez, E. Carrasco, J. de Pablo, C. Fornas, R. Rodriguez-Roisin, and J. M. Montserrat. Evidence of the effectiveness of continuous positive airway pressure in the treatment of sleep apnea/hypopnea syndrome. *Am J Respir Crit Care Med* 159: 495-501, 1999.

16. Bixler, E. O., A. N. Vgontzas, H. M. Lin, T. Ten Have, B. E. Leiby, A. Vela-Bueno, and A. Kales. Association of hypertension and sleep-disordered breathing. *Arch Intern Med* 160: 2289-95, 2000.

17. Bixler, E. O., A. N. Vgontzas, H. M. Lin, T. Ten Have, J. Rein, A. Vela-Bueno, and A. Kales. Prevalence of Sleep-disordered Breathing in Women. Effects of gender. *Am J Respir Crit Care Med* 163: 608-613, 2001.

18. Bixler, E. O., A. N. Vgontzas, T. Ten Have, K. Tyson, and A. Kales. Effects of age on sleep apnea in men: I. Prevalence and severity. *Am J Respir Crit Care Med* 157: 144-8, 1998.

19. Bonora, M., G. I. Shields, S. L. Knuth, D. Bartlett, Jr., and W. M. St. John. Selective depression by ethanol of upper airway respiratory motor activity in cats. *Am Rev Respir Dis* 130: 156-61, 1984.

20. Bonora, M., W. M. St. John, and T. A. Bledsoe. Differential elevation by protriptyline and depression by diazepam of upper airway respiratory motor activity. *Am Rev Respir Dis* 131: 41-5, 1985.

21. Bradley, T. D., I. G. Brown, N. Zamel, E. A. Phillipson, and V. Hoffstein. Differences in pharyngeal properties between snorers with predominantly central sleep apnea and those without sleep apnea. *Am Rev Respir Dis* 135: 387-91, 1987.

22. Bradley, T. D., W. T. McNicholas, R. Rutherford, J. Popkin, N. Zamel, and E. A. Phillipson. Clinical and physiologic heterogeneity of the central sleep apnea syndrome. *Am Rev Respir Dis* 134: 217-21, 1986.

23. Brooks, L. J., and K. P. Strohl. Size and mechanical properties of the pharynx in healthy men and women. *Am Rev Respir Dis* 146: 1394-7, 1992.

24. Brown, I. G., T. D. Bradley, E. A. Phillipson, N. Zamel, and V. Hoffstein. Pharyngeal compliance in snoring subjects with and without obstructive sleep apnea. *Amer Rev Resp Dis* 132: 211-5, 1985.

25. Brown, I. G., N. Zamel, and V. Hoffstein. Pharyngeal cross-sectional area in normal men and women. *J Appl Physiol* 61: 890-5, 1986.

26. Cao, K. Y., C. W. Zwillich, M. Berthon-Jones, and C. E. Sullivan. Increased normoxic ventilation induced by repetitive hypoxia in conscious dogs. *J Appl Physiol* 73: 2083-8, 1992.

27. Carley, D. W., R. Applebaum, R. C. Basner, E. Onal, and M. Lopata. Respiratory and arousal responses to acoustic stimulation. *Chest* 112: 1567-71, 1997.

28. Carlson, D. M., D. W. Carley, E. Onal, M. Lopata, and R. C. Basner. Acoustically induced cortical arousal increases phasic pharyngeal muscle and diaphragmatic EMG in NREM sleep. *J Appl Physiol* 76: 1553-9, 1994.

29. Chervin, R. D. Sleepiness, fatigue, tiredness, and lack of energy in obstructive sleep apnea. *Chest* 118: 372-9, 2000.

30. Ciscar, M. A., G. Juan, V. Martinez, M. Ramon, T. Lloret, J. Minguez, M. Armengot, J. Marin, and J. Basterra. Magnetic resonance imaging of the pharynx in OSA patients and healthy subjects. *Eur Respir J* 17: 79-86, 2001.

31. Cistulli, P. A., D. J. Barnes, R. R. Grunstein, and C. E. Sullivan. Effect of short-term hormone replacement in the treatment of obstructive sleep apnoea in postmenopausal women. *Thorax* 49: 699-702, 1994.

32. Cook, W. R., J. J. Benich, and S. A. Wooten. Indices of severity of obstructive sleep apnea syndrome do not change during medroxyprogesterone acetate therapy. *Chest* 96: 262-6, 1989.

33. Crocker, B. D., L. G. Olson, N. A. Saunders, M. J. Hensley, J. L. McKeon, K. M. Allen, and S. G. Gyulay. Estimation of the probability of disturbed breathing during sleep before a sleep study. *Am Rev Respir Dis* 142: 14-8, 1990.

34. Davies, A. M., J. S. Koenig, and B. T. Thach. Upper airway chemoreflex responses to saline and water in preterm infants. *J Appl Physiol* 64: 1412-20, 1988.

35. Deegan, P. C., and W. T. McNicholas. Pathophysiology of obstructive sleep apnoea. *Eur Respir J* 8: 1161-78, 1995.

36. Dempsey, J. A., E. B. Olson, and J. B. Skatrud. Hormones and neurochemicals in the regulation of breathing. In: *The respiratory system*, edited by N. S. Cherniack and J. G. Widdicombe. Bethesda, MD: American Physiological Society, 1986, p. 181-221.

37. Dempsey, J. A., and J. B. Skatrud. Apnea following mechanical ventilation may be caused by nonchemical neuromechanical influences. *Am J Respir Crit Care Med* 163: 1297-8, 2001.

38. Douglas, N. J., D. P. White, J. V. Weil, C. K. Pickett, R. J. Martin, D. W. Hudgel, and C. W. Zwillich. Hypoxic ventilatory response decreases during sleep in normal men. *Am Rev Respir Dis* 125: 286-9, 1982.

39. Douglas, N. J., D. P. White, J. V. Weil, C. K. Pickett, and C. W. Zwillich. Hypercapnic ventilatory response in sleeping adults. *Am Rev Respir Dis* 126: 758-62, 1982.

40. Duran, J., S. Esnaola, R. Rubio, and A. Iztueta. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. *Am J Respir Crit Care Med* 163: 685-9, 2001.

41. Eldridge, F. L. Central neural respiratory stimulatory effect of active respiration. *J Appl Physiol* 37: 723-35, 1974.

42. Eldridge, F. L. Posthyperventilation breathing: different effects of active and passive hyperventilation. *J Appl Physiol* 34: 422-30, 1973.

43. Eldridge, F. L., and P. Gill-Kumar. Central neural respiratory drive and afterdischarge. *Respir Physiol* 40: 49-63, 1980.

44. Eldridge, F. L., and D. E. Millhorn. Oscillation , gating and memory in the respiratory control system. In: *The respiratory system*, edited by N. S. Cherniack and J. G. Widdicombe. Bethesda, MD: American Physiological Society, 1986, p. 93-114.

45. Fuller, D. D., K. B. Bach, T. L. Baker, R. Kinkead, and G. S. Mitchell. Long term facilitation of phrenic motor output. *Respir Physiol* 121: 135-46, 2000.

46. Fuller, D. D., T. L. Baker, M. Behan, and G. S. Mitchell. Expression of hypoglossal long-term facilitation differs between substrains of Sprague-Dawley rat. *Physiol Genomics* 4: 175-81, 2001.

47. Gallman, E. A., and D. E. Millhorn. Two long-lasting central respiratory responses following acute hypoxia in glomectomized cats. *J Physiol (Lond)* 395: 333-47, 1988.

48. Georgopoulos, D., Z. Bshouty, M. Younes, and N. R. Anthonisen. Hypoxic exposure and activation of the afterdischarge mechanism in conscious humans. *J Appl Physiol* 69: 1159-64, 1990.

49. Georgopoulos, D., E. Giannouli, V. Tsara, P. Argiropoulou, D. Patakas, and N. R. Anthonisen. Respiratory short-term poststimulus potentiation (after-discharge) in patients with obstructive sleep apnea. *Am Rev Respir Dis* 146: 1250-5, 1992.

50. Gottlieb, D. J., C. W. Whitney, W. H. Bonekat, C. Iber, G. D. James, M. Lebowitz, F. J. Nieto, and C. E. Rosenberg. Relation of sleepiness to respiratory disturbance index: the Sleep Heart Health Study. *Am J Respir Crit Care Med* 159: 502-7, 1999.

51. Guilleminault, C., M. A. Quera-Salva, M. Partinen, and A. Jamieson. Women and the obstructive sleep apnea syndrome. *Chest* 93: 104-9, 1988.

52. Harik-Khan, R. I., R. A. Wise, and J. L. Fleg. The effect of gender on the relationship between body fat distribution and lung function. *J Clin Epidemiol* 54: 399-406, 2001.

53. Harms, C. A., Y. J. Zeng, C. A. Smith, E. H. Vidruk, and J. A. Dempsey. Negative pressure-induced deformation of the upper airway causes central apnea in awake and sleeping dogs. *J Appl Physiol* 80: 1528-39, 1996.

54. Horner, R. L., J. A. Innes, and A. Guz. Reflex pharyngeal dilator muscle activation by stimuli of negative airway pressure in awake man. *Sleep* 16: S85-6, 1993.

55. Horner, R. L., J. A. Innes, M. J. Morrell, S. A. Shea, and A. Guz. The effect of sleep on reflex genioglossus muscle activation by stimuli of negative airway pressure in humans. *J Physiol (Lond)* 476: 141-51, 1994.

56. Horner, R. L., M. P. Rivera, L. F. Kozar, and E. A. Phillipson. The ventilatory response to arousal from sleep is not fully explained by differences in CO_2 levels between sleep and wakefulness. *J Physiol* 534: 881-90, 2001.

57. Horner, R. L., S. A. Shea, J. McIvor, and A. Guz. Pharyngeal size and shape during wakefulness and sleep in patients with obstructive sleep apnoea. *Q J Med* 72: 719-35, 1989.

58. Hudgel, D. W., K. R. Chapman, C. Faulks, and C. Hendricks. Changes in inspiratory muscle electrical activity and upper airway resistance during periodic breathing induced by hypoxia during sleep. *Am Rev Respir Dis* 135: 899-906, 1987.

59. Hudgel, D. W., and P. Devadatta. Decrease in functional residual capacity during sleep in normal humans. *J Appl Physiol* 57: 1319-22, 1984.

60. Hudgel, D. W., E. A. Gordon, S. Thanakitcharu, and E. N. Bruce. Instability of ventilatory control in patients with obstructive sleep apnea. *Am J Respir Crit Care Med* 158: 1142-9, 1998.

61. Hudgel, D. W., and C. Hendricks. Palate and hypopharynx--sites of inspiratory narrowing of the upper airway during sleep [published erratum appears in Am Rev Respir Dis 1989 Aug;140(2):569]. *Am Rev Respir Dis* 138: 1542-7, 1988.

62. Innes, J. A., M. J. Morrell, I. Kobayashi, R. D. Hamilton, and A. Guz. Central and reflex neural control of genioglossus in subjects who underwent laryngectomy. *J Appl Physiol* 78: 2180-6, 1995.

63. Issa, F. G., P. Edwards, E. Szeto, D. Lauff, and C. Sullivan. Genioglossus and breathing responses to airway occlusion: effect of sleep and route of occlusion. *J Appl Physiol* 64: 543-9, 1988.

64. Javaheri, S., T. J. Parker, L. Wexler, S. E. Michaels, E. Stanberry, H. Nishyama, and G. A. Roselle. Occult sleep-disordered breathing in stable congestive heart failure. *Ann Intern Med* 122: 487-92, 1995.

65. Jiang, C., G. S. Mitchell, and J. Lipski. Prolonged augmentation of respiratory discharge in hypoglossal motoneurons following superior laryngeal nerve stimulation. *Brain Res* 538: 215-25, 1991.

66. Jonah, B. A. Sensation seeking and risky driving: a review and synthesis of the literature. *Accid Anal Prev* 29: 651-65, 1997.

67. Jordan, A. S., P. G. Catcheside, R. S. Orr, F. J. O'Donoghue, N. A. Saunders, and R. D. McEvoy. Ventilatory decline after hypoxia and hypercapnia is not different between healthy young men and women. *J Appl Physiol* 88: 3-9, 2000.

68. Kay, A., J. Trinder, G. Bowes, and Y. Kim. Changes in airway resistance during sleep onset. *J Appl Physiol* 76: 1600-7, 1994.

69. Khoo, M. C. Determinants of ventilatory instability and variability. *Respir Physiol* 122: 167-182, 2000.

70. Khoo, M. C., S. S. Koh, J. J. Shin, P. R. Westbrook, and R. B. Berry. Ventilatory dynamics during transient arousal from NREM sleep: implications for respiratory control stability. *J Appl Physiol* 80: 1475-84, 1996.

71. Khoo, M. C., R. E. Kronauer, K. P. Strohl, and A. S. Slutsky. Factors inducing periodic breathing in humans: a general model. *J Appl Physiol* 53: 644-59, 1982.

72. Khoo, M. C., J. J. Shin, M. H. Asyali, T. S. Kim, and R. B. Berry. Ventilatory dynamics of transient arousal in patients with obstructive sleep apnea. *Respir Physiol* 112: 291-303, 1998.

73. Kim, H. C., T. Young, C. G. Matthews, S. M. Weber, A. R. Woodward, and M. Palta. Sleep-disordered breathing and neuropsychological deficits. A population-based study. *Am J Respir Crit Care Med* 156: 1813-9, 1997.

74. Kripke, D. F., S. Ancoli-Israel, M. R. Klauber, D. L. Wingard, W. J. Mason, and D. J. Mullaney. Prevalence of sleep-disordered breathing in ages 40-64 years: a population-based survey. *Sleep* 20: 65-76, 1997.

75. Krol, R. C., S. L. Knuth, and D. Bartlett, Jr. Selective reduction of genioglossal muscle activity by alcohol in normal human subjects. *Am Rev Resp Dis* 129: 247-50, 1984.

76. Lansing, R., and J. Savelle. Chest surface recording of diaphragm potentials in man. *Electroencephalogr Clin Neurophysiol* 72: 59-68, 1989.

77. Leech, J. A., E. Onal, C. Dulberg, and M. A. Lopata. A comparison of men and women with occlusive sleep apnea syndrome. *Chest* 94: 983-8, 1988.

78. Leiter, J. C., and J. A. Daubenspeck. Selective reflex activation of the genioglossus in humans. *J Appl Physiol* 68: 2581-7, 1990.

79. Leiter, J. C., S. L. Knuth, R. C. Krol, and D. Bartlett, Jr. The effect of diazepam on genioglossal muscle activity in normal human subjects. *Am Rev Resp Dis* 132: 216-9, 1985.

80. Loube, D. I., J. S. Poceta, M. C. Morales, M. D. Peacock, and M. M. Mitler. Self-reported snoring in pregnancy. Association with fetal outcome. *Chest* 109: 885-9, 1996.

81. Ludbrook, J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc Res* 28: 303-11, 1994.

82. Malhotra, A., R. B. Fogel, R. Kikinis, S. A. Shea, and D. P. White. Influence of aging and gender on upper airway structure and function (Abstract). *Sleep* 22: S128, 1999.

83. Malhotra, A., G. Pillar, R. B. Fogel, J. Beauregard, J. K. Edwards, D. I. Slamowitz, S. A. Shea, and D. P. White. Genioglossal but not palatal muscle activity relates closely to pharyngeal pressure. *Am J Respir Crit Care Med* 162: 1058-62, 2000.

84. Martin, R. J., R. D. Ballard, D. W. Hudgel, and P. L. Hill. The effects of weight and chemosensitivity on respiratory sleep abnormalities: a family study. *Int J Obes* 10: 283-92, 1986.

85. Martin, S. E., R. Mathur, I. Marshall, and N. J. Douglas. The effect of age, sex, obesity and posture on upper airway size. *Europ Resp J* 10: 2087-90, 1997.

86. McArdle, N., G. Devereux, H. Heidarnejad, H. M. Engleman, T. W. Mackay, and N. J. Douglas. Long-term use of CPAP therapy for sleep apnea/hypopnea syndrome. *Am J Respir Crit Care Med* 159: 1108-14, 1999.

87. McEvoy, R. D., R. M. Popovic, N. A. Saunders, and D. P. White. Effects of sustained and repetitive isocapnic hypoxia on ventilation and genioglossal and diaphragmatic EMGs. *J Appl Physiol* 81: 866-75, 1996.

88. McNamara, S. G., M. J. Aarts, H. Becker, K. Cao, O. J. Polo, and C. E. Sullivan. Ventilatory responses to repetitive hypoxia in subjects with Obstructive Sleep Apnea (Abstract). *Am J Crit Care Med* 151: A100, 1995.

89. Mezzanotte, W. S., D. J. Tangel, and D. P. White. Influence of sleep onset on upper-airway muscle activity in apnea patients versus normal controls. *Am J Respir Crit Care Med* 153: 1880-7, 1996.

90. Mezzanotte, W. S., D. J. Tangel, and D. P. White. Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). *J Clin Invest* 89: 1571-9, 1992.

91. Mitchell, G. S., T. L. Baker, S. A. Nanda, D. D. Fuller, A. G. Zabka, B. A. Hodgeman, R. W. Bavis, K. J. Mack, and E. B. Olson, Jr. Invited review: Intermittent hypoxia and respiratory plasticity. *J Appl Physiol* 90: 2466-75, 2001.

92. Mitchell, G. S., F. L. Powell, S. R. Hopkins, and W. K. Milsom. Time domains of the hypoxic ventilatory response in awake ducks: episodic and continuous hypoxia. *Respir Physiol* 124: 117-28, 2001.

93. Mohsenin, V. Gender differences in the expression of sleep-disordered breathing : role of upper airway dimensions. *Chest* 120: 1442-7, 2001.

94. National Center for Health Statistics. Prevalence of Overweight and Obesity among adults in the United States.

http://www.cdc.gov/nchs/products/pubs/pubd/hestats/3and4/overweight.htm US Department of Health and Human Services, 1997.

95. Naughton, M., D. Benard, A. Tam, R. Rutherford, and T. D. Bradley. Role of hyperventilation in the pathogenesis of central sleep apneas in patients with congestive heart failure. *Am Rev Respir Dis* 148: 330-8, 1993.

96. Nieto, F. J., T. B. Young, B. K. Lind, E. Shahar, J. M. Samet, S. Redline, R. B. D'Agostino, A. B. Newman, M. D. Lebowitz, and T. G. Pickering. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. *JAMA* 283: 1829-36, 2000.

97. O'Connor, C., K. S. Thornley, and P. J. Hanly. Gender differences in the polysomnographic features of obstructive sleep apnea. *Am J Respir Crit Care Med* 161: 1465-72, 2000.

98. Okabe, S., W. Hida, Y. Kikuchi, H. Kurosawa, J. Midorikawa, T. Chonan, T. Takishima, and K. Shirato. Upper airway muscle activity during sustained hypoxia in awake humans. *J Appl Physiol* 75: 1552-8, 1993.

99. Olson, E. B., Jr., C. J. Bohne, M. R. Dwinell, A. Podolsky, E. H. Vidruk, D. D. Fuller, F. L. Powell, and G. S. Mitchel. Ventilatory long-term facilitation in unanesthetized rats. *J Appl Physiol* 91: 709-16, 2001.

100. Olson, L. G., M. T. King, M. J. Hensley, and N. A. Saunders. A community study of snoring and sleep-disordered breathing. Prevalence. *Am J Respir Crit Care Med* 152: 711-6, 1995.

101. Olson, L. G., M. T. King, M. J. Hensley, and N. A. Saunders. A community study of snoring and sleep-disordered breathing. Symptoms. *Am J Respir Crit Care Med* 152: 707-10, 1995.

102. Onal, E., D. L. Burrows, R. H. Hart, and M. Lopata. Induction of periodic breathing during sleep causes upper airway obstruction in humans. *J Appl Physiol* 61: 1438-43, 1986.

103. Onal, E., and M. Lopata. Periodic breathing and the pathogenesis of occlusive sleep apneas. *Am Rev Respir Dis* 126: 676-80, 1982.

104. Onal, E., M. Lopata, and T. O'Connor. Pathogenesis of apneas in hypersomnia-sleep apnea syndrome. *Am Rev Respir Dis* 125: 167-74, 1982.

105. Onal, E., M. Lopata, and T. D. O'Connor. Diaphragmatic and genioglossal electromyogram responses to CO2 rebreathing in humans. *J Appl Physiol* 50: 1052-5, 1981.

106. Onal, E., M. Lopata, and T. D. O'Connor. Diaphragmatic and genioglossal electromyogram responses to isocapnic hypoxia in humans. *Am Rev Respir Dis* 124: 215-7, 1981.

107. Orem, J. The wakefulness stimulus for breathing. In: *Sleep and Breathing* (2nd ed.), edited by N. A. Saunders and C. E. Sullivan. New York: Marcel Dekker, 1994, p. 113-156.

108. Pae, E. K., A. A. Lowe, K. Sasaki, C. Price, M. Tsuchiya, and J. A. Fleetham. A cephalometric and electromyographic study of upper airway structures in the upright and supine positions. *Am J Orthod Dentofacial Orthop* 106: 52-9, 1994.

109. Phillipson, E. A., and G. Bowes. Control of breathing during sleep. In: *The Respiratory System*, edited by N. S. Cherniack and J. G. Widdicombe. Bethesda, MD: American Physiological Society, 1986, p. 649-689.

110. Pillar, G., A. Malhotra, R. Fogel, J. Beauregard, R. Schnall, and D. P. White. Airway mechanics and ventilation in response to resistive loading during sleep: influence of gender. *Am J Respir Crit Care Med* 162: 1627-32, 2000.

111. Popovic, R. M., and D. P. White. Influence of gender on waking genioglossal electromyogram and upper airway resistance. *Am J Resp Crit Care Med* 152: 725-31, 1995.

112. Popovic, R. M., and D. P. White. Upper airway muscle activity in normal women: influence of hormonal status. *J Appl Physiol* 84: 1055-62, 1998.

113. Powell, F. L., W. K. Milsom, and G. S. Mitchell. Time domains of the hypoxic ventilatory response. *Respir Physiol* 112: 123-34, 1998.

114. Rajagopal, K. R., P. H. Abbrecht, and B. Jabbari. Effects of medroxyprogesterone acetate in obstructive sleep apnea. *Chest* 90: 815-21, 1986.

115. Rechtschaffen, A., and, and A. E. Kales. A manual of standardised terminology, techniques and scoring systems for sleep stages of human subjects. UCLA Los Angeles, USA: National Institute of Health, 1968.

116. Redline, S., K. Kump, P. V. Tishler, I. Browner, and V. Ferrette. Gender differences in sleep disordered breathing in a community-based sample. *Am J Respir Crit Care Med* 149: 722-6, 1994.

117. Regensteiner, J. G., W. D. Woodard, D. D. Hagerman, J. V. Weil, C. K. Pickett, P. R. Bender, and L. G. Moore. Combined effects of female

hormones and metabolic rate on ventilatory drives in women. *J Appl Physiol* 66: 808-13, 1989.

118. Reyes del Paso, G. A., and J. Vila. Respiratory influences on the cardiac defense response. *Int J Psychophysiol* 15: 15-26, 1993.

119. Riha, R. L., S. E. Martin, B. Izci, M. Vennelle, W. A. Liston, and N. J. Douglas. Acoustic reflectance in pregnancy - a case-control study (Abstract). *Respirology* 7: A59, 2002.

120. Rowley, J. A., X. Zhou, I. Vergine, M. A. Shkoukani, and M. S. Badr. Influence of gender on upper airway mechanics: upper airway resistance and Pcrit. *J Appl Physiol* 91: 2248-54, 2001.

121. Schwab, R. J., W. B. Gefter, E. A. Hoffman, K. B. Gupta, and A. I. Pack. Dynamic upper airway imaging during awake respiration in normal subjects and patients with sleep disordered breathing. *Am Rev Respir Dis* 148: 1385-400, 1993.

122. Sforza, E., C. Petiau, T. Weiss, A. Thibault, and J. Krieger. Pharyngeal critical pressure in patients with obstructive sleep apnea syndrome. Clinical implications. *Am J Respir Crit Care Med* 159: 149-57, 1999.

123. Shahar, E., C. W. Whitney, S. Redline, E. T. Lee, A. B. Newman, F. Javier Nieto, G. T. O'Connor, L. L. Boland, J. E. Schwartz, and J. M. Samet. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med* 163: 19-25, 2001.

124. Shepard, J. W., Jr., M. Garrison, and W. Vas. Upper airway distensibility and collapsibility in patients with obstructive sleep apnea. *Chest* 98: 84-91, 1990.

125. Sin, D. D., F. Fitzgerald, J. D. Parker, G. Newton, J. S. Floras, and T. D. Bradley. Risk factors for central and obstructive sleep apnea in 450 men and women with congestive heart failure. *Am J Respir Crit Care Med* 160: 1101-6, 1999.

126. Sin, D. D., R. L. Jones, and G. C. Man. Hypercapnic ventilatory response in patients with and without obstructive sleep apnea: do age, gender, obesity, and daytime $PaCO_2$ matter? *Chest* 117: 454-9, 2000.

127. Smith, C. A., K. S. Henderson, L. Xi, C. Chow, P. R. Eastwood, and J. A. Dempsey. Neural-mechanical coupling of breathing in REM sleep. *J Appl Physiol* 83: 1923-32, 1997.

128. St John, W. M., and T. A. Bledsoe. Comparison of respiratory-related trigeminal, hypoglossal and phrenic activities. *Respir Physiol* 62: 61-78, 1985.

129. St. John, W. M., D. Bartlett, Jr., K. V. Knuth, S. L. Knuth, and J. A. Daubenspeck. Differential depression of hypoglossal nerve activity by alcohol. Protection by pretreatment with medroxyprogesterone acetate. *Am Rev Respir Dis* 133: 46-8, 1986.

130. Stewart, D., M. Berthen-Jones, S. McNamara, R. Grunstein, and C. Sullivan. Ventilatory responses to repetitive hypoxia in normal males (Abstract). *Am J Resp Crit Care Med* 149: A260, 1994.

131. Takano, N. Change in time course of posthyperventilation hyperpnea during menstrual cycle. *J Appl Physiol* 64: 2631-5, 1988.

132. Tangel, D. J., W. S. Mezzanotte, E. J. Sandberg, and D. P. White. Influences of NREM sleep on the activity of tonic vs. inspiratory phasic muscles in normal men. *J Appl Physiol* 73: 1058-66, 1992.

133. Tatsumi, K., C. K. Pickett, C. R. Jacoby, J. V. Weil, and L. G. Moore. Role of endogenous female hormones in hypoxic chemosensitivity. *J Appl Physiol* 83: 1706-10, 1997.

134. Thurnheer, R., P. K. Wraith, and N. J. Douglas. Influence of age and gender on upper airway resistance in NREM and REM sleep. *J Appl Physiol* 90: 981-8, 2001.

135. Thut, D. C., A. R. Schwartz, D. Roach, R. A. Wise, S. Permutt, and P. L. Smith. Tracheal and neck position influence upper airway airflow dynamics by altering airway length. *J Appl Physiol* 75: 2084-90, 1993.

136. Trinder, J., A. Kay, J. Kleiman, and J. Dunai. Gender differences in airway resistance during sleep. *J Appl Physiol* 83: 1986-97, 1997.

137. Trinder, J., M. Padula, D. Berlowitz, J. Kleiman, S. Breen, P. Rochford, C. Worsnop, B. Thompson, and R. Pierce. Cardiac and respiratory activity at arousal from sleep under controlled ventilation conditions. *J Appl Physiol* 90: 1455-63, 2001.

138. Turner, D. L., and G. S. Mitchell. Long-term facilitation of ventilation following repeated hypoxic episodes in awake goats. *J Physiol (Lond)* 499: 543-50, 1997.

139. Van de Graaff, W. B. Thoracic influence on upper airway patency. *J Appl Physiol* 65: 2124-31, 1988.

140. van Klaveren, R. J., and M. Demedts. Determinants of the hypercapnic and hypoxic response in normal man. *Respir Physiol* 113: 157-65, 1998.

141. Vgontzas, A. N., D. A. Papanicolaou, E. O. Bixler, K. Hopper, A. Lotsikas, H. M. Lin, A. Kales, and G. P. Chrousos. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 85: 1151-8, 2000.

142. Ware, J. C., R. H. McBrayer, and J. A. Scott. Influence of sex and age on duration and frequency of sleep apnea events. *Sleep* 23: 165-170, 2000.

143. Wheatley, J. R., W. S. Mezzanotte, D. J. Tangel, and D. P. White. Influence of sleep on genioglossus muscle activation by negative pressure in normal men. *Am Rev Respir Dis* 148: 597-605, 1993.

144. White, D. P. Occlusion pressure and ventilation during sleep in normal humans. *J Appl Physiol* 61: 1279-87, 1986.

145. White, D. P., N. J. Douglas, C. K. Pickett, J. V. Weil, and C. W. Zwillich. Hypoxic ventilatory response during sleep in normal premenopausal women. *Am Rev Respir Dis* 126: 530-3, 1982.

146. White, D. P., N. J. Douglas, C. K. Pickett, J. V. Weil, and C. W. Zwillich. Sexual influence on the control of breathing. *J Appl Physiol* 54: 874-9, 1983.

147. White, D. P., J. K. Edwards, and S. A. Shea. Local reflex mechanisms: Influence on basal genioglossal muscle activation in normal subjects. *Sleep* 21: 719-728, 1998.

148. White, D. P., R. M. Lombard, R. J. Cadieux, and C. W. Zwillich. Pharyngeal resistance in normal humans: influence of gender, age, and obesity. *J Appl Physiol* 58: 365-71, 1985.

149. Xie, A., F. Rankin, R. Rutherford, and T. D. Bradley. Effects of inhaled CO2 and added dead space on idiopathic central sleep apnea. *J Appl Physiol* 82: 918-26, 1997.

150. Xie, A., R. Rutherford, F. Rankin, B. Wong, and T. D. Bradley. Hypocapnia and increased ventilatory responsiveness in patients with idiopathic central sleep apnea. *Am J Respir Crit Care Med* 152: 1950-5, 1995.

151. Xie, A., B. Wong, E. A. Phillipson, A. S. Slutsky, and T. D. Bradley. Interaction of hyperventilation and arousal in the pathogenesis of idiopathic central sleep apnea. *Am J Respir Crit Care Med* 150: 489-95, 1994.

152. Younes, M. Apnea following mechanical ventilation may not be caused by neuromechanical influences. *Am J Respir Crit Care Med* 163: 1298-301, 2001.

153. Younes, M. The physiological basis of central apnea and periodic breathing. *Curr Pulmonol* 10: 265-326, 1989.

154. Younes, M., M. Ostrowski, W. Thompson, C. Leslie, and W. Shewchuk. Chemical control stability in patients with obstructive sleep apnea. *Am J Respir Crit Care Med* 163: 1181-90, 2001.

155. Young, T. Analytic epidemiology studies of sleep disordered breathing--what explains the gender difference in sleep disordered breathing? *Sleep* 16: S1-2, 1993.

156. Young, T., J. Blustein, L. Finn, and M. Palta. Sleep-disordered breathing and motor vehicle accidents in a population- based sample of employed adults. *Sleep* 20: 608-13, 1997.

157. Young, T., and L. Finn. Epidemiological insights into the public health burden of sleep disordered breathing: sex differences in survival among sleep clinic patients. *Thorax* 53: S16-9, 1998.

158. Young, T., R. Hutton, L. Finn, S. Badr, and M. Palta. The gender bias in sleep apnea diagnosis. Are women missed because they have different symptoms? *Arch Intern Med* 156: 2445-51, 1996.

159. Young, T., M. Palta, J. Dempsey, J. Skatrud, S. Weber, and S. Badr. The occurrence of sleep-disordered breathing among middle-aged adults. *New Engl J Med* 328: 1230-5, 1993.

160. Young, T., P. Peppard, M. Palta, K. M. Hla, L. Finn, B. Morgan, and J. Skatrud. Population-based study of sleep-disordered breathing as a risk factor for hypertension. *Arch Intern Med* 157: 1746-52, 1997.

161. Young, T., E. Shahar, F. J. Nieto, S. Redline, A. B. Newman, D. J. Gottlieb, J. A. Walsleben, L. Finn, P. Enright, and J. M. Samet. Predictors of sleep-disordered breathing in community-dwelling adults: the Sleep Heart Health Study. *Arch Intern Med* 162: 893-900, 2002.

162. Zabka, A. G., M. Behan, and G. S. Mitchell. Selected contribution: Time-dependent hypoxic respiratory responses in female rats are influenced by age and by the estrus cycle. *J Appl Physiol* 91: 2831-8, 2001.

163. Zabka, A. G., M. Behan, and G. S. Mitchell. Serotonin-dependent long-term facilitation of respiratory motor output decreased with age in male rats. *J Physiol (Lond)* 531: 509-514, 2001.

164. Zhou, X. S., S. Shahabuddin, B. R. Zahn, M. A. Babcock, and M. S. Badr. Effect of gender on the development of hypocapnic apnea/hypopnea during NREM sleep. *J Appl Physiol* 89: 192-199, 2000.