

The Role of Surface Tension of Upper Airway
Lining Liquid and Breathing Route in
Sleep Disordered Breathing

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Preface

The work described in this thesis was carried out in the Ludwig Engel Centre for Respiratory Research, Westmead Millennium Institute, The University of Sydney at Westmead Hospital under the supervision of Professor John Wheatley and Associate Professor Terence Amis. The submission of this thesis fulfils the requirements for the Degree of Doctor of Philosophy.

I am entirely responsible for the work presented in this thesis, although it would not have been possible without the assistance of many people. Most of the work in this thesis has been previously presented at the annual scientific meetings of The Australasian Sleep Association and the American Thoracic Society.

Dedication

This work is dedicated to my wife, Jennifer, and my children, Lucinda and Xavier

Acknowledgements

Completing a PhD is never done without the help and encouragement of others. I have been fortunate to be surrounded by friends and colleagues who have helped and encouraged me along the way. I am grateful for the opportunities given to me to present my data at multiple local and overseas conferences on behalf of the Ludwig Engel Centre for Respiratory Research and the University of Sydney.

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Finally, I would like to thank my wife, Jennifer, and my children Lucinda and Xavier. Without their love and support this work would not have been possible.

Abstract

This thesis examines the relationship between surface tension in the upper airway liquid, breathing route, and sleep disordered breathing. Although anatomical factors play the predominant role in sleep disordered breathing, surface tension forces may influence upper airway patency.

The range of surface tension values for upper airway liquid was examined by collection of upper airway liquid samples and salivary samples across the day for healthy subjects. This demonstrated that surface tension values for upper airway liquid were relatively stable across the day for healthy subjects, but depending on the time of day, surface tension of saliva may not accurately reflect the surface tension of upper airway liquid, although values for both were similar.

Following on from the study examining the range of surface tension values for healthy subjects, evening samples of upper airway liquid and saliva were collected, and surface tension values examined in subjects with obstructive sleep apnoea. The results demonstrated a wide range of values in these subjects, in addition to a positive association between older subjects with severe OSA and increased surface tension in upper airway liquid.

The relationship between uncontrolled route of breathing and surface tension was examined in subjects with severe obstructive sleep apnoea. Nasal route of breathing occurred in all subjects, and was the predominant breathing route, while oronasal breathing route was observed in most subjects. There was no association found between breathing route and the surface tension of upper airway liquid in this study, however, breathing route was not enforced.

Enforced oral breathing route in healthy subjects was shown to induce sleep disordered breathing. Two separate sleep study nights were undertaken, one with enforced nasal breathing route and one with enforced oral breathing route. Enforced oral breathing route not only induced sleep disordered breathing, but also increased the surface tension of upper airway liquid.

To determine whether the enforced oral breathing route induced sleep disordered breathing via surface tension mediated mechanisms, these healthy subjects were given exogenous surfactant with enforced oral route of breathing during sleep. Lowering the surface tension of upper airways lining liquid reduced, but did not eliminate oral breathing induced sleep disordered breathing. Therefore oral breathing induced sleep disordered breathing in healthy subjects appears to be partially mediated by increases in surface tension of upper airway liquid.

Finally, exogenous surfactant and normal saline were administered to subjects with mild to moderate obstructive sleep apnoea on separate nights, following an initial diagnostic sleep study confirming the presence of mild to moderate obstructive sleep apnoea. Although a reduction in upper airway surface tension was seen on the exogenous surfactant night, there was no associated reduction in the severity of obstructive sleep apnoea. Individual subjects demonstrated a substantial variability in the severity of obstructive sleep apnoea between the initial diagnostic sleep study, the normal saline night and the exogenous surfactant night. This study concluded that although exogenous surfactant resulted in a decrease in surface tension of upper airway liquid, the variable response in sleep apnoea severity suggests that a suitable patient phenotype may need to be defined for exogenous surfactant therapy.

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List of Abbreviations

AASM	American Academy of Sleep Medicine
AHI	Apnoea – Hypopnoea Index
AI	Arousal Index
ANOVA	Analysis of Variance
ASDA	American Sleep Disorders Association
BMI	Body Mass Index
CPAP	Continuous Positive Airway Pressure
CV	Coefficient of Variation
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electroocculomyogram
ES	Exogenous Surfactant
m	Metre
mg	Milligram
min	Minute
MW	Mucosal Wetness
nM	Newton Metre
NS	Normal Saline
OSA	Obstructive Sleep Apnoea

Pcrit	Upper Airway Critical Closing Pressure
PSG	Polysomnography
RDI	Respiratory Disturbance Index
REM	Rapid Eye Movement
s	Second
SD	Standard Deviation
SDB	Sleep Disordered Breathing
SEM	Standard Error of the Mean
SpO2	Oxygen Saturation via Pulse Oximetry
ST	Surface Tension
STUAL	Surface Tension Upper Airway Liquid
TSE	Total Sleep Epochs
TST	Total Sleep Time
UAL	Upper Airway Liquid

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Publications and Abstracts

Chien J, Verma M, Fish V, Wheatley J, Amis T. Daytime Variation in the Surface Tension (ST) of Upper Airway Lining Liquid (UAL) in Healthy Awake Subjects. *Sleep and Biological Rhythms*, Vol 5 Supp 1, A81 (2007)

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Chien J, Lee S, Verma M, Wheatley J, Amis T. Surface Tension of Upper Airway Lining Liquid in Patients with Obstructive Sleep Apnoea Hypopnoea Syndrome (OSAHS). *Am. J. Respir. Crit. Care Med.*, May 2007, Vol 175, A274

Chien J, Verma M, Tyler L, Wheatley J, Amis T. Enforced Oral Breathing Induces Sleep Apnoea, and Increases Surface Tension in Upper Airway Lining Liquid in Healthy Subjects. Abstract 924. American Thoracic Society International Conference, New Orleans, 2010.

Chien J, Verma M, Tyler L, Wheatley J, Amis T. Oral Breathing (OB) Route Induced Sleep Disordered Breathing (OSDB) Is Not Primarily Mediated By Effects On Surface Tension Of Upper Airway Lining Liquid (STUAL). Abstract 16392. American Thoracic Society International Conference, Denver, 2011

Chapter 1: General Introduction

Sleep disordered breathing encompasses a wide spectrum of sleep related breathing abnormalities, and includes snoring and obstructive sleep apnoea (OSA). The basis of sleep related breathing abnormalities are due to a number of pathophysiological factors. The recurrent narrowing, airflow limitation, and closure of the upper airway during sleep may be due to a number of factors. These include structural defects and anatomical narrowing due to bony structures , changes in soft tissue mass compromising or collapsing the upper airway, inadequate neuromuscular responses from upper airway dilating muscles, abnormal arousal responses (or threshold to wake up) due to upper airway narrowing, abnormalities in the sensitivity of the ventilatory control system leading to ventilatory instability, and more recently, surface tension of the liquid on mucosal surfaces of the upper airway has been recognised as a factor influencing upper airway patency.

Previous work done in our laboratory by Kirkness et al, has examined the role of surface tension of liquid lining the upper airway in obstructive sleep apnoea (Kirkness et al., 2003c). A new method of measuring surface tension was devised call the “Pull Off Force” Technique and this involved measuring the force required to separate two silica discs bridged by a microliter volume of the liquid being examined. The force required to result in disc separation was taken as the surface tension of the liquid measured

(Kirkness et al., 2005a). Previous studies have examined the influence of exogenous surfactant agents on the patency of the upper airway in humans (Van der Touw et al., 1997, Morrell et al., 2002), and the series of experiments undertaken by Kirkness et al extensively described the measurement of surface tension forces, and the effect of instilled exogenous surfactant on lowering surface tension, and the influence of this lowered surface tension on upper airway patency (Kirkness et al., 2003a, Kirkness et al., 2003b, Kirkness et al., 2005b).

An animal model using anaesthetized rabbits demonstrated that surfactant administered into the upper airway lowered the surface tension of the upper airway liquid (STUAL) and this correlated strongly with decreases in upper airway opening and closing pressures (Kirkness et al., 2003a).

A similar study was performed in anaesthetised human subjects and this study similarly demonstrated that a decrease in STUAL using instilled exogenous surfactant resulted in decreases in upper airway resistance, opening pressures and closing pressures. The authors concluded that lowering the STUAL with exogenous surfactant into the upper airway increases the upper airway patency and augments reopening of a collapsed airway (Kirkness et al., 2003b).

Finally, Kirkness and his co-workers instilled exogenous surfactant into the upper airway of subjects with OSA. This demonstrated that instilled exogenous surfactant lowered the STUAL and reduced the severity of OSA,

leading the authors to conclude that manipulation of surface tension may be a potential therapeutic intervention for sleep disordered breathing (Kirkness et al., 2005b).

Further studies carried out in our laboratory have demonstrated oral route of breathing increases STUAL in awake subjects, with significant increases in STUAL seen following 2 hours of enforced oral route of breathing (Verma et al., 2006). Furthermore, oral route of breathing is observed to occur during sleep (Madronio et al., 2004). Hence, changes in route of breathing during sleep may provide a mechanism where alterations in STUAL with breathing route may potentially influence the severity of sleep disordered breathing.

This thesis will explore the role of STUAL and breathing route in sleep disordered breathing. Chapters 2 - 3 represents a literature review on the anatomical and pathophysiological causes of upper airways collapse in sleep disordered breathing, in addition to the role of surface tension forces and the measurement of STUAL. Chapter 4 will review the literature regarding Obstructive Sleep Apnoea, the characteristics, epidemiology, burden of disease, and current therapeutic options. Previous literature examining the role of surface tension forces in obstructive sleep apnoea will also be reviewed here.

The experimental chapters 5 and 6 will describe the STUAL in healthy subjects and subjects with OSA. Chapters 7 to 9 will investigate the role of

enforced and non-enforced breathing route, with and without exogenous surfactant, on the severity of sleep disordered breathing. Chapter 10 is a clinical trial investigating the effect of exogenous surfactant on sleep disordered breathing in patients with mild to moderate obstructive sleep apnoea.

Chapter 2: Upper Airway Anatomy

The upper airway includes the anatomical structures from the nares and oral cavity, and caudally to the point where the airway divides into the laryngeal and oesophageal structures. The nose is comprised of the external nasal structures and the nasal cavity.

Externally, the nares are supported by cartilage, fibrous connective tissue, and the levator labii superioris alaeque nasi provide muscular dilatation. The cavity of the nose is separated by a nasal septum. On each side there is a roof, floor, medial and lateral wall. The medial wall is formed by the nasal septum. On each side there is an inferior, middle and superior turbinate that increases surface area to facilitate humidification and warming of inspired air. Posterior to this, the divided nasal cavities on each side meet to form the airway lumen of the nasopharynx. The Eustachian tubes originate from the nasopharynx and the origin is located in the lateral nasopharyngeal walls.

The velopharynx is the portion of upper airway bounded by the soft palate anteriorly, and the lateral and posterior pharyngeal walls. This joins with the oral cavity at the oropharynx. The mouth and lips form the opening to the oral cavity. Superiorly, the hard palate forms the roof, and the bony hard palate extends posteriorly until it forms the muscular soft palate, ending in a small, posterior, central conical projection of tissue called the uvula.

The tongue forms the floor of the oral cavity. The posterior boundary of the oral cavity is formed by the palatoglossal arch, which joins the lateral walls of the soft palate to the tongue laterally, and distal to this is the oropharynx. Here, the oropharynx divides into two regions, the velopharynx or retropalatal oropharynx, which extends from the hard palate to the caudal margin of the soft palate, and the retroglossal oropharynx. The hypopharynx extends from the tip of the epiglottis to the inferior border of the cricoid cartilage (Figure 2.1).

The pharyngeal muscles that are important in maintaining patency can be divided into anatomical/ functional groups and include the extrinsic muscles of the tongue, the muscles controlling palate shape and function, the muscles influencing hyoid bone position, and the pharyngeal constrictor muscles.

The muscles of the tongue include the Genioglossus which protrudes and depresses the tongue, the Styloglossus, which raises and retracts the tongue, while the Hyoglossus depresses and retracts the tongue. Activation of the Genioglossus widens the oropharynx in the anterior-posterior dimension and has a major role in opening the pharyngeal airway.

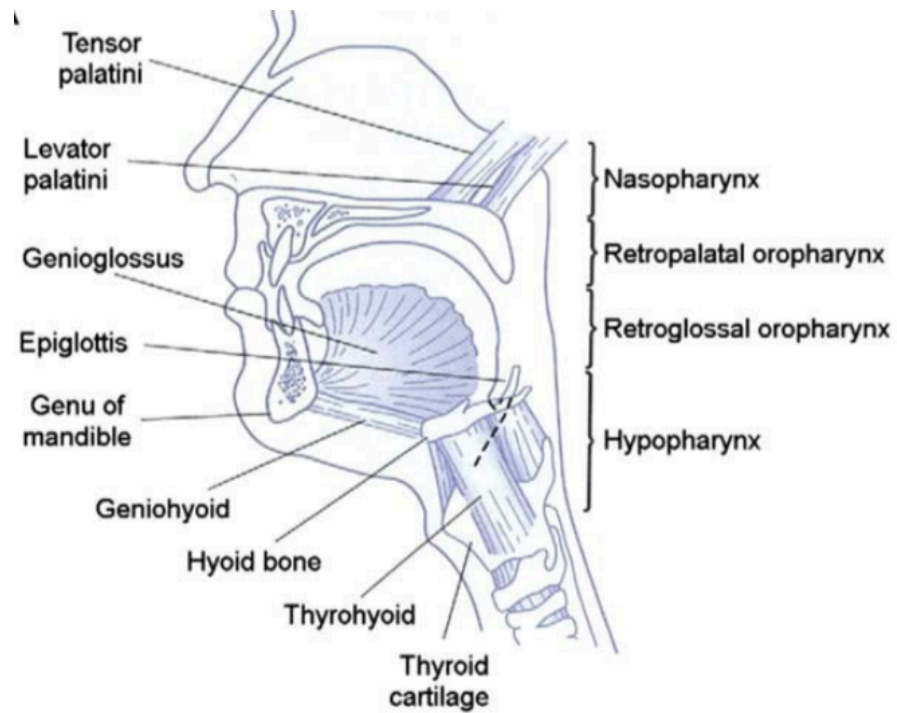


Figure 2.1 Mid sagittal view of the anatomy and musculature of the upper airway

This demonstrates the 4 subdivisions of the upper airway which include the nasopharynx, the retropalatal oropharynx or velopharynx, the retroglottal oropharynx, and the hypopharynx. Adapted from Kryger, Roth and Dement 2005.

Muscles responsible for palatal shape and position include the Tensor Palatini, which tenses the soft palate, Palatoglossus, which elevates and pulls the palate onto the posterior tongue, the Palatopharyngeus which lifts the pharyngeal wall and moves the soft palate anteriorly, and the Musculus Uvulae, which raises the uvula.

The hyoid bone position is influenced by insertion and contraction of multiple muscles including the Geniohyoid, Mylohyoid, Thyrohyoid, Sternohyoid, Stylohyoid, and Omohyoid muscles. These work to elevate, depress, or to stabilise the Hyoid bone. The pharyngeal constrictor muscles consist of the Superior, Middle, and Inferior Constrictor muscles which have origins in the skull, mandible, hyoid, and larynx. They insert into various structures in the anterior pharynx including the tongue, and at high lung volumes can act as constrictors while at lower lung volumes dilate to maintain airway patency.

Chapter 3: Upper Airway Physiology and Airway Closure

3.1 Functions of the Upper Airway

In humans, the pharynx serves as a conduit for airflow from the nose and mouth to the larynx. It also serves other functions including secretory, phonation, and swallowing. Its patency during wakefulness is due to continual neuromuscular control by the higher nervous system which supervises the action of the pharyngeal muscles and ensures a patent pharynx. During sleep, this pharyngeal control is altered and may compromise airway patency. When the neurological changes in sleep occur in the presence of anatomical abnormalities, severe narrowing or closure may occur, leading to sleep hypopnoea or apnoea. The pharynx consists of four anatomical subsegments, the nasopharynx, velopharynx, oropharynx, and hypopharynx.

During inspiration, pharyngeal structures are pulled inward by sub atmospheric luminal pressure. The pharyngeal structures return to resting position during expiration. The pharyngeal muscles receive phasic activation during inspiration which promotes patency of the pharyngeal lumen. The most important dilator muscles in the upper airway promoting airways patency include the genioglossus, levator palatini, and hyoid muscles. The tensor palatini enhances the patency of the most rostral part of the airway,

while activation of the genioglossus muscle increases the stiffness of the mid portions of the pharynx, with the hyoid muscles maintain patency of the caudal portion of the pharynx, and also pulls the epiglottis anteriorly/ventrally.

In the upper airway, the anatomical and neurological interactions that allow it to change shape and calibre to enable functions such as swallowing and phonation, also makes it vulnerable to collapse, due to alterations in the anatomical structures that make up non-rigid and collapsible portions , and changes in neurological input particularly during the sleep state.

3.2 Anatomical Box Model of the Upper Airway

The propensity for the upper airway to collapse may be determined by several interacting anatomical factors. The size of the upper airway boundary, or “bony enclosure”, the amount of soft tissue within this enclosure, and the intraluminal pressure within the airway surrounded by soft tissue, may interact to determine airway collapsibility (Watanabe et al., 2002). An alteration in any of these factors i.e. a small bony enclosure, an increase in extraluminal soft tissue, and a reduction in intraluminal pressure may lead to increased collapsibility of the upper airway (Figure 3.1).

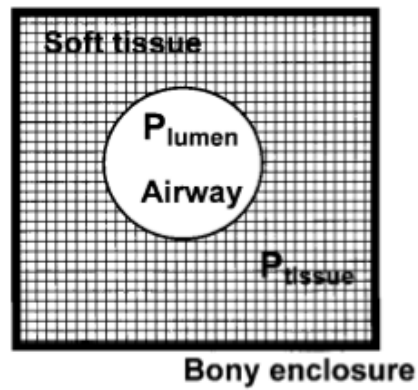


Figure 3.1 Mechanical model of the pharyngeal airway

The size of the pharyngeal lumen (circle labelled "Airway") can be viewed as being dependent on the size of the bony enclosure (box with solid line) and the amount of soft tissue within it (shaded grid within the box). Adapted from Watanabe et al. 2003.

Upper airway collapse in OSA has been associated with anatomical factors such as tonsillar enlargement, retrognathia, and facial bone abnormalities/ craniofacial structure (Miyazaki et al., 1989). These factors lead to a smaller luminal area in the upper airway which predisposes upper airway collapse.

Similarly, head position and mandible position may influence the collapsibility of the upper airway by potentially altering the “bony enclosure” size. Neck flexion and bite opening decreases the size and increased collapsibility of the velopharynx and oropharynx while neck extension increases the oropharyngeal airway size (Isono et al., 2004).

Obesity leads to increased fat deposition in the tongue, parapharyngeal fat pads, lateral pharyngeal walls and soft palate. This again, leads to a smaller pharyngeal luminal area, with greater predisposition to collapse (Welch et al., 2002). However, weight loss can reverse these anatomical changes and improve OSA severity (Sampol et al., 1998).

3.3 Balance of Forces Concept

This concept proposes that the size of the pharyngeal lumen is dependent on a balance between the outward forces produced by contracting muscles, and the inward collapsing forces from extraluminal tissue pressure, and subatmospheric intraluminal pressures during inspiration (Remmers, 1978).

Intraluminal Pressure

The collapsing force of negative pressure generated by the diaphragm would be transmitted to the upper airway, causing a narrowing of the airway, the degree of which would be dependent on the compliance of the airway wall as well as the opposing dilating forces from pharyngeal muscles.

However, this mechanism does not explain the pathogenesis of airways obstruction in the presence of positive intraluminal pressures. Schneider et al showed that dynamic changes in respiratory phase and airflow could modulate upper airway collapsibility (Schneider et al., 2002).

Extraluminal Tissue Pressure

The pharynx can be viewed as a collapsible tube. Extraluminal tissue pressure (ETP) exerts a collapsing force upon the pharyngeal airway that is dependent upon the amount of soft tissue within an “anatomical box” bound by the mandible and spinal column (Isono et al, Watanabe et al). Moreover, upper airway collapsibility can vary with mandibular size and position, and

the opening and closing pressures in the upper airway may be manipulated by change in mandibular position (Kairaitis et al., 2007).

Extraluminal tissue pressure alters the transmural pressure and hence can influence the degree of airway collapse. The transmural pressure is the difference between the intraluminal airway pressure and the extraluminal tissue pressure. The transmural pressure determines the degree of airways collapse. A positive transmural pressure maintains upper airway patency, while a negative transmural pressure (e.g. by applying subatmospheric intraluminal pressure) promotes airways collapse(Schwartz et al., 2006). In OSA patients, the airway can occlude at above atmospheric pressures, suggesting a higher extraluminal tissue pressure in OSA patients contributing to airways collapse (Smith et al, 1988).

Reduction in ETP by mandibular advancement in a rabbit model results in a reduction in extraluminal tissue pressures, while mouth opening, which can reduce the size of the bony enclosure and causes compression of extraluminal tissues by the mandible, is associated with an increase in upper airway collapsibility. Mandibular advancement results in the opposite, with a decrease in lateral peripharyngeal tissue pressure, and an improvement in airway patency (Kairaitis et al., 2006).

3.4 The Starling Resistor model of the Upper Airway

The pharyngeal airway can be described as a Starling resistor because the pressure flow dynamics are the same as in other collapsible biological conduits (Pride et al., 1967). The term is used to describe a highly collapsible tube having infinite compliance, changing from completely open to completely closed with little change in intraluminal pressure near that at which complete collapse occurs. The luminal pressure at which the airway shifts from fully open to fully closed is determined by the extramural pressure and is known as the critical pressure (P_{crit}). P_{crit} can be extrapolated by varying upstream pressure (for example by CPAP) under conditions of inspiratory flow limitation, and by monitoring pressure associated with zero flow. The model describes the conditions where changes in the pressure flow profile can lead to upper airway occlusion (Figure 3.2).

3.5 Lung Volume

Lung volume may interact with the upper airway to influence patency and collapsibility. Changes in lung volume can influence upper airway patency. Caudal traction exerts longitudinal tension on all components of the pharyngeal wall to reduce the propensity for upper airway collapse (Kairaitis et al., 2007, Schwartz et al., 2006). Decreased lung volumes may lead rostral movement of the diaphragm and thorax, and a loss of caudal traction hence increasing upper airway collapsibility (Figure 3.3).

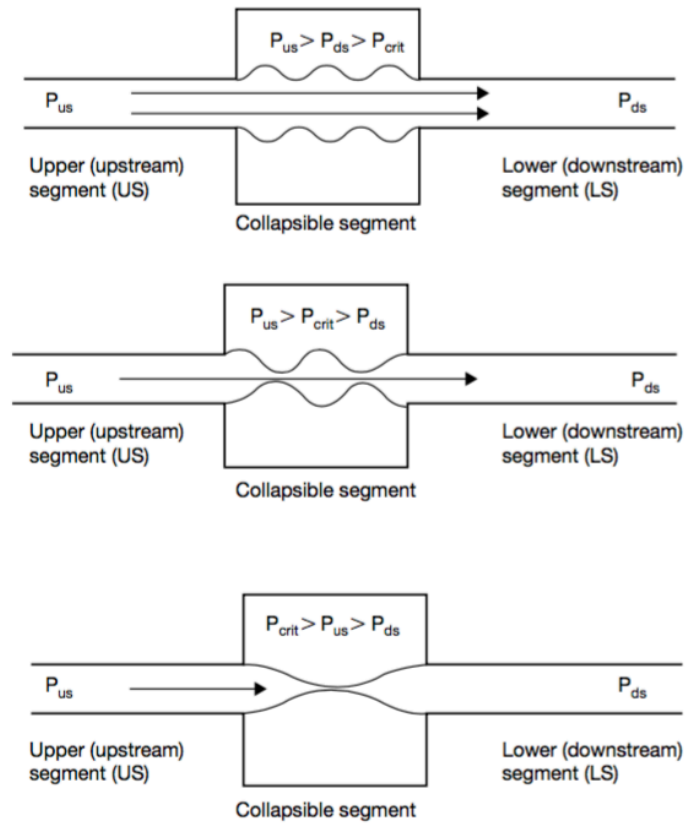


Figure 3.2 Starling Resistor Model of the Upper Airway

In this model, the airway can be represented as a rigid tube with a collapsible segment. Pressures in the fixed, rigid upstream and downstream segments are represented by P_{us} and P_{ds} respectively. The collapsible segment is subject to the surrounding pressure, and the pressure required to collapse it is represented by P_{crit} . Normal breathing occurs when $P_{us} > P_{ds} > P_{crit}$. When P_{crit} is greater than the downstream pressure, collapse occurs in the upper airway, but airflow still occurs. However, when the P_{crit} exceeds both the upstream and downstream pressures, complete occlusion of the airway occurs, with no airflow. Adapted from Kirkness et al. 2006

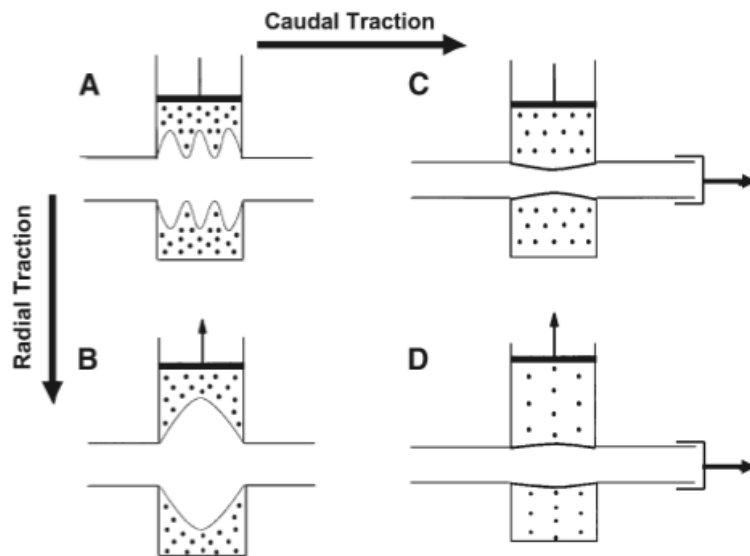


Figure 3.3 Tracheal traction and airway patency

Effects of caudal and radial traction on mucosal tension and extraluminal tissue pressure. Caudal traction can “stiffen” the airway wall via increased mucosal tension whilst radial traction leads to decompression of surrounding soft tissues. Adapted from Schwartz et al. 2006.

3.6 Neuromuscular and Neuroventilatory Factors

The upper airway's patency is strongly influenced by the dilating function of the pharyngeal musculature. A reduction in pharyngeal muscle activity during sleep induces pharyngeal narrowing and is a significant mechanism in the pathogenesis of OSA. The largest pharyngeal dilator muscle in humans is the genioglossus. In most animals, tracheal obstruction/occlusion or subatmospheric pressures causes a neurologically mediated activation of the pharyngeal muscles. In addition, neuromuscular reflexes are present in upper airway cause pharyngeal muscle dilation with inspiration and prevent upper airway closure.

Subjects with Obstructive Sleep Apnoea have increased EMG and genioglossus activity in wakefulness (Mezzanotte et al., 1992), but a blunting of this activity and reflexes during sleep may predispose to pharyngeal airway collapse (Wheatley et al., 1993). Weiner et al demonstrated that upper airway reflexes also occurred in the presence of chemical stimuli in the upper airways. Increased activity was observed in the phrenic, hypoglossal, and pharyngeal nerves of dogs when hypoxia or hypercapnia was induced in anaesthetised, paralysed dogs, in addition to the increased activity seen with inspiration (Weiner et al., 1982).

Recently, single motor unit recording techniques have been used to determine the activity of individual motor units in the genioglossus muscle, and its role in OSA (Bailey et al., 2007). A better understanding may potentially lead to therapies targeting neuromuscular activity, thereby reducing the severity of OSA in some patients. However, due to the multifactorial influences on upper airway patency, modifying upper airways neuromuscular output alone may not lead to an improvement for all OSA patients. Interestingly, Puhan et al demonstrated that upper airway muscle training with 4 months of didgeridoo playing reduced the severity of sleep disordered breathing in OSA patients (Puhan et al., 2006), suggesting a training effect in strengthening or improving upper airway muscle tone may decrease the propensity for the upper airway to collapse during sleep.

3.7 Route of Breathing

Nasal, oral, and oronasal breathing may occur overnight during sleep. Oronasal breathing in sleep may be increased in older individuals (Madronio et al., 2004). Oral route of breathing leads to increased upper airways resistance and increases the severity of sleep disordered breathing (Fitzpatrick et al., 2003). The mechanism may be due to changes in upper airways geometry with the open jaw position, and possibly related changes in length/ tension relationships of upper airway muscles with an open jaw position. However, even though there is a relationship between the increase in tendency to sleep apnoea and nasal obstruction, this may not necessarily be due to mouth breathing, but may be due to a component of continued nasal breathing through a higher resistance nasal airway.

Chronic nasal obstruction may play a role in upper airway collapse in OSA by reducing intra luminal pressure, thereby predisposing to upper airway vibration (snoring) and partial collapse, resembling a Starling resistor with flow limitation. In patients enrolled in the Wisconsin sleep cohort study, nocturnal nasal congestion frequency was associated with increased snoring frequency (Young et al., 2001). There is some evidence that improving nasal breathing during sleep by medical intervention, for example with nasal steroids for rhinitis, and surgical therapy for correction of turbinate hypertrophy, septal deviation, and nasal polyposis , may reduced nasal resistance and reduce the severity of sleep disordered breathing (Kohler et al., 2007).

In patients with allergic rhinitis, treating severe nasal obstruction results in reduced nasal resistance, reduced oral breathing during sleep, and a reduction in the severity of obstructive sleep apnoea (McLean et al., 2005).

The effect of bite opening and mandibular position has also been studied and may contribute to upper airway collapse. Isono et al 2004 described the influence of head posture and jaw position on upper airway collapsibility (Isono et al., 2004), while time spent with an open mouth during sleep was greater in patients with OSA than in normal subjects (Miyamoto et al., 1999). Mouth opening changes mandibular position, which may cause i) posterior displacement of the tongue at the velopharynx, increasing the amount of soft tissue enclosed by the maxilla and ii) reduce the size of the “bony box” due to posterior displacement of the mandible.

An increased propensity to upper airway collapse may also occur in oral breathing due to changes in surface tension with route of breathing. Enforced oral route of breathing in awake subjects during daytime demonstrated an increase in surface tension after 2 hours of enforced oral breathing from 64.4 ± 2.7 mN/m to 77.4 ± 1.1 mN/m (Verma et al., 2006). The authors concluded that an enforced oral breathing route may impede the maintenance of a low surface tension in the upper airway lining liquid. The implication of this is oral breathing and the associated increase in surface tension may increase the severity of sleep disordered breathing.

3.8 The Upper Airway Mucosal Surface

Surface tension is defined as the force exerted across the surface of a liquid at a liquid - gas interface. This is caused by the attraction of molecules of the liquid. Liquid molecules are pulled equally by other liquid molecules. At the surface of the liquid (at the liquid-gas interface), the surface liquid molecules are pulled inwards towards the centre of the liquid. This inward pull acts to decrease the surface area, and hence the surface of the liquid acts as a tensed elastic membrane. (An example is a drop of water, where a smallest possible surface area causes it to assume a characteristic spherical shape). The interface between a liquid and a solid wall is also subject to surface tension forces, an example being the meniscus (contact angle) seen at the glass/ water interface.

Pulmonary Surfactant is a naturally occurring surface active phospholipid which exerts surface active forces in the alveoli, lower airways and upper airways.

Pulmonary surfactant contains 90% lipids and approximately 10% protein, of which half has surfactant properties with the rest being contaminant from lung tissue or plasma (Halliday, 2005).

The main surfactant phospholipid in the respiratory system is dipalmitoylphosphatidylcholine, secreted from phospholipid lamellar bodies in type II alveolar cells. Similar cells producing surfactant have also been found in upper airways (Bradford, 2005), suggesting a possible physiological role in

the upper airway. The hydrophilic head group remains on the liquid side of the interface while the hydrophobic tails extend into the gas. In the alveolus, surfactant serves to reduce surface tension forces that would otherwise collapse alveoli, thereby maintaining alveolar size and improving lung compliance (West).

Surfactants (surface acting agents) lower the surface tension at the liquid-gas interface. These agents are described as being amphipathic, with both a polar and non polar portion (or a hydrophilic and hydrophobic portion).

Four surfactant proteins have been identified: SP-A, SP-B, SP-C, and SP-D. SP-B and SP-C have surface tension lowering effects with SP-B having secondary effects on surfactant protein synthesis. SP-C is important in facilitating film formation, promoting film stability and respreading during compression and expansion that occurs with respiration. SP-A and SP-D both have immunological roles in host defence against micro-organism invasion (Halliday, 2005, Griese, 1999).

The study of pulmonary surfactant arose from research into neonatal respiratory distress syndrome, previously known as hyaline membrane disease. Prior to surfactant replacement therapy, premature infants who died from this condition were found to have collapsed alveoli, with respiratory surfaces having increased adhesiveness. There was no effective treatment until exogenous surfactant was instilled into neonatal airways (Fujiwara and Adams, 1980).

The role of exogenous surfactant in the treatment of neonatal respiratory distress syndrome by stabilising alveolar surface tension, and preventing alveolar collapse is now well established (Raghavendran et al., 2011).

It is also becoming clear that surface effects in the upper airway play a role in airways collapse. In the upper airways, surface tension is a force that contributes to airways closure by modulating the opening and closing pressures. The increased “stickiness” of the mucosal surface needs to be overcome in a collapsed airway to facilitate airway re-opening and is the reason opening pressures are greater than closing pressures.

Surfactant lamellar bodies similar to those found in type II pneumocytes, are also present in sinus mucosa (Bradford, 2005) indicating a source of surfactant production in the upper airways mucosa.

Human saliva has surface active properties and exhibits a lower surface tension than water, the primary component of saliva. Human saliva has a mean surface tension of approximately 53.1 to 57 mN/m (Braddock et al., 1970) with values from more recent data demonstrating a surface tension of 59 mN/m (Kirkness et al., 2005b), with the surfactant effect due to surface active phospholipids present in saliva, while water exhibits a surface tension of 71 mN/m.

Previous investigators (Reed et al., 1985, Wilson et al., 1980) reported collapsed airways in post-mortem studies in infant cadavers, as well as in living infants, showing that airways re-opening required a greater pressure than that for airways collapse.

A link between surface tension of upper airways and airways obstruction was first described in a study investigating the effects of upper airway lubrication and surface active agents on the opening of the obstructed airway in dogs (Widdicombe and Davies, 1988).

Surface active agents (surfactant) instilled into the upper airways lower the opening pressures in the upper airway (Kirkness et al., 2003b, Kirkness et al., 2003a, Morrell et al., 2002). It has therefore been proposed that intraluminal surface tension and adhesive forces play a role in upper airways patency, and that modulating the surface tension in the upper airway may change patency, and opening and closing pressures (Van der Touw et al., 1997).

The usual range for surface tension in normal subjects ranges from 52 to 59 mN/m with nasal breathing. This increases to 64 to 77mN/m with enforced oral breathing suggesting that oral breathing may lead to loss/evaporation of the lower surface tension liquid layer in the upper airway (Kirkness et al., 2005b). Subsequent studies in OSA patients have shown that the reference range for STUAL is similar in OSA patients, ranging from 50 to 72 mN/m in both saliva and UAL. The STUAL and ST saliva also

correlate well suggesting the fluid lining the upper airway is very similar to swallowed saliva. Possible explanations accounting for small differences may be postnasal drip/ nasal sinus secretions and possibly even refluxed surfactant containing fluid from the lower respiratory tract.

In a series of studies, Kirkness firstly developed a new method of measuring surface tension in microliter volumes of fluid collected from the upper airway using the “Pull-off force” technique (Kirkness et al., 2005a). The apparatus consists of two , cylindrically polished silica discs, with the top disc mounted on a stable platform, with the lower disc mounted on a double cantilever spring (Figures 3.4 and 3.5).

The surface tension of microliter volumes of liquid could be measured by this method. Microliter volumes (approximately 0.2 microliters) of the liquid sample were placed on the lower silica disc, which was then raised up until the two silica discs met with the liquid bridging the two silica discs. The lower silica disc was mounted to the double cantilever spring, which was lowered via the micrometer screw gauge driven by the DC motor. The force required to separate the two silica discs bridged by the liquid was measured and the surface tension calculated from this.

In a rabbit model, Kirkness et al demonstrated that upper airway opening and closing pressures were affected by changes in surface tension. When saline was applied to the upper airway, the increased surface tension resulted in an increase in both opening and closing pressures, while reducing

the surface tension by applying exogenous surfactant reduced both the opening and closing pressures. The surface tension of liquid lining the upper airway, is hence a factor which may modulate upper airway collapsibility and re-opening, particularly airway walls that are in close apposition (Kirkness et al., 2003a).

Exogenous surfactant was studied by Kirkness et al in an anaesthetised human model (Kirkness et al., 2003b). This demonstrated a reduction in STUAL when exogenous surfactant was instilled into the upper airway, with the reduction in surface tension from approximately 62mN/m to 50mN/m. The reduction in STUAL also correlated with reductions in upper airway resistance and opening pressures (Figure 3.6).



Figure 3.4 “Pull-off” force apparatus

(Refer to schematic in Figure 3.5).

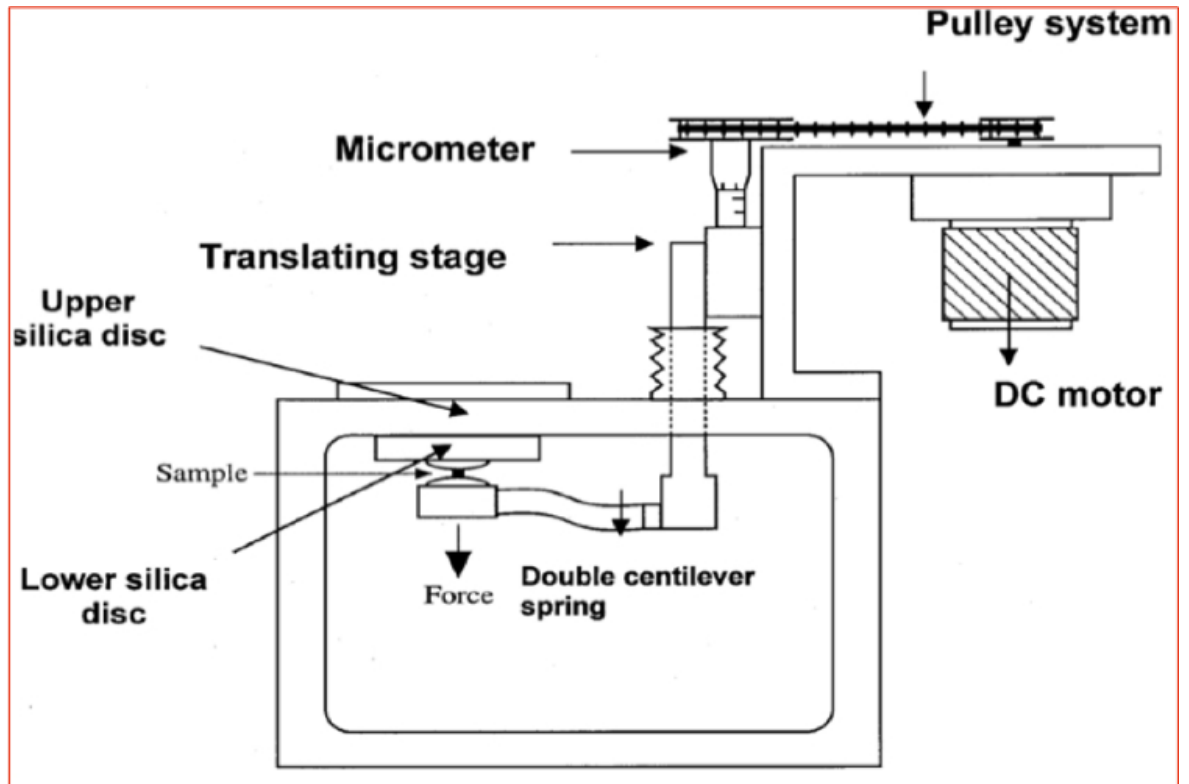


Figure 3.5 Schematic diagram of the “pull-off” force apparatus

“Pull off force” technique device used for measuring Surface Tension in micro litre volumes. The fluid sample is placed between 2 silica discs as a fluid “bridge”. The DC motor is connected to a micrometer and translating stage which moves the double cantilever spring downwards. The force required to separate the discs was taken to be the surface tension of the liquid.

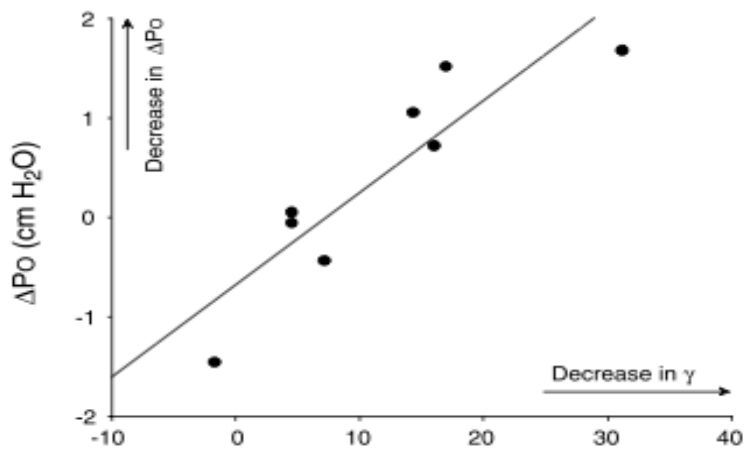
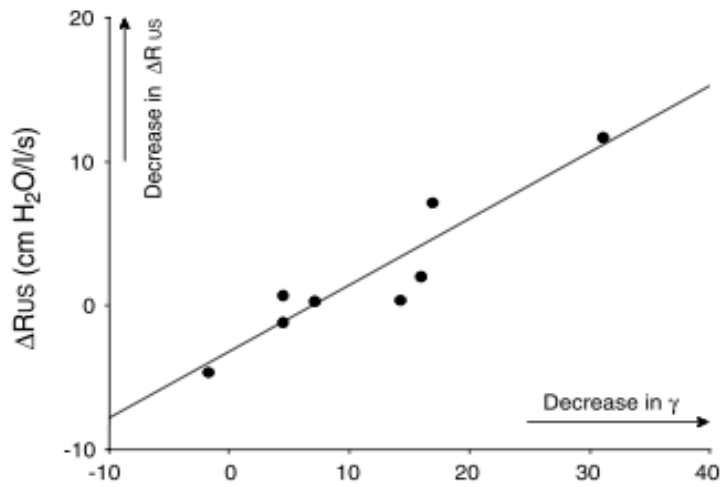


Figure 3.6 STUAL, upper airway re-opening pressure, and upper airways resistance

Effect of changing STUAL on upper airway reopening pressure and upper airways resistance. A decrease in STUAL resulted in a reduction in both re-opening pressure and upper airways resistance. Adapted from Kirkness et al 2003.

The surface tension in upper airway lining liquid may also be manipulated with exogenous surfactant to decrease the severity of sleep disordered breathing in patients with obstructive sleep apnoea.

The values for surface tension of upper airway lining liquid (STUAL) were compared between a small cohort of healthy subjects, and subjects with obstructive sleep apnoea, and a small increase in surface tension was found in the OSA group when compared with the healthy subject group. The occurrence of nasal breathing during sleep was associated with a fall in STUAL in both groups (Kirkness et al., 2005b)

Surface tension forces play an important part in modulating upper airway patency. In an anaesthetised rabbit model, decreasing the surface tension by instillation of exogenous surfactant closed and re-opened the upper airway at lower intraluminal pharyngeal pressures (Lam et al., 2008, Kirkness et al., 2003a). Similarly, in anaesthetised human subjects, there is a relationship between the surface tension in the upper airway and its passive mechanical properties (Kirkness et al., 2003b). Lowering the surface tension in the upper airway lining liquid reduced re-opening pressures, and upper airway resistance. In a single blind crossover placebo- controlled study, Morrell et al demonstrated that exogenous surfactant instilled into the upper airway reduced the upper airway resistance and respiratory disturbance index in sleeping human subjects (Morrell et al., 2002).

Chapter 4: Obstructive Sleep Apnoea

4.1 Definition

In early research into upper airway collapse , episodic apnoeas were detected by oronasal thermistors and chest and abdominal strain gauges/ bands. This, in combination with EEG monitoring, allowed sleep related obstructive events to be identified (Guilleminault et al , Ann Rev Med 1976). Respiratory events and apnoeas at ≥ 5 events per hour of sleep were described as abnormal, with obstructive apnoeas defined by cessation of airflow on nasal pressure transducer or thermistor of > 10 s. The scoring of sleep stages, EEG arousals, and respiratory events were the culmination of early consensus guidelines used for sleep staging (Rechtschaffen and Kales 1968), and the American Academy of Sleep Medicine consensus reports which have evolved into the current American Academy of Sleep Medicine criteria for scoring (Berry et al., 2012).

An apnoea is defined as a $\geq 90\%$ reduction in airflow for ≥ 10 seconds. It is classified as obstructive if respiratory effort is present and central if no respiratory effort is present. Hypopnoeas are defined by a $\geq 30\%$ reduction in airflow for ≥ 10 seconds associated with a $\geq 4\%$ oxygen desaturation. The alternative hypopnoea definition requires a $\geq 50\%$ reduction in airflow for

≥ 10 seconds associated with a $\geq 3\%$ oxygen desaturation or electroencephalogram (EEG) arousal. Increased respiratory effort and arousals related to these may contribute to sleep fragmentation and are currently scored as Respiratory Effort Related Arousals or RERAs. (Berry et al., 2012).

Hypopnoeas have also previously been described according to the “Chicago Criteria” based on a 1999 consensus report published by the American Academy of Sleep Medicine (AASM, 1999) . These guidelines defined standardized scoring criteria for 2 types of hypopnoeas. The first were events with a 50% decrease in airflow (using a valid measure of airflow), without oxygen desaturation or arousal, and the second type of hypopnoea was a lesser subjective airflow reduction with a $\geq 3\%$ oxygen desaturation or an arousal.

An Apnoea-Hypopnoea Index (AHI) is calculated as the total number of apnoeas and hypopnoeas divided by the Total Sleep Time (TST). The Respiratory Disturbance Index (RDI) is calculated as the total number of apnoeas, hypopnoeas, and RERAs divided by the TST. The AHI and RDI can be used to describe the frequency of respiratory events that can influence sleep fragmentation, and are currently used as markers for severity of sleep disordered breathing.

The Obstructive Sleep Apnoea Syndrome (OSAS) is defined as the presence of at least 5 obstructive respiratory events per hour of sleep in conjunction with excessive daytime sleepiness. Other frequently described

symptoms include snoring, gasping/ choking during sleep, unrefreshing sleep, sleep fragmentation, sleep maintenance insomnia, nocturia, morning headaches, decreased concentration, impaired short term memory, decreased libido, and irritability.

4.2 Epidemiology

A number of studies with large samples representative of the population provide more recent estimates for OSA in various countries including Australia. The prevalence of the condition with accompanying daytime sleepiness has been estimated at 3-7% for adult men, and 2-5% for women (Punjabi et al., 2008). There are differences in disease prevalence depending on obesity, race, gender, and age. Data from the Wisconsin Cohort Study looked at age and gender specific prevalence and showed a prevalence of having sleep disordered breathing with an AHI > 5 events per hour of sleep was 9% for women and 24% for men. The prevalence of OSA with AHI greater than 15 was 4% for women and 9% for men.

More recent data from the Swiss population, with PSG data from 2121 subjects demonstrated that the prevalence of moderate to severe OSA was 23.4% in women and 49.7% in men, with an AHI >20.6 events per hour of sleep associated independently with the presence of hypertension, diabetes, metabolic syndrome and depression (Heinzer et al., 2015).

The high prevalence of OSA in this population may be due increased sensitivity of current recording techniques and scoring criteria. The authors from this study suggested that the true prevalence in other Western Countries could be higher, since BMI is lower in Switzerland than in other Western Countries such as the USA.

There is an increase in the prevalence of OSA with ageing. In a random population of people aged > 65 years, 24% had an apnoea index > 5 events per hour of sleep, with 62% having an RDI \geq 10 events per hour of sleep (Ancoli-Israel et al., 1991) .

There is a strong predilection for a higher prevalence of OSA in males. This has been shown in numerous large cohort studies where men are more predisposed to OSA (Redline et al., 1994). The reasons of why this is so are not completely clear, and factors may include truncal and neck fat/ body fat distribution pattern in men, hormonal factors, and possibly differences in reporting patterns for symptomatic snoring and witnessed apnoeas. This is supported by evidence of increased prevalence of sleep disordered breathing in postmenopausal women (Bixler et al., 2001).

Prevalence may also differ depending on ethnicity and may be accounted for by differences in craniofacial anatomy. For example there is a higher prevalence among South East Asian and African populations that may be explained on the basis of differences in body fat distribution, habits, and craniofacial shape (Ralls and Grigg-Damberger, 2012).

4.3 Pathophysiologic Consequences and Burden of Disease

The consequence of OSA on mortality and morbidity have been investigated by observational cohort studies and population based studies. Increased mortality from cardiovascular complications was seen in the Sleep Heart Health Study (Shahar et al., 2001) while increased cardiovascular and cerebrovascular mortality was seen in untreated severe OSA patients (Marin et al., 2005). This study also demonstrated that CPAP therapy reduced mortality in severe OSA. The postulated mechanisms whereby OSA leads to an increase in cardiovascular risk may be vascular/ endothelial dysfunction, systemic inflammation and metabolic dysregulation. Tissue vibration from snoring may lead to endothelial dysfunction inducing vascular disease (Cho et al., 2011, Lee et al., 2008). Oxidative stress during sleep apnoea may induce a cascade of inflammatory pathways which may have an effect on the progression of vascular disease. There is increased insulin resistance, and diabetes mellitus amongst OSA patients and treatment with CPAP therapy may mitigate glucose intolerance, possibly by ameliorating sleep deprivation and sympathetic activation (Spiegel et al., 1999).

The associated cardiovascular diseases include hypertension, cardiac failure and cardiac arrhythmias. The causal relationship between OSA and hypertension was demonstrated by the Wisconsin Sleep Cohort Study (Shahar et al., 2001). Furthermore, increasing severity of OSA increases the risk for developing hypertension. Randomised studies have shown that

treating obstructive sleep apnoea in mild hypertension may have a small yet significant effect in decreasing blood pressure (Becker et al., 1987).

There is an association between OSA and cardiac failure, firstly with an increased prevalence of OSA in heart failure patients, and secondly that there may potentially be increased nocturnal upper airway oedema in patients with cardiac failure, predisposing them to OSA. The Sleep Heart Health study demonstrated that the presence of OSA conferred an increased relative risk of developing cardiac failure. Fluid overload, such as that seen with cardiac failure and redistribution of body fluid may also predispose to sleep apnoea. In patients with drug resistant hypertension, it has been found that a rostral shift of fluid overnight is associated with a greater severity of OSA (Friedman et al., 2010) (Friedman et al., 2010).

Upper airways collapse that predisposes to snoring may lead to vibration induced damage that, in turn, may increase the risk of cerebrovascular disease. Heavy snoring may increase the risk of carotid atherosclerosis, independent of other risk factors including the degree of overnight hypoxia and severity of sleep disordered breathing. The prevalence of atherosclerosis was greater for severe snoring (64%) as compared to moderate (32%) or mild snoring (20%), with the severity of snoring determined by the proportion of the night spent with snoring (Lee et al., 2008). The relationship between OSA and stroke risk has also been demonstrated in the Sleep Heart Health Study (Redline et al., 2010).

Two recent meta-analyses have been published which describe firstly the risks of all-cause mortality, and secondly the effect of CPAP on cardiovascular events and mortality in OSA. Patients with OSA exhibit an increased risk in all-cause mortality, with those having severe OSA demonstrating a significantly increased mortality (Pan et al., 2016). CPAP therapy had no significant effect on mortality, stroke, and cardiovascular events compared with control groups, however, CPAP therapy was associated with a non-significant trend to lower mortality rate, reduced rate of stroke (Guo et al., 2016).

The increased risk of motor vehicle accidents has been associated with OSA, with the relationship clearly identified through many studies. A meta-analysis of studies looking at the relationship between OSAS and collisions found an overall estimated risk (odds ratio) of 2.5 (Sassani et al., 2004). Besides the increased motor vehicle accident risk, OSA is associated with increased accidents in the household and in the workplace (Hortsmann et al., 2000). Sleep related breathing disorders are also associated with depression, with an increase in the severity of OSA from mild to moderate being associated with a 1.8 fold increased adjusted odds for development of depression (Peppard et al., 2006).

4.4 Current Therapeutic Options

The current treatment modalities for OSA include weight loss, treatment or avoidance of factors that can precipitate sleep apnoea, devices such as CPAP or mandibular advancement devices, and surgery. The choice of treatment is predominantly determined by the severity of obstructive sleep apnoea.

Conservative measures for the management of OSA include weight loss, positional therapy, avoidance of alcohol and sedatives, in addition to smoking cessation.

Measures such as avoidance of alcohol and sedating agents, may reduce the severity of OSA. Weight loss may improve indices of OSA, but response to dieting and lifestyle modification has a delayed effect. The reduction in weight, especially central adiposity, may reduce the adipose tissue around the upper airway, improve lung volumes, and ameliorate the blunting of neuromuscular response found in obesity, to reduce the severity of sleep disordered breathing (Schwartz et al., 2008).

Oral devices may be preferable in some patients with mild to moderate OSA and work by preventing or ameliorating retroglossal collapse. A mandibular advancement splint may be effective as an alternative to treatment for snoring, and mild to moderate obstructive sleep apnoea. Oral

appliances can improve subjective sleepiness and reduce the severity of OSA, but may be less effective than CPAP therapy (Lim et al., 2006).

CPAP therapy was first described as an effective means of preventing upper airways collapse in 1981 (Sullivan et al., 1981). CPAP works as a “pneumatic splint” for the upper airway, with intramural pressure exceeding the surrounding tissue pressure to maintain airway patency. CPAP therapy may alleviate the symptoms of OSA and improve symptoms of excessive daytime somnolence, and nocturnal snoring. The patients who are most symptomatic are more likely to have better long term compliance to treatment with CPAP therapy (Kohler et al., 2010). CPAP therapy has also benefits in being a cost effective management in reducing cardiovascular and cerebrovascular risk (Marin et al., 2005).

Surgery remains an option for patients with snoring and sleep apnoea, but the magnitude of improvement in sleep disordered breathing is usually small. The options for surgical treatment includes uvulopalatopharyngoplasty, tongue reduction surgery, maxillary and mandibular advancement. An aggressive, radical surgical approach achieves a “cure” (AHI < 5 events per hour) rate of 43%, and remains an option for selected patients (Holty and Guilleminault, 2010). Tracheostomy completely bypasses the site of obstruction in the upper airway, but such an invasive approach may not appeal with less invasive options available.

Hypoglossal nerve stimulation has emerged as an experimental surgical therapy but the clinical application of this procedure has not yet clearly been defined (Schwartz et al., 1993, Schwartz et al., 2001, Eastwood et al., 2011).

4.5 Summary of Studies Investigating Surface Tension and Upper Airway Collapse

Currently, exogenous surfactants are not utilised as mainstream therapy for obstructive sleep apnoea. Some over the counter sprays are available, and may reduce the surface tension in upper airway lining liquid, but their efficacy in the treatment of snoring and obstructive sleep apnoea has not been shown in any randomised controlled trials.

Earlier studies have shown that exogenous surfactant may reduce upper airway collapsibility and maintain upper airway patency in awake human subjects. A study which investigated the effect of instillation of surfactant vs saline into the upper airway of 5 subjects observed a decrease in opening pressures after exogenous surfactant (5ml Exosurf neonatal, Burroughs Wellcome Australia). Supraglottic airway measurements were taken by measuring the anteroposterior intraluminal diameter on X-ray fluoroscopy. Saline instillation did not produce any consistent reduction in closing pressure, while surfactant instillation resulted in a consistent reduction in closing pressure. Similarly, although there was no consistent

increase in the upper airway opening pressure with saline, there was a reduction in opening pressure with surfactant. In addition, it was observed that positive pressure was required to reopen a closed upper airway in the control or saline instillation patients, while no positive pressure was required to re-open a collapsed upper airway after surfactant instillation (Van der Touw et al., 1997).

The role of a soft tissue lubricant in sleep apnoea was previously investigated using phosphocholinamin (Sonarite; Guardian chemicals, New York, NY) in 10 subjects with previous polysomnography demonstrating mild to moderate OSA. The surface tension of this soft tissue lubricant was 25.4mN/m at 37 degrees centigrade. 0.4 ml was introduced nasally by pipette at lights out, and also at 3.5 hours while placebo was administered on the other (control) night. There was a significant reduction in the AHI on the soft tissue lubricant night compared to the placebo night with all 10 subjects demonstrating a lower AHI. The AHI decreased from a mean AHI of 24 events per hour of sleep on the placebo night to 14 events per hour of sleep on the lubricant night. This effect was seen in both supine (AHI reduction from 33 to 20 events/hour) and lateral sleep (AHI reduction from 18 to 11 events/hour). There was a difference in effect of soft tissue lubricant between REM and NREM sleep however, with soft tissue lubricant resulting in a significant reduction of AHI in NREM sleep (25 to 14 events/hour), while there was no reduction with soft tissue lubricant in REM sleep (Jokic et al., 1998).

Exogenous surfactant may decrease critical closing pressures by 3cm H₂O post instillation (Kirkness et al., 2003c), and has been shown to reduce the frequency of hypopnoeas, although there is no significant reduction in frequency of apnoeas. Similar decreases in critical pressures have previously been demonstrated with postural change, with lateral sleep reducing opening pressures compared to supine sleep opening pressures (Neill et al., 1997).

The most recent series of studies investigating the role of surface tension in upper airway collapse, and obstructive sleep apnoea, are the studies performed by Kirkness and his co-workers. This series of studies have demonstrated that a higher surface tension in upper airways lining liquid promotes upper airway collapsibility, that lowering surface tension reduces the propensity to airway closure, and reduces opening pressures in a collapsed airway. In studies performed on anaesthetised rabbits, and subsequently on anaesthetised humans, lowering surface tension in the upper airway with exogenous surfactant administration, led to decreased critical opening pressures, reduced upstream resistance, promoting upper airway patency (Kirkness et al., 2003a, Kirkness et al., 2003b). In patients with obstructive sleep apnoea, instillation of exogenous surfactant reduced STUAL from 60.9 ± 3.1 mN/m to 45.2 ± 2.5 mN/m, whilst reducing the Pcrit from 1.19 ± 1.14 cm H₂O to -0.56 ± 1.15 cm H₂O. There was a reduction in the RDI which correlated to the reduction in surface tension, with the main reduction in respiratory events due to a reduction in obstructive hypopnoeas (Kirkness et al., 2005b). There was a wide variation seen in the STUAL of these subjects, and proposed mechanisms included changes in salivary flow

across the night, changes in route of breathing, and pharyngeal mucosal properties.

The relationship between STUAL and route of breathing was investigated in awake subjects. Verma et al examined relationships between breathing route, oral mucosal wetness, and STUAL. The subjects were randomised to oral only or nasal only route of breathing for a period of 120minutes, with sampling for measurement of upper airway mucosal “wetness” and STUAL taken at the beginning, and at 15 minute intervals. With enforced oral breathing route, there was a significant reduction in mucosal “wetness” , and concurrently a significant increase in STUAL over 120 minutes by approximately 14 mN/m. Enforced nasal route of breathing, however, resulted in no significant change in mucosal wetness, and a decrease in STUAL of approximately 10 mN/m (Verma et al., 2006). This relationship between STUAL and route of breathing may potentially be important in determining upper airway patency, with increased oral route of breathing and increased surface tension promoting upper airway collapse.

The role of increased surface tension in upper airway collapse has also been investigated in patients with primary Sjogren’s Syndrome. Patients with this condition suffer from symptomatic xerostomia, potentially leading to increased surface tension. These patients also demonstrate increased levels of sleepiness and fatigue, and despite their increased STUAL compared to normal control subjects, they do not appear to have any significant difference in upper airway collapsibility index (Hilditch et al., 2008). The same research

team subsequently reported an increased prevalence of OSA amongst Sjogren's syndrome patients, with primary Sjogren's syndrome patients having twice the frequency of obstructive apnoeas and hypopnoeas compared with control subjects (Usmani et al., 2012).

In summary, OSA is characterized by repeated episodes of partial or complete upper airways collapse. Anatomical factors and muscle activation play major roles, but other factors such as surface tension may also play a significant role in upper airway collapsibility. There is evidence that surface tension forces play a role in upper airway collapsibility with changes in surface tension affecting upper airway collapsibility. Lowering the surface tension in upper airway liquid reduces closing and re-opening pressures in the upper airway, and instillation of exogenous surfactant is a method in which the surface tension forces of the airway may be manipulated. Other factors which include route of breathing, may also have a role in modifying the surface tension in upper airway liquid via local effects such as evaporation.

Chapter 5: Surface Tension of Upper Airway Liquid in Healthy Subjects

5.1 Introduction

Surface tension arises from cohesive forces between liquid molecules at a liquid-gas interface. Surface tension forces are present in the collapsible upper airways, and the STUAL influences upper airway opening and closing pressures. In previous work performed in our laboratory, the STUAL in healthy subjects and in OSA patients was studied. OSA patients were found to have a higher STUAL than healthy subjects (Mean [95% confidence interval] 59.9 mN/m [53.8, 58.8] vs 56.3 mN/m [57.7, 62.1]) (Kirkness et al., 2005b). No overnight difference in STUAL was found when evening and morning samples were compared in either group, although a reduction in STUAL was observed in those subjects with > 50% of total sleep epochs with nasal breathing. This study observed a range of surface tension in both saliva and UAL in a small group of healthy subjects and OSA patients. However, it is not known if STUAL varies during the course of a 24 hour period, or if it follows any biological (i.e. circadian or diurnal) rhythm. The hypothesis raised was that saliva production would vary over a 24 hour cycle and follow a circadian rhythm, thereby potentially influencing measured STUAL values across the day.

5.2 Aims

The aim of the first part of this project is to establish, in healthy subjects, the range of baseline values for ST of Saliva and STUAL. The second aim is to determine the variability of STUAL and ST Saliva over a 24 hour period.

5.3 Methods

5.3.1 Subjects

Healthy subjects were recruited from amongst staff members from Westmead Hospital and from the Ludwig Engel Centre for Respiratory Research. Subjects were screened by the MAP Sleep Symptom Frequency Questionnaire (Maislin et al., 1995) to exclude sleep disordered breathing, with all subjects scoring a MAP index < 0.6 . Subjects were excluded if they had a history of allergic rhinitis or snoring. No inhaled or oral medications were taken by any of the subjects for 12 hours prior to the study.

16 healthy volunteers were recruited for this study (10 female, 6 male; mean age \pm SD: 33 ± 10.4 years, range 20 - 54 years; BMI $24.8 \pm 3.2\text{kg/ m}^2$, range 21.0 - 31.8kg/m²).

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Ethics Committee.

5.3.2 Measurements

Surface Tension

Surface Tension of Upper Airway Lining Liquid was measured by the “Pull Off Force Technique” as discussed in more detail previously in chapter 3 (figure 3.5) . Microliter volumes of upper airway lining liquid are placed between two silica discs, and the surface tension measured as the force required to separate the discs.

Upper Airway Lining Liquid and Saliva Sampling

Upper airway lining liquid and saliva were sampled from the posterior oropharyngeal wall and sublingually respectively, with approximately 0.2 μ L sampled from each site. Samples were aspirated into polyethylene tubing (Tyco Electronics; external diameter 0.8mm; internal diameter 0.5mm), using a 23g needle (BD Precision Glide 0.6mm x 32mm) attached to a 3ml syringe (Terumo Medical Corporation 3cc / ml). Surface tension of UAL or saliva was measured by the “Pull Off Force Technique”.

5.3.3 Protocol

All subjects abstained from any food or drink for 30 minutes prior to the study. Nasal only route of breathing was enforced (mouth taped) for 15 minutes prior to sampling of UAL and saliva. Sample collection was performed with the subject in the upright seated position, with salivary or posterior pharyngeal wall samples collected as described above.

UAL samples were obtained at 3 hourly intervals from 0900 hrs to 1800 hrs, with sampling the following morning at 0900 hrs.

Salivary samples were obtained at 3 hourly intervals from 0900 hrs to 2100 hrs with sampling the following morning at 0600 and 0900 hrs. The 0600 hrs salivary collection was performed by the subject either at, or as close to 0600 hrs as possible.

All subjects remained nil by mouth for 30 minutes with 15 minutes of enforced nasal breathing before each sampling. Subjects were, however, permitted to eat and drink outside of these periods. Route of breathing was not enforced outside of sampling periods.

5.3.4 Data Analysis

Data were expressed as mean \pm SEM and compared using repeated measures ANOVA. Single comparisons were made using paired t-test. Variance was expressed as coefficient of variation (CV). Relationships between STUAL, ST Saliva, and time of day were statistically modelled using linear mixed effects modelling. Correlations were examined using linear regression. $P < 0.05$ was considered significant.

5.4 Results

The overall range of surface tension values obtained for both STUAL and ST Saliva for all subjects across all time periods is demonstrated in Figure 5.1. The surface tension for saliva and UAL ranges from 56 mN/m to 71.4 mN/m.

The ST of saliva was positively correlated with the STUAL across all time periods (Figure 5.2). Additionally, there were no significant differences between STUAL and ST of Saliva when data were compared across all common time points. However, there was a large variability in differences between ST of saliva and STUAL of up to approximately 9mN/m within the same time point (Figure 5.3).

The values of STUAL across all time periods demonstrated a mean of 63.7 mN/m, with a range from 56.5 mN/m to 70.4 mN/m. The mean values at sequential time points were: 0900 Hrs = 63.8 ± 0.9 mN/m, 1200 Hrs = 62.9 ± 0.9 mN/m, 1500 Hrs = 63.6 ± 0.9 mN/m, 1800 Hrs = 64.5 ± 0.8 mN/m, 0900 Hrs (following morning) = 63.7 ± 0.9 mN/m (Figure 5.4).

Across all time periods, the mean intra-subject coefficient of variation was 3.3% with a range of 1.4% to 7.6%. The mean inter-subject coefficient of variation was 5.5% with a range of 5.1 to 5.8% .

For ST of Saliva, the values across all time periods demonstrated a mean of 63.9 mN/m, with a range from 56.0 to 71.4 mN/m. Mean values at sequential time points were: 0900 Hrs = 62.7 ± 1.0 mN/m, 1200 Hrs = 63.5 ± 0.9 mN/m, 1500 Hrs = 63.7 ± 0.7 mN/m, 1800 Hrs = 65.1 ± 0.9 mN/m, 2100 Hrs = 64.3 ± 0.9 mN/m, 0600 Hrs = 64.4 ± 1.0 mN/m, 0900 Hrs (following morning) = 63.6 ± 1.1 mN/m. (Figure 5.5).

Across all time periods, the mean intra-subject coefficient of variation was 3.4% with a range of 1.8% to 5.5%. The mean inter-subject coefficient of variation was 5.9% with a range of 4.6% to 5.8%

The variation in STUAL and ST Saliva was also analysed using a Mixed Effects Model to look for any significant trend during the sampling period undertaken. The STUAL appeared unaffected by the time of day, while ST Saliva varied with time of day, with ST values reaching a peak in the evening at the 1800 Hrs sampling time (Figure 5.6).

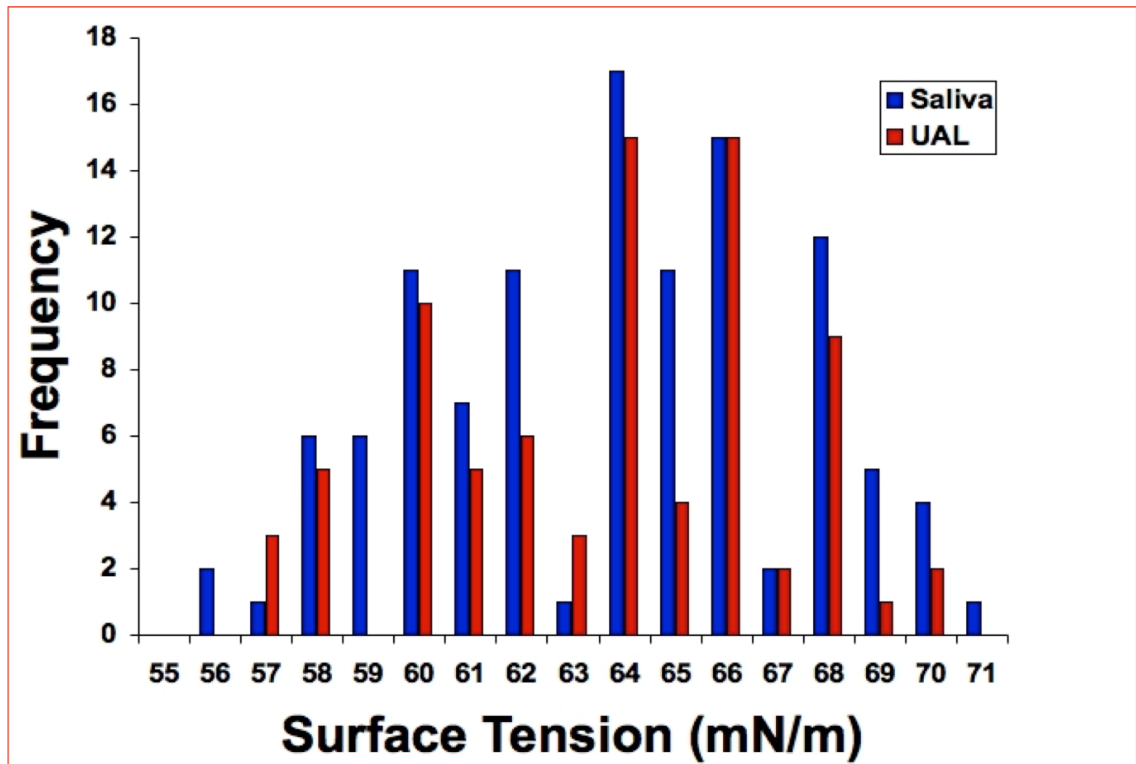


Figure 5.1 Range of Surface Tension values for Saliva and UAL

Frequency histogram with the count of ST values in 1 mN/m bins for the full range of both STUAL and ST Saliva values for all subjects and all collection times. UAL = Upper Airway Liquid.

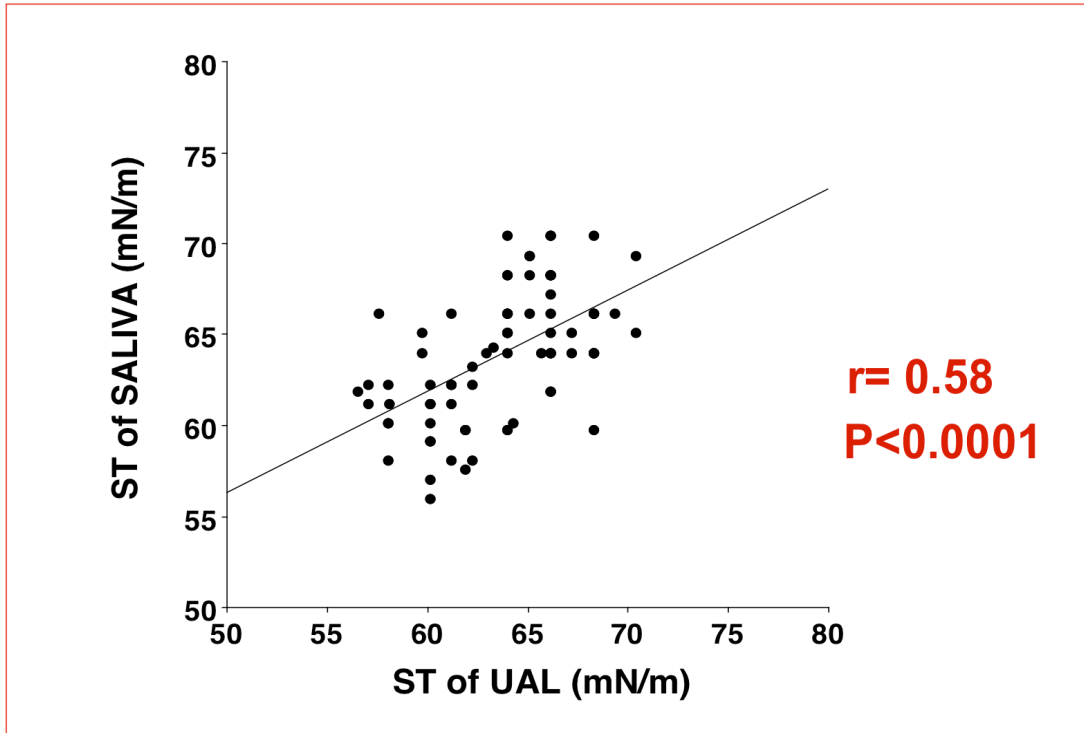


Figure 5.2 STUAL vs ST of Saliva

STUAL correlated well with ST Saliva across all time periods. Line = regression line. ST = Surface Tension; UAL = Upper Airway Liquid; STUAL = Surface Tension Upper Airway Liquid.

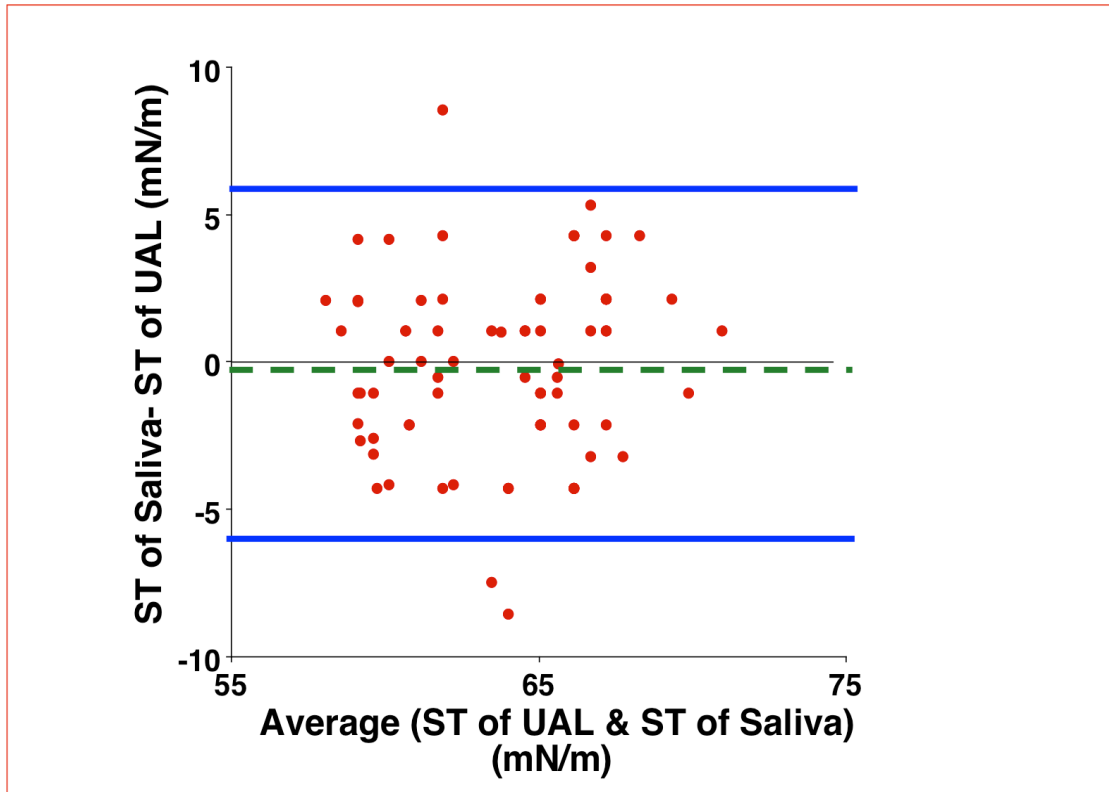


Figure 5.3 Bland-Altman graph of STUAL and ST Saliva

Bland-Altman graph of Average ST of UAL and ST of Saliva vs the difference between ST of Saliva and ST of UAL. No overall difference between STUAL and ST Saliva at all common time points. There was some variability seen in differences between ST of Saliva and ST of UAL (up to ~9mN/m) but generally less than 5 mN/m. Blue lines = 95% Confidence Interval limits. Dashed green line = mean difference. Thin black line = zero difference. ST= surface tension; UAL = Upper Airway Liquid

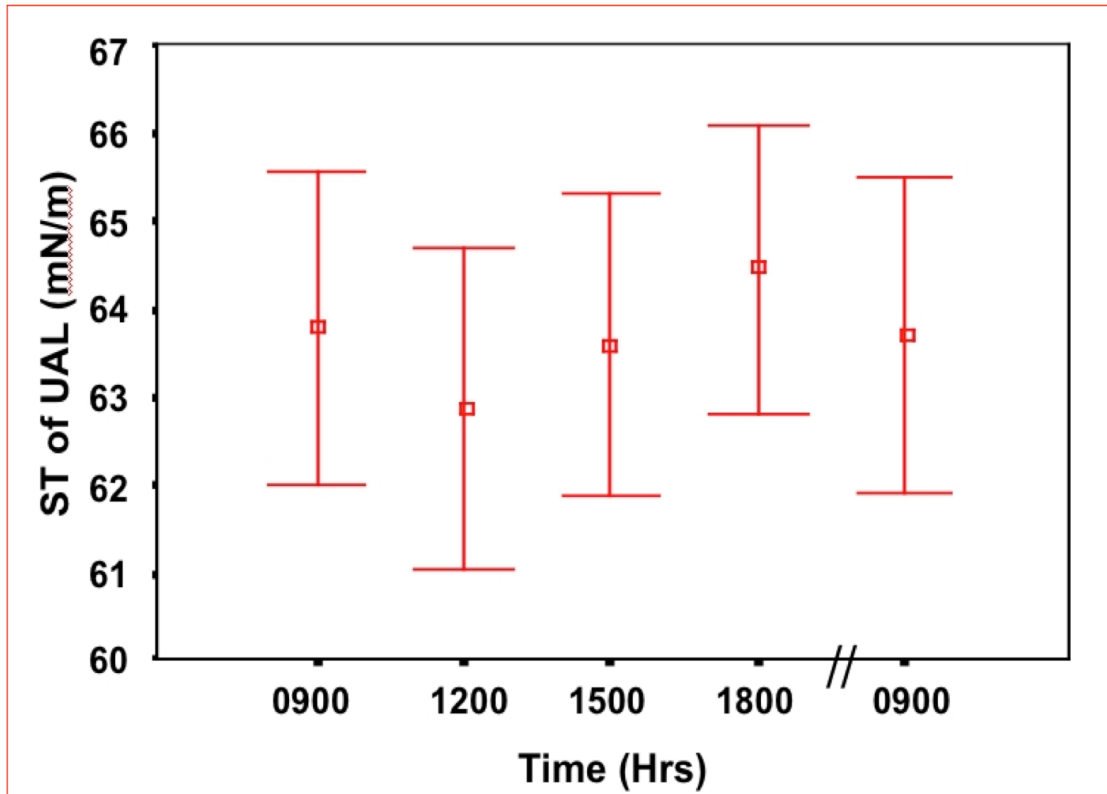


Figure 5.4 Group mean (\pm SEM) values for STUAL at sequential time points

There was little change in ST of UAL values across the time points sampled. The mean values at sequential time points were: 0900 Hrs = 63.8 ± 0.9 mN/m, 1200 Hrs = 62.9 ± 0.9 mN/m, 1500 Hrs = 63.6 ± 0.9 mN/m, 1800 Hrs = 64.5 ± 0.8 mN/m, 0900 Hrs (following morning) = 63.7 ± 0.9 mN/m. The horizontal bars represent 95% Confidence Interval limits. ST = Surface Tension; UAL = Upper Airway Liquid.

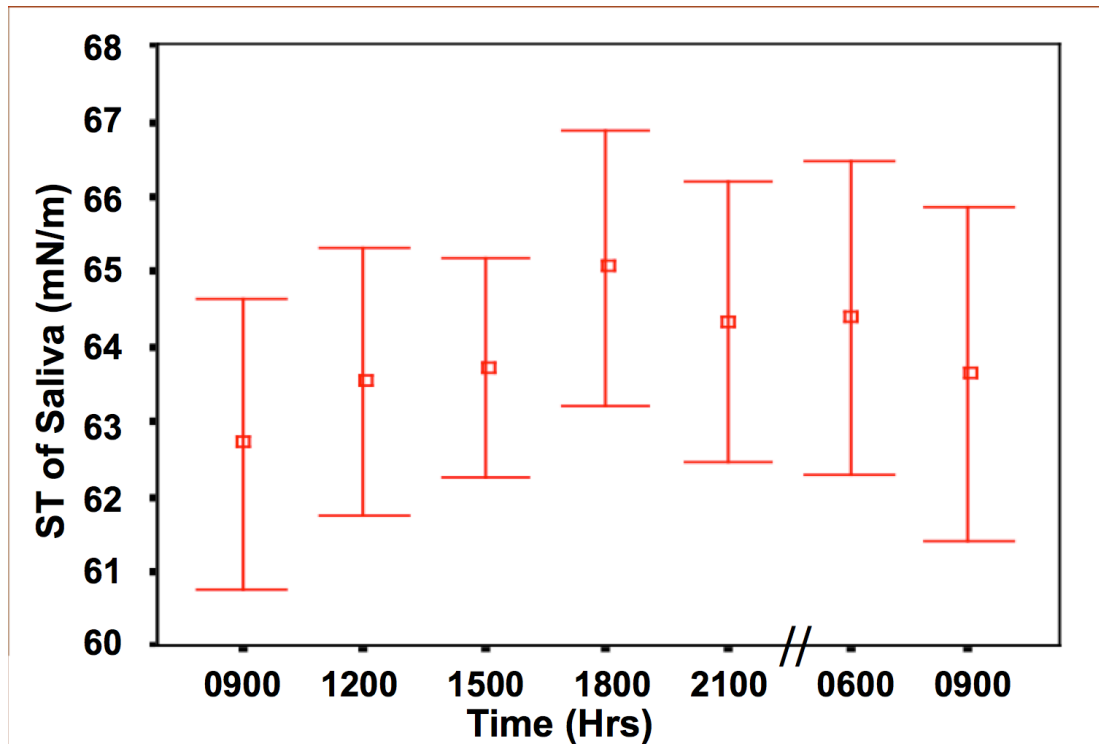


Figure 5.5 Group mean (\pm SEM) values for ST of Saliva at sequential time points

Mean values at sequential time points were: 0900 Hrs = 62.7 ± 1.0 mN/m, 1200 Hrs = 63.5 ± 0.9 mN/m, 1500 Hrs = 63.7 ± 0.7 mN/m, 1800 Hrs = 65.1 ± 0.9 mN/m, 2100 Hrs = 64.3 ± 0.9 mN/m, 0600 Hrs = 64.4 ± 1.0 mN/m, 0900 Hrs (following morning) = 63.6 ± 1.1 mN/m. The horizontal bars represent 95% Confidence Interval limits. ST = Surface Tension.

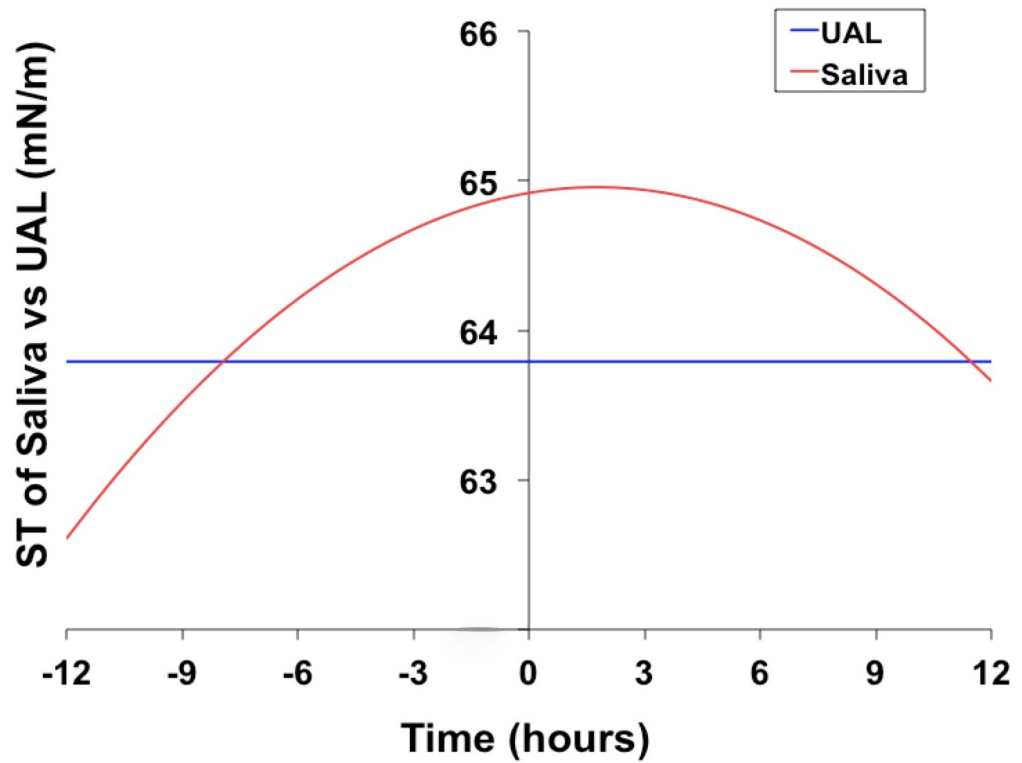


Figure 5.6 Mixed Effects Models for STUAL and ST of Saliva

Mixed Effects Models for ST of Saliva (red line = quadratic) and STUAL (blue line = linear) versus time. ST of UAL did not vary with time. ST of Saliva varied with time of day according to the relationship: $ST\ of\ Saliva = 64.9 + 0.04 (time) - 0.01 (time)^2$ ($p < 0.05$).

Zero time = 2100 hours

5.5 Discussion

This study demonstrated a range of normal surface tensions for UAL and saliva in healthy subjects, and established a range of surface tension values over a 24 hour period for saliva and UAL in these subjects. Whilst there was a trend towards higher ST Saliva in the evening, the STUAL did not change across the day and evening when route of breathing is controlled.

5.5.1 Critique of Methods

The ST Saliva and UAL were measured using the previously described “Pull Off Force technique”. Compared to the values obtained by Kirkness et al, the mean values for ST of both UAL and Saliva appeared higher. The mean ST of UAL in anaesthetized human subjects is 62 mN/m (Kirkness et al., 2003b), whilst the mean ST of UAL in this study was 63.7 mN/m. In the Kirkness et al study looking at ST values and OSA, both normal subjects and OSA patients were studied with sampling performed at 10pm in the evening and between 5.30-6.00 am the following morning (Kirkness et al., 2005b). Overnight, there were no meals between evening and morning sampling, and since the subjects were asleep for the most part there was no vocalization or conversation as would be expected from normal activities of subjects in the daytime. In the present study, sampling times were different, with five sampling times for UAL and seven sampling times for Saliva. During the day, in between sampling times, the subjects were permitted to talk, eat, or drink with the exception of 30 minutes before sampling time. Variable oral route of breathing, in addition to food and drink intake across the day in-

between sampling times, may potentially effect the surface tension of both UAL and Saliva and account for the apparent differences in ST values in this study compared with previous studies.

Despite multiple samplings of UAL and Saliva, there was a prolonged period overnight where sampling was not performed. There was a sampling for ST Saliva, but not STUAL at 6.00 am in the morning (the ST Saliva sample was self-collected by the subject at 6.00 am). Route of breathing was not controlled during this period of time, and oral route of breathing may potentially have evaporative effects leading to changes in surface tension overnight which we have not measured.

Otherwise, the methodology used in the sampling and processing of UAL and Saliva sample for the purpose of measuring ST was identical to that used previously in our laboratory.

5.5.2 *ST Saliva and STUAL*

The ST of Saliva tended to be slightly higher in the evening. Linear effects model of the data for ST of Saliva demonstrated a quadratic relationship with time, with increased values in the evening, and with maximal recorded values at approximately 2100 hours. The postulated mechanisms for this may include uncontrolled route of breathing during the day between sampling times, in addition to oral intake of food and liquids

potentially affecting subsequent measured surface tension of sampled fluids. In this study, the subjects were kept nil by mouth for 30 minutes prior to sampling, however, it remains unclear regarding the magnitude and duration of effect of food or drink on surface tension. Factors such as eating or drinking food or liquid with higher surface tensions, talking (with increased associated oral breathing route), and possibly changes salivary production with diurnal rhythm may potentially lead to the gradual increase in surface tension of saliva seen by the evening.

There is a diurnal rhythm in salivary production, with decreased levels of saliva seen overnight. Unstimulated whole saliva does demonstrate a significant circadian effect, particularly with flow, rate, as well as sodium and chloride content (Dawes, 1975), and this may have an influence on the surface tension of saliva, potentially contributing to a circadian pattern of surface tension values over a 24 hour period.

Saline has a relatively high surface tension in comparison to water (approximately 82 mN/m vs 70 mN/m). Most western diets have a significant salt content, with a typical daily intake of approximately 10 g/day (Brown et al., 2009). This may potentially account for some of the increase in ST Saliva seen across the day as meals are consumed. Since the salivary sample was taken sublingually, to some degree it may reflect the surface tension of fluids / salt content of ingested food and liquids across the day.

Interestingly, STUAL did not demonstrate any significant change over the course of the day. The reasons and potential for increased oro-nasal route of breathing during working hours are identical to those which may be responsible for the increase in ST of saliva, however, whilst ST saliva changes with time of day, STUAL remains stable. The sampling of STUAL is from the posterior pharyngeal wall rather than sublingually for ST Saliva, and may be less affected by ingested food or drink. While the ST of sublingual samples may be affected by the surface tension of ingested food/ liquids, the posterior pharyngeal samples represent fluid that may be a composite of sublingual, submandibular, and parotid glands, in addition to surfactant containing mucus and liquid from the nasopharyngeal mucosa. Hence, although the STUAL and ST Saliva were positively correlated, there are possible reasons why the surface tension values may differ related to both the origin of the fluid and to different environmental exposures during the course of the day.

5.6 Conclusion:

In conclusion, for healthy subjects, ST of Saliva tends to be slightly higher in the evening than the morning. The reasons for this may relate to the influence of route of breathing and oral intake of food and drink on the surface tension of saliva in the sublingual sampling site, and/or to the origin of the actual fluid.

The STUAL appears to be relatively stable across the day. This implies that the origin of the fluid may differ or that the environmental influences in the posterior pharynx are different to those in the mouth.

In this study, we have been able to establish a range of surface tension values over a 24 hour period for healthy subjects. Although there is considerable variability in individual measures of STUAL, the values remain relatively constant over a 24 hour period. Overall, ST of Saliva and STUAL values are very similar, with their means being 63.7 and 63.9 mN/m respectively. However, dependent upon the time of day, the ST of saliva may not accurately reflect the ST of UAL. Therefore, studies investigating the surface tension properties of the upper airway should continue to take samples from the posterior pharyngeal airway and not use saliva as a substitute.

Chapter 6: Surface Tension of Upper Airway Liquid in Obstructive Sleep Apnoea Subjects

6.1 Introduction

In the previous chapter, a range of surface tension values for both saliva and UAL was demonstrated for healthy subjects with no history of OSA. A range of surface tension values for UAL has been previously published by Kirkness et al who examined the surface tension of UAL in both healthy subjects and patients with OSA before and after sleep (Kirkness et al., 2005b). The results were described for 11 healthy adult subjects (5 men, 6 women), and 15 patients with OSA (14 men, 1 woman). The mean STUAL for the OSA group in this study was 59.9 mN/m, compared with 56.3 mN/m in the healthy subject group (pooled AM and PM samples). These values are lower compared with the healthy subjects studied in the previous chapter, but fall within the broad range of surface tension values described.

For other patient groups, the surface tension for human saliva has previously been described in cystic fibrosis patients and in the dental literature (Braddock et al., 1970), and more recently in patients with Primary Sjogren's Syndrome (Hilditch et al., 2008). In contrast, the surface tension of UAL has only been described in a limited number of OSA subjects.

Previous measurements of STUAL in OSA subjects have demonstrated an increase in ST when compared with healthy subjects, of approximately 3.5 mN/m (Kirkness et al., 2005b). The authors suggested this result should be interpreted with caution in light of the small difference and the small sample size.

6.2 Aims

The aim of the work in this chapter is to determine the range of surface tension values in a larger cohort of subjects diagnosed with untreated OSA. The secondary aim is to examine the OSA patient cohort for any factors associated with the STUAL including, but not limited to, age and the severity of sleep disordered breathing.

6.3 Methods

6.3.1 Subjects

Study subjects were recruited from the Westmead Hospital Sleep Laboratory between June 2008 and June 2011. Subjects were recruited from patients referred to the Sleep Laboratory for investigation of possible sleep disordered breathing. Subjects who were current smokers, aged less than 18 years or older than 80 years were excluded from the study. None of the subjects had prior sleep studies nor did they have any history of prior CPAP use. Of the 95 subjects recruited, 22 were females and 73 were males. Age ranged from 23 years to 82 years with a mean \pm SD of 52 ± 13.8 years. BMI ranged from 21.5 kg/m^2 to 65.6 kg/m^2 with a mean of $31.7 \pm 7.3 \text{ kg/m}^2$.

Subjects were also excluded if they were current smokers, had a current upper respiratory tract infection, any inflammatory condition or medical illness affecting the mouth or upper aerodigestive tract mucosa, previous head and neck irradiation, and any history of airways disease requiring inhaled anticholinergic or inhaled steroid medication. Inhaled anticholinergic agents such as tiotropium or ipratropium and inhaled steroids were identified as medications which could potentially affect salivary or upper airways secretion production, which may, in turn affect ST values.

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Ethics Committee.

6.3.2 Measurements

Surface Tension

Surface Tension of Upper Airway Lining Liquid (STUAL) was measured by the “Pull Off Force Technique”. This technique is the same as that used in the measurement of ST in previous chapter and is described in further detail previously in chapter 3 (Figure 3.5). Following sampling of UAL and Saliva from the subject, microliter volumes of upper airway lining liquid are placed between two silica discs, and the force required to separate the discs with the microliter volume of liquid bridging both disc surfaces, is taken to be the surface tension.

Upper Airway Lining Liquid and Saliva

Upper airway lining liquid was sampled from the posterior oropharynx and saliva sampled sublingually. Samples were aspirated into polyethylene tubing (Tyco Electronics; external diameter 0.8 mm; internal diameter 0.5 mm), using a 23g needle (BD Precision Glide 0.6mm x 32mm) attached to a 3ml syringe (Terumo Medical Corporation 3cc / mL).

Samples (microlitre volumes) were then applied onto the surface of a silica disc for measurement of surface tension in the surface force measurement device using the “Pull Off Force” technique.

Polysomnography

The study was conducted over a single night, and all polysomnographic studies were undertaken at the Westmead Hospital Sleep Laboratory. The variables monitored overnight included EEG (electroencephalogram), ECG (electrocardiogram), submental and diaphragmatic EMG (Electromyogram), left and right EOG (electro-oculogram), respiratory inductance plethysmography (for thoraco-abdominal movement), arterial oxygen saturation, sound, and body position, whilst oral and nasal airflow was monitored using nasal pressure cannulae (1600 Nasal Cannula; Salter Labs Inc., Arvin, CA, USA) and oro-nasal thermistors (F-ONT2A; Grass, West Warwick, RI, USA). Video monitoring was recorded for all subjects. All signals were recorded on a Compumedics Profusion 3 software system. Sleep staging was performed on the overnight EEG using Rechtschaffen and Kales rules (Rechtschaffen and Kales 1968) to score sleep staging, while obstructive events were scored using “Chicago” criteria (AASM, 1999). Arousals from sleep were scored based on American Sleep Disorders Association criteria (Bonnet M, 1992).

6.3.3 Protocol

The study was conducted over one night at the Westmead Hospital sleep laboratory. Subjects arrived at the Westmead Hospital Sleep Laboratory between 1700 and 1900 hours on the night of their diagnostic sleep study. Upon arrival in the sleep laboratory, the subjects were set up by the sleep laboratory sleep technicians after patient identification and PSG documentation was checked. Subjects were then set up with PSG monitoring which included EEG with electrode placement based on the international 10-20 system (Klem et al., 1999), EMG, EOG, ECG, respiratory inductance plethysmography, finger oximeter probe, nasal pressure cannulae and oro-nasal thermistors. Signals were then verified by the sleep laboratory technician. Sampling of Saliva and UAL occurred following patient set-up and calibration and was performed in the sleep laboratory.

Subjects remained nil by mouth for one hour prior to sampling time, which was between 2100 and 2200 hours. The subject spent a minimum of 15 minutes of nasal only breathing prior to sampling. Sampling of both UAL and Saliva was performed whilst the subject was awake and in the seated position, with UAL and salivary sampling performed using the polyethylene tube aspiration method as described above. Samples contained within the polyethylene tubes were taken within 1-2 hours of sampling for measurement ST using the "Pull Off Force" technique on the evening of the study.

6.3.4 Data Analysis

Sleep Parameters

Sleep studies were scored for sleep stage using Rechtschaffen and Kales criteria (Rechtschaffen and Kales 1968). The Respiratory Disturbance Index (RDI) was derived from flow signals using a nasal pressure transducer and a nasal thermistor to determine the aggregate number of apnoeas and hypopnoeas per hour of sleep. Respiratory Events were identified using American Academy of Sleep Medicine “Chicago” Criteria (AASM Task Force; Sleep 1999). An obstructive apnoea was defined as a 10 second or greater absence of airflow with oximetry demonstrating a 3% or greater reduction in oxygen saturation in comparison to baseline oximetry. Two types of obstructive hypopnoea (duration of which being 10 seconds or greater) were defined according to AASM “Chicago Criteria”, 1) as greater than a 50% reduction in a valid measure of airflow or 2) those with a lesser airflow reduction together with oximetry demonstrating a 3% or greater reduction in oxygen saturation (compared to baseline) or terminating in an EEG arousal (AASM, 1999).

The overall Respiratory Disturbance Index (RDI) was calculated as the total number of both obstructive apnoeas and hypopnoeas divided by the total sleep time during overnight polysomnography. The Arousal Index (AI) was calculated as the total number of EEG arousals divided by the Total Sleep Time (TST). The Sleep Efficiency (SE) was calculated by dividing the total number of minutes of sleep divided by the number of minutes in bed.

Statistical Analysis

All data were expressed as mean \pm SD and compared using repeated measures ANOVA. Single comparisons were made using paired t-test. Correlations were examined using linear regression.

Statistical data were graphically represented and analysed using Graphpad Prism version 6.0. A p-value < 0.05 was considered significant.

6.4 Results

6.4.1 *STUAL vs ST Saliva*

The mean evening STUAL for all subjects was 61.4 ± 5.2 mN/m with the range being 45.1 mN/m to 73.7 mN/m (Figure 6.1). The mean evening ST Saliva for all subjects was 61.3 ± 5.1 mN/m with values ranging from 49.5 mN/m to 77.2 mN/m (Figure 6.2).

Similar to the previous chapter for healthy subjects, the values for STUAL and ST Saliva in subjects with OSA demonstrated no significant overall difference (Bland-Altman, Figure 6.3). The group mean difference between ST of UAL and Saliva was 0.1 ± 0.7 mN/m and there was no significant difference in the mean values. There was a strong correlation between STUAL values and ST Saliva values with $r = 0.78$ ($p < 0.0001$) (figure 6.4).

6.4.2 STUAL and ST Saliva vs age

For the group as a whole, there was no correlation between STUAL and age (Figure 6.5). However, when only subjects aged ≥ 40 years with an RDI ≥ 30 events per hour were considered (32 out of 95 subjects), there was an association between STUAL and age in this sub-group with severe OSA, with STUAL demonstrating an increase with ageing (Figure 6.6).

Frequency Histogram of STUAL (pm)

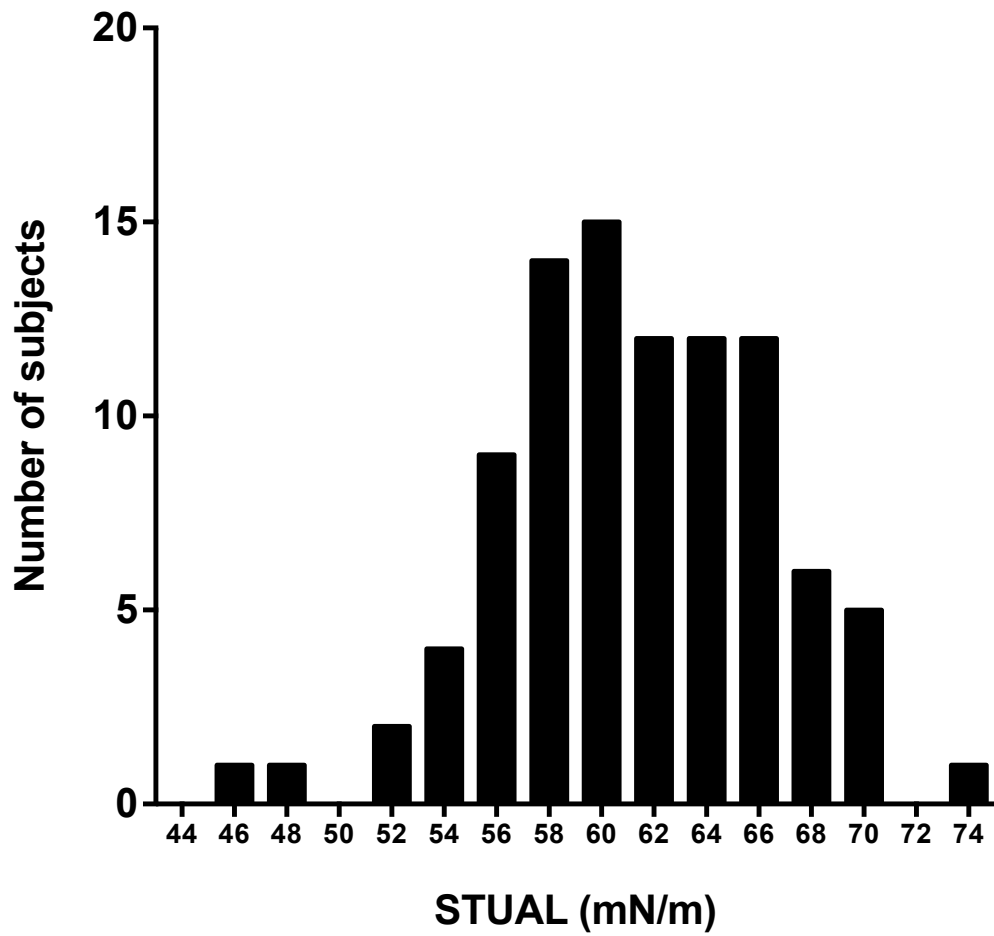


Figure 6.1 Frequency Histogram for STUAL in OSA subjects

Frequency Histogram (bins of 2 mN/m) for STUAL (mN/m) obtained from 95 OSA subjects.

The mean evening STUAL for all subjects was 61.4 ± 5.2 mN/m. STUAL = Surface Tension

Upper Airway Liquid.

Frequency Histogram of ST Saliva (pm)

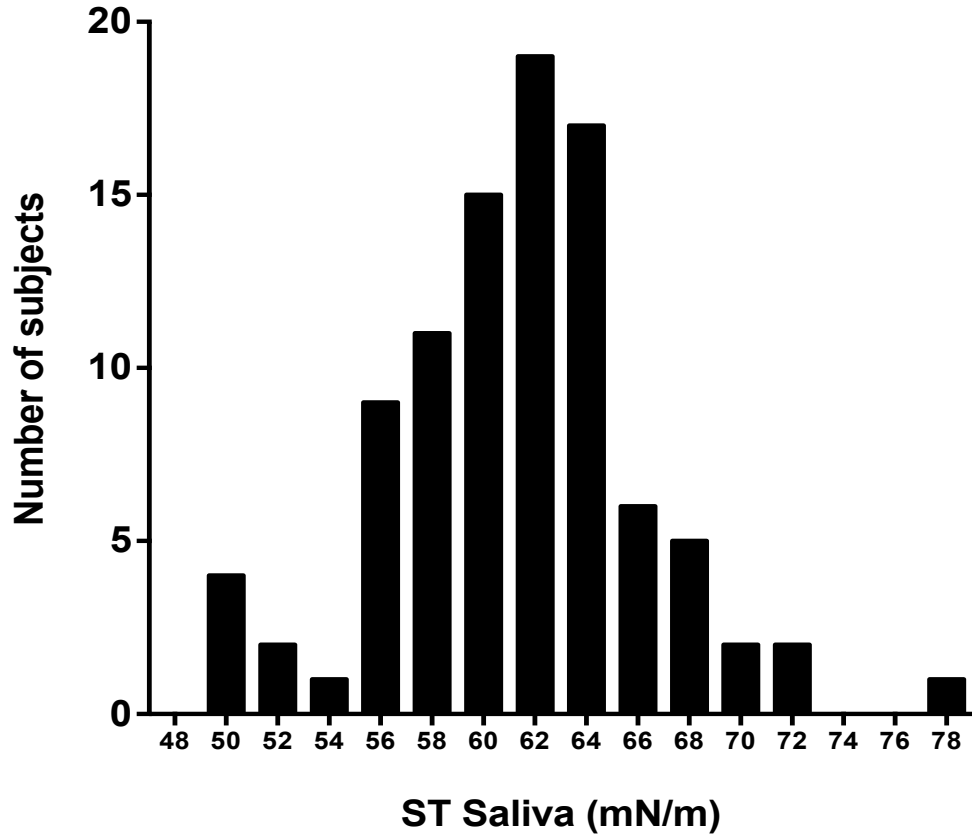


Figure 6.2 Frequency Histogram for ST Saliva in OSA subjects

Frequency Histogram (bins of 2 mN/m) for ST Saliva (mN/m) obtained from 95 OSA subjects. Mean evening ST Saliva for all subjects was 61.3 ± 5.1 mN/m. ST = Surface Tension

STUAL vs ST Saliva

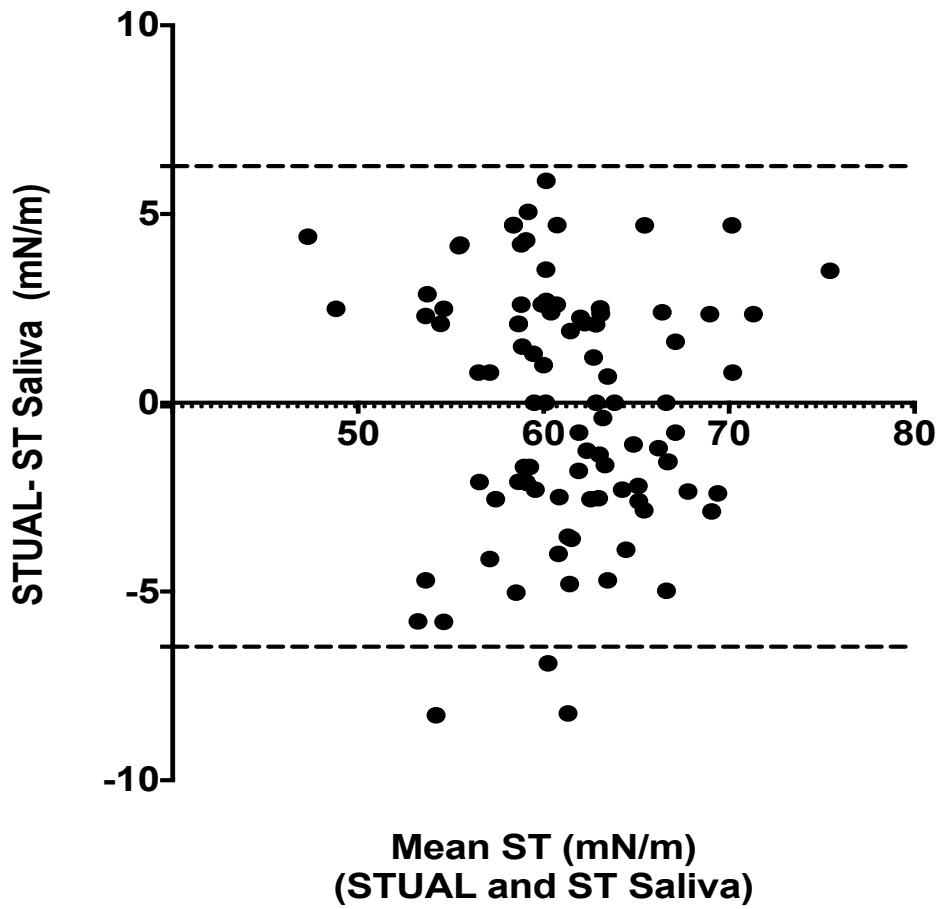


Figure 6.3 Bland-Altman graph for STUAL vs ST Saliva in OSA subjects

Bland Altman graph of average STUAL and ST Saliva vs the difference between STUAL and ST Saliva. No significant difference was observed between STUAL and ST Saliva (mean difference was 0.1 ± 0.7 mN/m = dotted black line). Dashed lines = 95% Confidence Interval limits. ST = Surface Tension; STUAL = Surface Tension Upper Airway Liquid.

ST Saliva vs STUAL

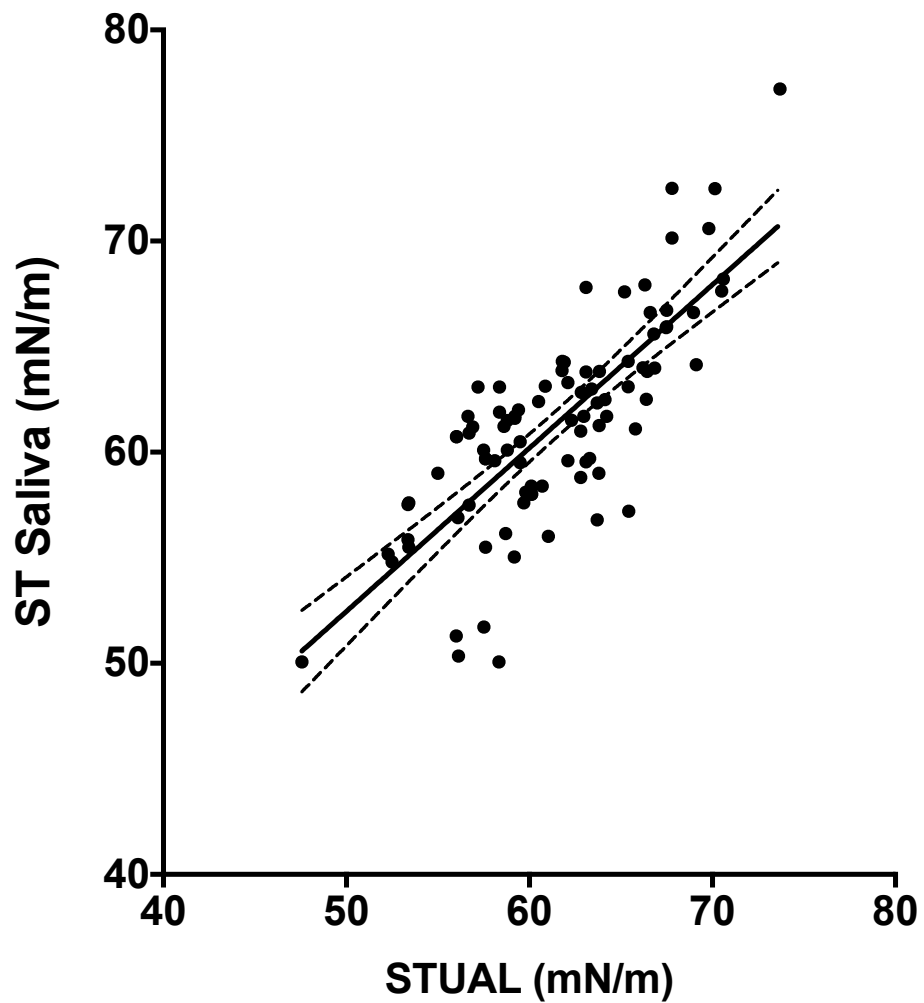


Figure 6.4 Relationship between ST Saliva and STUAL

There was a strong correlation between ST Saliva and STUAL values ($r = 0.78$, $p < 0.0001$).

Solid line = linear regression line. Dashed lines = 95% Confidence Interval limits. ST = Surface Tension. STUAL = Surface Tension Upper Airway Liquid.

STUAL vs age (all subjects)

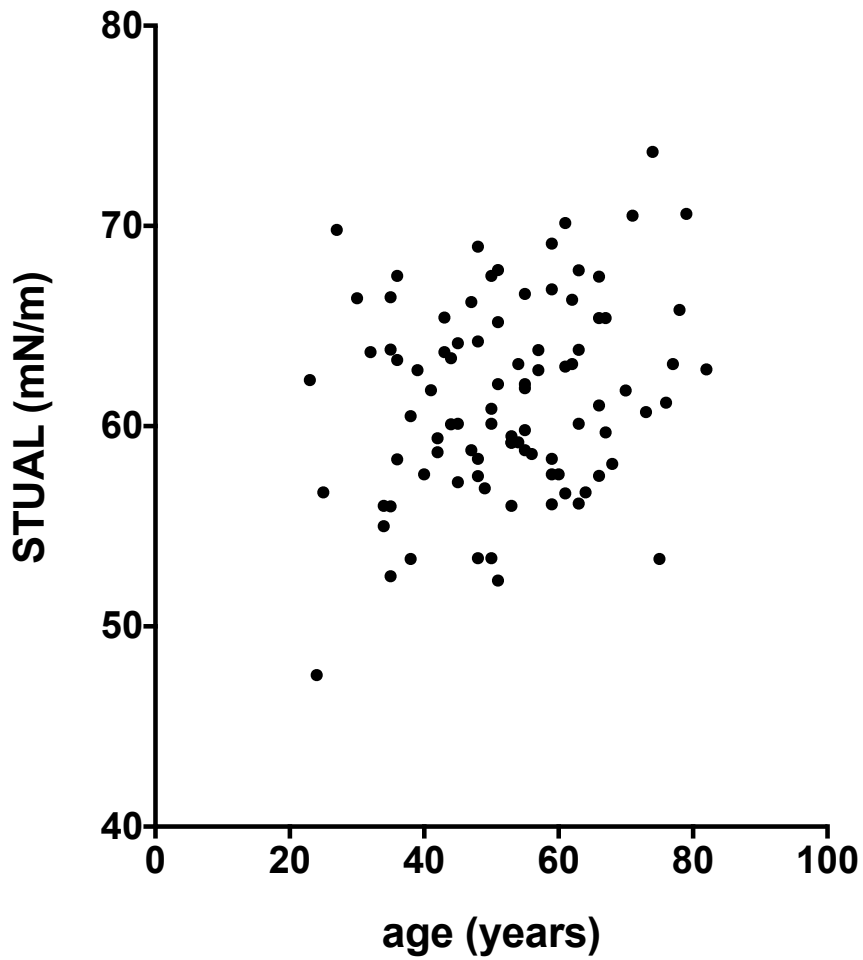


Figure 6.5 STUAL vs age for OSA subjects

Surface tension of the upper airway lining liquid (STUAL) for the whole OSA group as a function of increasing age. There was no association between age and STUAL when the subject group was observed as a whole. STUAL = Surface Tension Upper Airway Liquid.

STUAL vs age (≥ 40 years with severe OSA)

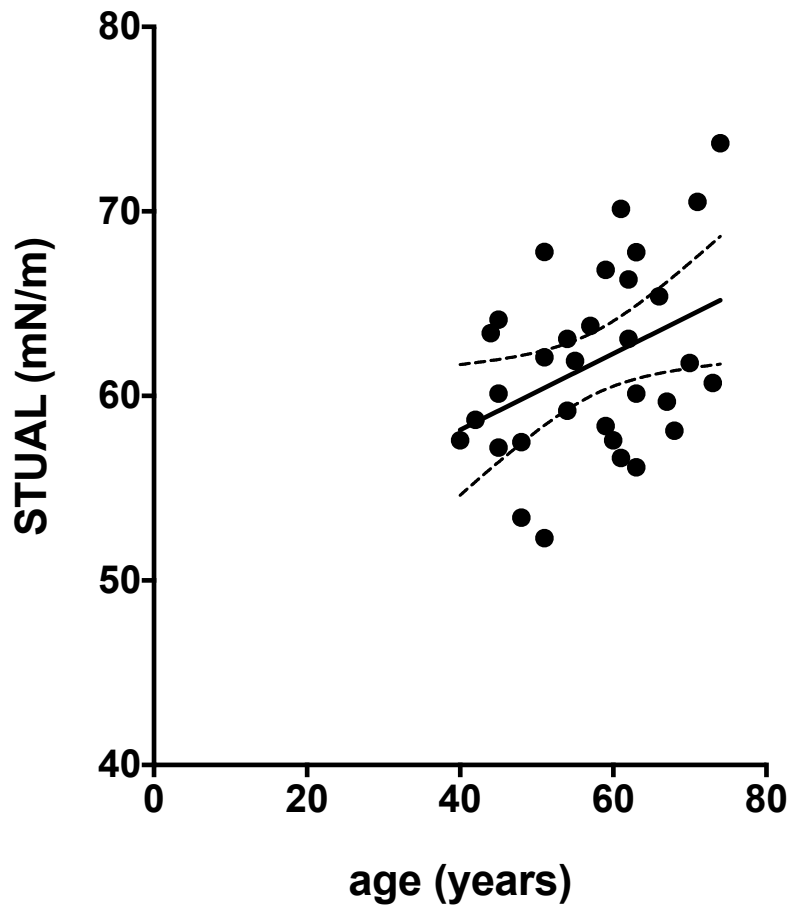


Figure 6.6 STUAL vs age ≥ 40 years (OSA subjects)

Relationship between STUAL and Age for subjects aged ≥ 40 yrs with severe OSA (RDI ≥ 30 events per hour). For patients with severe OSA, increasing age was associated with increasing STUAL ($r = 0.39$, $p = 0.03$). Solid line = linear regression line. Dashed lines = 95% confidence interval limits. STUAL = Surface Tension Upper Airway Liquid.

6.4.3 STUAL vs RDI

The mean RDI for the group was 30.9 ± 22.5 events per hour, with the range varying between 2 to 97 events per hour of sleep (Figure 6.7).

The data were examined for other univariate relationships besides RDI and STUAL. There was no significant correlation found between the RDI and ST of Saliva. Other sleep parameters including sleep efficiency and arousal index were examined for an association with STUAL and ST Saliva, however, no significant associations were found.

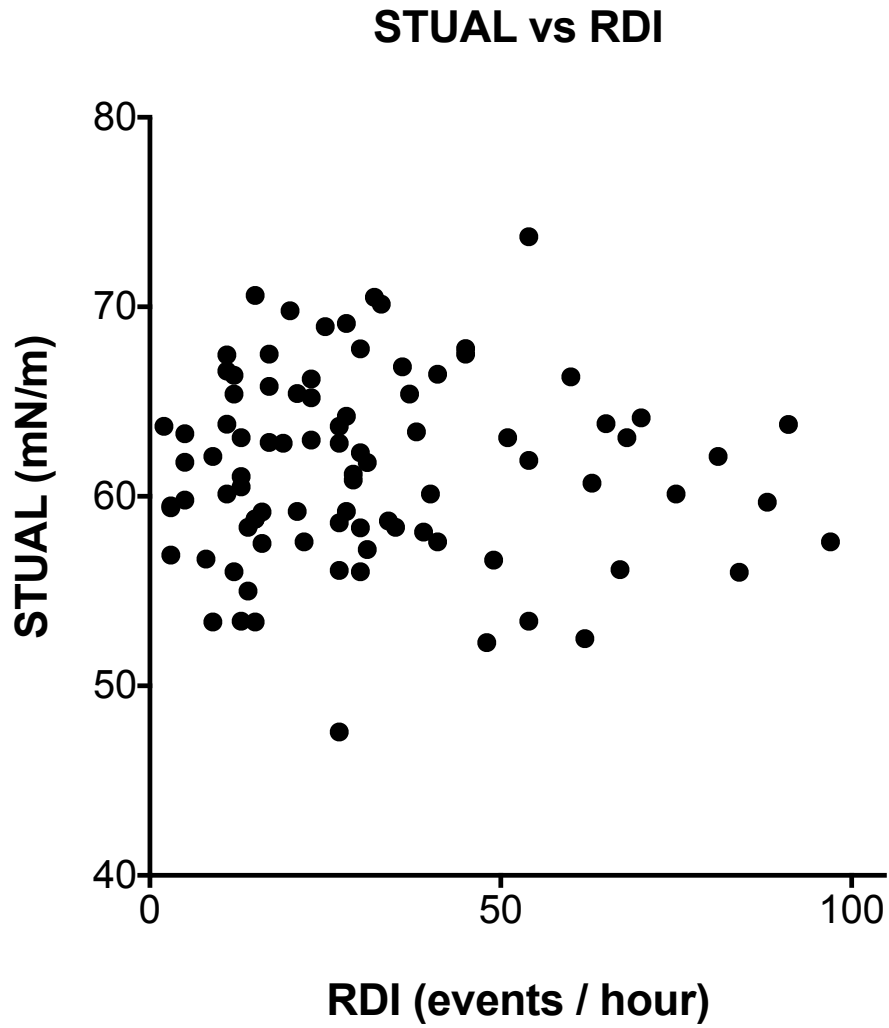


Figure 6.7 STUAL vs RDI in OSA subjects

STUAL for the whole OSA group as a function of increasing RDI. STUAL = Surface Tension Upper Airway Liquid. RDI = Respiratory Disturbance Index.

6.5 Discussion

This study demonstrated a wide range of surface tension values in subjects with OSA. There was a strong correlation between STUAL and ST of Saliva in this group of OSA subjects. In general, age did not influence STUAL in OSA patients. However, in older subjects with severe OSA, increasing age was weakly associated with increasing STUAL.

6.5.1 Critique of Methods

Although all the subjects in this study were kept nil by mouth in the hour before sampling, they were not controlled for food or liquid intake prior to this. Similarly, the route of breathing was not controlled prior to the enforced period of nasal breathing for 15 minutes before sampling time. It remains unclear as to the magnitude and duration of effect that ingested food or liquids will have on STUAL. Furthermore, despite criteria which excluded subjects with smoking, inhalers, medical conditions or inflammatory conditions which may affect the nature and volume of saliva produced, many of the prescribed drugs in use by our patients for common medical conditions have the potential to either be present in saliva, or may affect salivary output (Wolff et al., 2008), hence potentially affecting both ST of Saliva and STUAL. This could potentially account for some of the variation in STUAL values.

Previous published work from our laboratory also demonstrates a significant effect of breathing route upon STUAL (Verma et al., 2006). In that study a significant difference in STUAL between oral and nasal route of breathing was not observed until 90 minutes into the enforced route of breathing challenge. In the present study, enforced nasal route of breathing was undertaken for only 15 minutes prior to the evening sample time for practical reasons. It remains unclear the degree to which the route of breathing in the hours prior to sampling in this study may have influenced evening STUAL and ST Saliva values.

Additionally, the single sample of both UAL and Saliva collected in the evening prior to “lights out” reflects the STUAL and ST Saliva at the time of collection. This value for STUAL and ST Saliva may not be representative of the values overnight or across the day (Chapter 5), and may potentially be influenced by the other factors described above.

6.5.2 STUAL and ST Saliva

Values measured for STUAL and ST Saliva were not significantly different with the group mean difference being 0.1 ± 0.7 mN/m. As such, based on the results of this current study, and with the liquid lining of the oral cavity and the posterior oropharyngeal wall presumably predominantly comprised of saliva, under appropriately controlled circadian timing and environmental conditions STUAL and ST Saliva may be regarded as having essentially the same surface tension.

The mean evening STUAL in this group of OSAHS patients is very similar to previous results published in a smaller group of patients with OSAHS (60.1 mN/m vs 61.4 mN/m[current study]) (Kirkness et al., 2005b). There was a wide range of values observed in this study, with the lowest evening STUAL being 45.1 mN/m and the highest value being 73.7 mN/m. The minimum value seen in this study is similar to that observed in subjects given exogenous surfactant (Kirkness et al., 2003c), and there were two subjects with STUAL values below 50 mN/m. These values are inconsistent with surface tension values seen in human STUAL and ST Saliva, and may possibly be due to ingested food or drink in the hours prior to the period of nil by mouth and enforced nasal breathing prior to evening sampling.

Food or liquid containing alcohol, or foods which contain certain phospholipids may affect the surface tension of upper airways lining liquid

(van der Touw T et al. Retention of ingested phospholipids on the oral mucosa, *Respirology*, 2009), and the duration of the effect upon surface tension may potentially be longer than the period of “nil by mouth” the subjects underwent in this current study.

The highest surface tension values for UAL seen in this study are the same as the surface tension seen in normal saline (0.9% w/v of NaCl), which has one of the highest surface tension values of any liquid with the exception of liquid mercury. Overall, there were 4 subjects who had evening STUAL values greater than 70 mN/m, and values of 70 to 73mN/m are usually observed in saline solution (dependent upon concentration and temperature). Again whether these “high range” evening STUAL values are due to external influences such as ingested food or liquid prior to the “nil by mouth” period specified in the study protocol, remains unclear.

6.5.3 *STUAL and Age*

For the group as a whole, there was no correlation seen between STUAL and age. However, for the subgroup of OSA subjects ≥ 40 years of age with a RDI ≥ 30 events per hour of sleep (i.e. older OSA subjects with severe disease), there was an association between increasing STUAL with increasing age.

The association demonstrated in this study for higher STUAL with increasing age in subjects with severe OSA may potentially be due to the effect of route of breathing in older subjects. Oral breathing, nasal breathing, and oro-nasal breathing all occur during sleep in variable proportions. Verma, et al. demonstrated that increased oral breathing during daytime hours increases STUAL, and the same effect is likely to occur with increased oral breathing during sleep (Verma et al., 2006).

There is evidence of a positive association between age and oro-nasal route of breathing during sleep, with a strong correlation between oral breathing and age in males in a small study (Gleeson et al., 1986), with a larger study in healthy subjects without OSA demonstrating an association between age \geq 40 years and oro-nasal route of breathing, but only a weak association was observed (Madronio et al., 2004). In this study, by Madronio, et al. the authors found that subjects \geq 40 years of age were approximately six times more likely than younger subjects to spend $>$ 50% of the night with oro-nasal breathing route.

The present study does not address the question of whether or not route of breathing may influence the STUAL. However, the association demonstrating higher STUAL with increasing age in subjects with severe OSA raises the question of whether or not this outcome is, at least partially, influenced by the breathing route. There are no data demonstrating that STUAL changes simply due to increasing age, but a potential mechanism for any increase in STUAL may be via changes in breathing route with increasing age.

6.6 Conclusion

This study demonstrates a wide range of STUAL values in the largest group of subjects with OSA tested to date. The mean surface tension values are similar to previous studies performed in this laboratory by Kirkness et al. (2005), but the present study demonstrates a wider range in values, with some values as low as those seen with exogenous surfactant, and some higher range values similar to the surface tension of normal saline. Additionally, the ST saliva and STUAL values demonstrated a strong correlation.

The finding of a positive association between older subjects with severe OSA (aged ≥ 40 years) and increased STUAL is unexpected and the mechanisms which may potentially underlie this remain unknown, but the route of breathing during sleep is a major plausible mechanism. The following chapter will investigate the relationship between STUAL and overnight route of breathing with the aim of exploring potential underlying mechanisms.

Chapter 7: Non-enforced Breathing Route and Surface Tension of Upper Airway Liquid in Subjects with Obstructive Sleep Apnoea

7.1 Introduction

Although there are data regarding the route of breathing during sleep in subjects without evidence of obstructive sleep apnoea (Madronio et al., 2004) there are limited observational data regarding the route of breathing during sleep in subjects with OSA. Furthermore, there are few data regarding the effect of route of breathing upon overnight STUAL, and whether or not this may, in turn, affect the collapsibility of the upper airway in a subject with OSA.

Breathing route may potentially affect the collapsibility of the upper airway in sleep disordered breathing. The degree of jaw or “bite opening” may be an anatomical factor in upper airway collapsibility in OSA (Meurice et al., 1996), whilst patients with a greater proportion of the night spent oral breathing may have more episodes of apnoea (Gleeson et al., 1986). Mouth opening may also increase the critical pressure for collapse at the velo- or oro-pharyngeal level without changing the airway patency upstream towards the nose, with anatomical and neuromuscular mechanisms accounting for upper airway collapsibility (Ayuse et al., 2004) i.e. posterior movement of the jaw may

cause a reduction in size of the upper airway predisposing to airways collapse at the level of the oropharynx. A change in length-tension relationship of upper airways muscles due to this posterior jaw translation may affect the ability of these upper airway muscles to maintain patency.

The breathing route may influence upper airway collapsibility via anatomical or neuromuscular mechanisms. In addition, breathing route may also influence upper airway patency by an effect upon STUAL. Saliva contains surface active phospholipids and this contributes to the surfactant effect. The previous chapters have examined STUAL and ST Saliva values in both healthy subjects and also in subjects with OSA. The mean value for STUAL and ST Saliva in OSA patients in the previous chapter was ~ 61 mN/m. Enforced oral route of breathing in normal subjects during the day has shown that the value of STUAL may increase up to 77 mN/m after 120 minutes of enforced oral breathing (Verma et al., 2006).

Therefore, route of breathing during sleep may also influence the collapsibility of the upper airway via surface tension mediated mechanisms, with nasal breathing reducing STUAL potentially leading to a reduction in the severity of OSA, and oral breathing increasing STUAL, potentially increasing the severity of OSA.

7.2 Aims

The aim of the study described in this chapter is to measure both the evening and morning STUAL values for subjects with OSA, and to determine if there is a relationship between the change in overnight STUAL and the route of breathing overnight.

The secondary aims include measuring any changes in both the mucosal wetness and salivary flow between evening and morning sampling to determine any relationship with route of breathing overnight.

7.3 Methods

7.3.1 Subjects

Study subjects were recruited from the Westmead Hospital Sleep laboratory between June 2008 and August 2010. These subjects were recruited from patients referred into the Westmead Hospital Sleep Laboratory for investigation of sleep disordered breathing with polysomnography. There were 43 subjects recruited, and of these, 13 were female and 30 were male. Age ranged from 24 years to 82 years with a mean of 52.3 ± 13.1 years. BMI ranged from 22 kg/m^2 to 58 kg/m^2 with a mean of $32.3 \pm 7.0 \text{ kg/m}^2$.

Exclusion criteria for the study included current smoking status, current respiratory tract infection on history, any inflammatory condition or medical illness affecting the mouth or upper aerodigestive tract, previous head and neck irradiation, and history of airways disease requiring inhaled steroid medication or inhaled anticholinergic agent.

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Ethics Committee.

7.3.2 Measurements

Surface Tension

UAL samples were aspirated from the posterior pharyngeal wall using fine bore polyethylene tubing (Tyco electronics; external diameter 0.8mm, internal diameter 0.5mm), attached to a 23g needle (BD Precisionglide 0.6mm x 32mm) and a 3 ml syringe (Terumo Medical Corporation 3cc / mL).

Small microlitre volumes were aspirated into the tubing and subsequently transferred with a 1 µl syringe (7500.5N, Hamilton Company, Reno, NV, USA) to the surface of a silica disc for measurement of Surface Tension using the “Pull off force” technique (Kirkness et al., 2005a) as described in Chapter 3 (figure 3.5).

This method measures the force required to separate two silica discs bridged by a droplet of the liquid, with the force required for disc separation taken as the liquid’s surface tension.

Salivary Flow Rate

Non-stimulated salivary flow was measured by collecting saliva into a polystyrene cup, with the subject sitting forward with an open mouth to drool saliva into the collection cup for a period of 5 minutes. The subject was asked to breathe nasally and not to swallow during this period of time. This method of “salivary flow rate” measurement (Navazesh and Christensen, 1982) was used to collect a volume of saliva which was subsequently

weighed and the flow rate expressed as ml / min salivary flow rate. The weight of collected saliva was determined by weighing the polystyrene cup before and after salivary collection with the difference in weight taken as the weight of collected saliva following the 5 minute collection period.

Mucosal Wetness

Mucosal “wetness” of the upper airway was measured using a timed gravimetric contact absorbent paper strip method described by Ciantar and Caruana in 1998. The absorbent paper strip (Sialo-Strip for saliva collection, Oraflow Inc, New York, USA) was placed centrally in contact with posterior part of the tongue for 5 seconds. The volume of fluid was calculated by comparing the weight of the paper strip before and after collection and expressed as microlitres / 5 s.

Polysomnography

All subjects underwent overnight polysomnography for investigation of sleep disordered breathing. Studies were all undertaken at the Westmead Hospital Sleep Laboratory.

Variables monitored during the overnight polysomnography included nasal pressure (1600 Nasal Cannula; Salter Labs Inc., Arvin, CA, USA), EEG (electroencephalogram), ECG (electrocardiogram), submental and diaphragmatic EMG (electromyogram), left and right EOG (electro-oculogram), respiratory impedance plethysmography (for thoraco-abdominal movement), oxygen saturation, sound, and body position. Monitored signals

were recorded on a Compumedics Profusion 3 software system for further sleep stage and respiratory event scoring and analysis. Sleep staging was performed on the overnight signals using Rechtschaffen and Kales scoring criteria (Rechtschaffen and Kales 1968). Arousals from sleep were scored according to the American Sleep Disorders Association criteria (Bonnet M, 1992).

Obstructive events were scored according to “Chicago” criteria using the American Academy of Sleep Medicine criteria (American Academy of Sleep Medicine task force, 1999).

Route of Breathing

The route of breathing was determined by using a dual channel oro-nasal thermocouple device (F-ONT2A; Grass, West Warwick, RI, USA). The thermocouple leads were placed into the nares and anterior to the oropharynx to detect flow, and secured to the patient’s face with surgical tape (3M™ Micropore™ Microporous Hypo-Allergenic Surgical Tape). The qualitative signals from the thermocouple device were checked recorded for nasal-only, oral-only, and oro-nasal flow at the start of the study. The signals were checked for cross contamination and to confirm route of breathing by having each subject perform periods (up to 10 breaths) of exclusive nasal, exclusive oral breathing, and oro-nasal breathing in the supine posture.

7.3.3 Protocol

The study was conducted over one night. Subjects arrived at the Westmead Hospital Sleep Laboratory by 6pm on the study night and were set up with PSG monitoring after patient identification and PSG documentation was checked. The PSG set up included EEG with electrode placement based on the international 10-20 system (Klem et al., 1999), EMG, EOG, ECG, respiratory inductance plethysmography, finger oximeter probe, nasal pressure cannulae, and dual channel oro-nasal thermistors. Nil by mouth was maintained for a minimum time of one hour prior to “lights out” time at approximately 2200 hours. Evening (pm) UAL samples for ST were taken from the posterior oropharynx, and saliva samples for ST were taken sublingually with the subject in the seated position, immediately following 15 minutes of nasal only breathing, and prior to “lights out”.

Mucosal wetness was then sampled immediately after ST sample collection, followed by salivary flow rate collection into a cup over a 5 minute period.

Following sampling, the subject was allowed to go to sleep. All PSG monitored parameters were recorded on a computer using a Compumedics Profusion software system. The subject was permitted to sleep in their preferred posture and route of breathing was monitored overnight. The placement of nasal pressure cannulae and oro-nasal thermistors was checked if movement during the night resulted in dislodgement. A re-calibration of the nasal pressure and oro-nasal flow signal was performed

during the night if required. The subject was allowed to sleep until 0600 hours, at which time they were awoken if not so already.

Morning (am) sampling of UAL ST, saliva ST, mucosal wetness, and salivary flow rate was repeated as soon as possible following awakening at approximately 0600 hours.

7.3.4 Data Analysis

Sleep Parameters

Sleep studies were sleep staged for each 30 second epoch using Rechtschaffen and Kales scoring criteria (Rechtschaffen and Kales 1968). Respiratory events were scored according to American Academy of Sleep Medicine criteria (American Academy of Sleep Medicine task force, 1999), with the respiratory disturbance index (RDI) calculated as the total number of both apnoeas and hypopnoeas divided by the total sleep time during overnight polysomnography.

Route of Breathing Analysis

The subject's route of breathing was determined by analysis of the nasal and oral thermocouple signals, and each 30 second epoch of sleep was scored as a nasal-only, oral-only, or oro-nasal breathing epoch. Nasal-only route of breathing epochs were defined as those which contained ≥ 3 consecutive breaths seen only on the nasal thermocouple signal. Oral-only route of breathing epochs were defined as those which contained ≥ 3

consecutive breaths seen only on the oral thermocouple signal. Oro-nasal route of breathing epochs were defined as those which contained ≥ 3 consecutive breaths seen on both the oral and nasal thermocouple signals. Epochs where the route of breathing could not be confirmed based on the above criteria were excluded from analysis e.g. a combination of less than 3 consecutive oral, nasal or oro-nasal breaths within the same epoch. This, however, was rarely observed.

Epochs which contained respiratory events, significant cortical arousals, movement or signal artifacts were excluded from route of breathing analysis. i.e. epochs containing obstructive apnoeas or hypopnoeas were excluded from analysis. The proportions of nasal, oral, or oro-nasal breathing route were expressed as a percentage of the total sleep epochs which were analysed for breathing route.

The occurrence of nasal-only, oral-only or oronasal route of breathing was expressed as a percentage of the total sleep epochs (TSE) analysed.

Statistical Analysis

All data were expressed as mean \pm SD. Statistical data were graphically represented and analysed using Graphpad Prism. Single comparisons were made using paired t-test. Linear regression analysis was used to search for univariate correlation between breathing route and the overnight change in: i) STUAL; ii) mucosal wetness; and iii) salivary flow rate. $P < 0.05$ was considered significant.

7.4 Results

7.4.1 STUAL

The mean pm STUAL for the group was 61.2 ± 5.0 mN/m while the mean am STUAL was 61.4 ± 4.3 mN/m. Overall, comparison with t-test demonstrated no significant difference ($p = 0.56$) in STUAL between pm and am values (Δ STUAL) (Figure 7.1).

The maximum and minimum pm STUAL values respectively were 73.2 mN/m and 47.6 mN/m, respectively. The maximum and minimum pm STUAL values were 72.4 mN/m and 47.5 mN/m, respectively. The STUAL decreased overnight in 22 subjects and increased in 21 subjects.

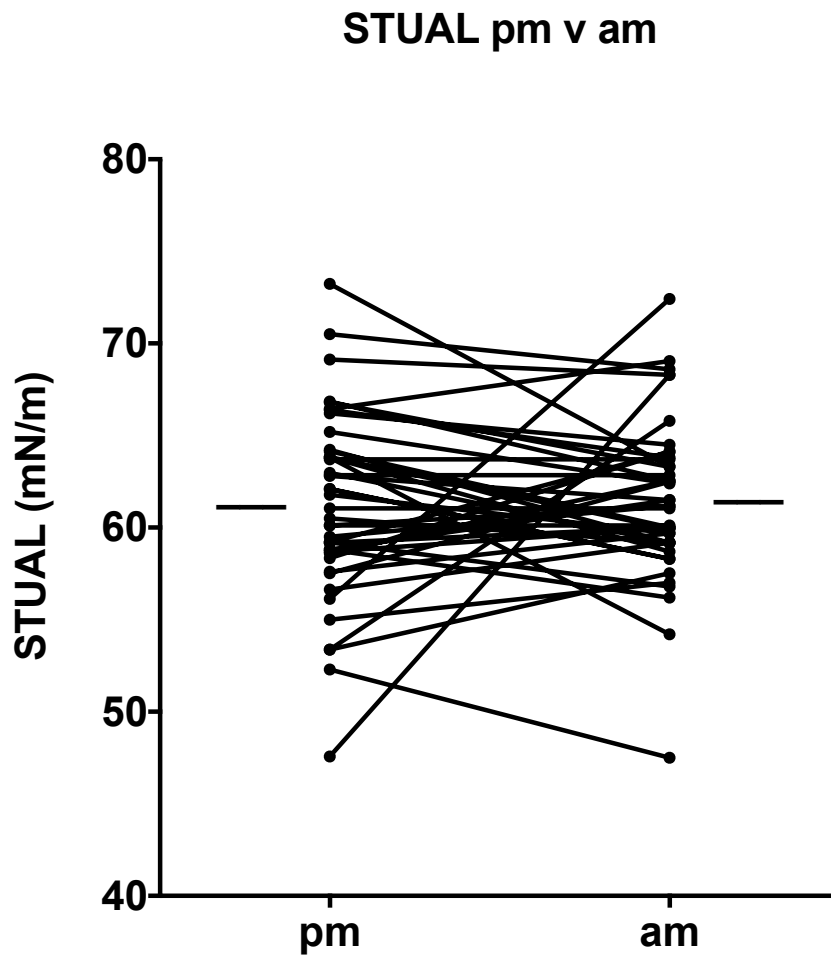


Figure 7.1 STUAL values pm vs am

Plot demonstrating the STUAL values in individual subjects ($n = 43$) with OSA both in the evening (pm) before, and in the morning (am) after overnight polysomnography. Individual data for STUAL are plotted as dots for both evening and morning values, with a line between each subject's pm and am value. Horizontal bars are the group mean data for pm and am STUAL. Overall, there was no significant difference in pm and am values (t-test, $p = 0.56$), but some individuals showed large increases and decreases in ST. STUAL = Surface Tension Upper Airway Liquid.

7.4.2 Salivary Flow Rate

The group mean pm salivary flow rate was 0.56 ± 0.36 ml/min while the am rate was 0.41 ± 0.31 ml/min (Figure 7.2). This represented a significant difference between pm and am salivary flow rate (mean difference of pm - am value [or mean Δ salivary flow rate]) of -0.15 ml/min (t-test, $p < 0.05$).

The maximum and minimum pm values for salivary flow rate were 1.58 ml/min and 0.03 ml/min, respectively. The maximum and minimum am values for salivary flow rate were 1.58 ml/min and 0.02 ml/min, respectively. The salivary flow rate decreased overnight in 34 subjects and increased in 9 subjects.

Salivary Flow Rate pm v am

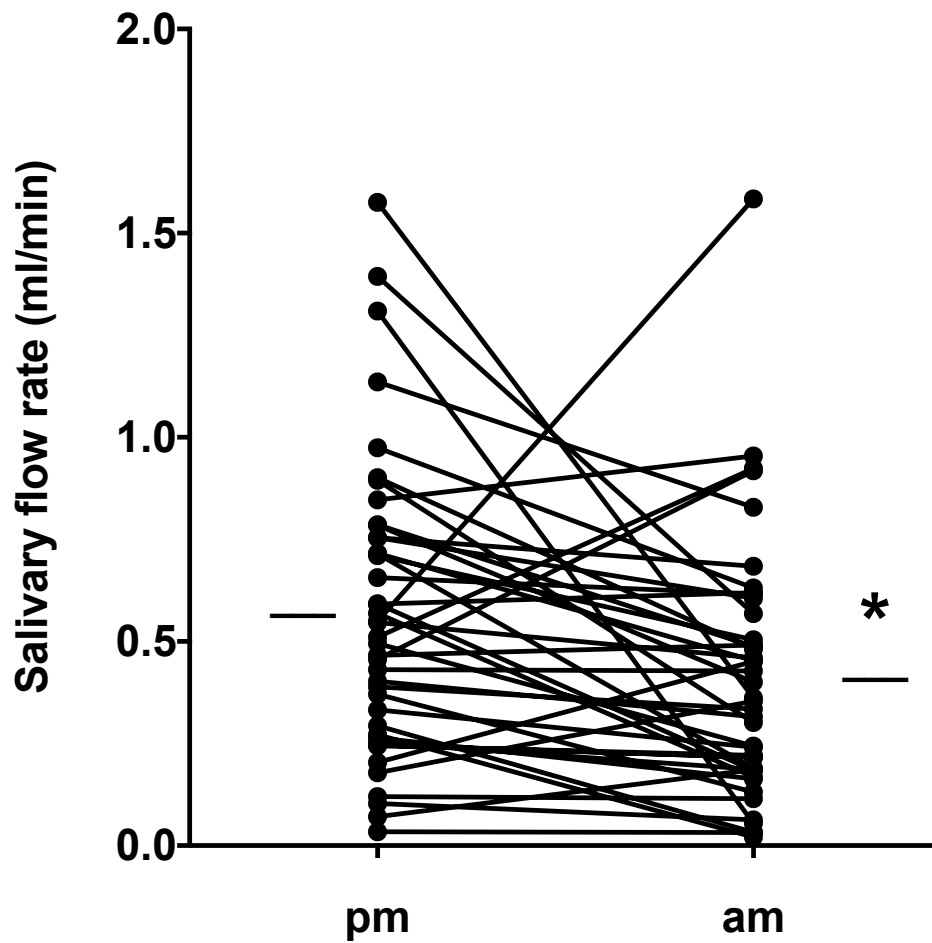


Figure 7.2 Salivary flow rate pm vs am

Salivary flow rate in individual subjects with OSA both before (pm) and after (am) overnight polysomnography. There was a significant decrease in am salivary flow rates when compared to pm salivary flow rates ($p < 0.05$). Individual data for salivary flow rate are plotted as dots for both evening and morning values, with a line between each subject's pm and am value. Horizontal bars denote mean values. * $p < 0.05$ (t-test).

7.4.3 Mucosal Wetness (MW)

The mean pm mucosal wetness for the group was $4.6 \pm 0.6 \mu\text{l (5s)}^{-1}$ while the am mucosal wetness was $3.8 \pm 0.6 \mu\text{l (5s)}^{-1}$ (Figure 7.3). There was a significant decrease in am mucosal wetness when compared to pm mucosal wetness (mean difference of pm - am value or Δ MW) of $0.8 \mu\text{l (5s)}^{-1}$ (t test, $p < 0.05$).

The maximum and minimum PM mucosal wetness values were $31.6 \mu\text{l (5s)}^{-1}$ and $0.3 \mu\text{l (5s)}^{-1}$, respectively. The maximum and minimum AM mucosal wetness values were $26.0 \mu\text{l (5s)}^{-1}$ and $0.0 \mu\text{l (5s)}^{-1}$, respectively.

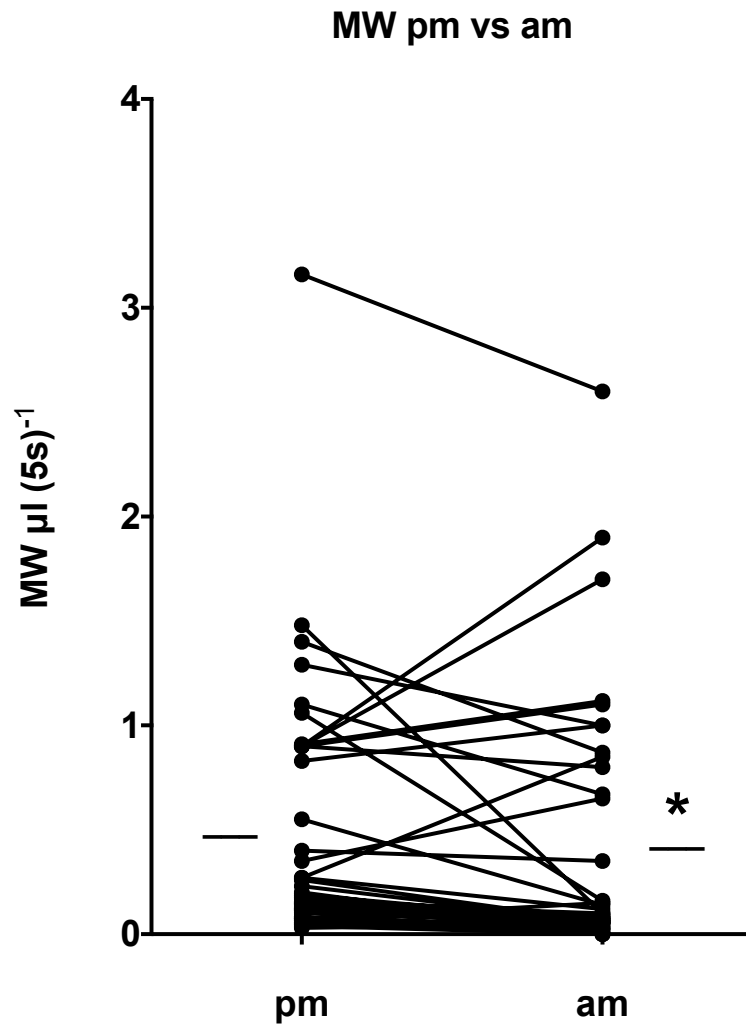


Figure 7.3 Mucosal Wetness pm vs am

Mucosal wetness in individual subjects with OSA before (pm) and after (am) polysomnography. In comparison to the pm mucosal wetness, there was a significant decrease in the am mucosal wetness. Individual data for mucosal wetness are plotted as dots for both evening and morning values, with a line between each subject's pm and am value. Horizontal bars denote mean values. * $p < 0.05$ (t-test). MW = Mucosal Wetness

7.4.4 RDI and Sleep Efficiency

The mean RDI for the group was 30.7 ± 21.2 events per hour of sleep with a minimum RDI of 9.0 and a maximum RDI of 91.0 events per hour of sleep. Of the 43 subjects, there were 9 with mild OSA (RDI 5 -14 events per hour of sleep), 20 with moderate OSA (RDI 15 - 30 events per hour of sleep), and 14 with severe OSA (RDI > 30 events per hour of sleep). The mean sleep efficiency for all subjects was normal at $77.5 \pm 10.9\%$, with a minimum sleep efficiency of 49% and a maximum sleep efficiency of 98%.

7.4.5 Route of Breathing During Sleep

The route of breathing overnight was expressed as a percentage of the night spent oronasal breathing. Following analysis of route of breathing in all subjects, there were no exclusively oral breathing epochs identified, although oronasal route of breathing was identified in variable proportions for all subjects overnight. Therefore, route of breathing was either oro-nasal or nasal-only in varying proportions for all subjects.

The mean percentage of overnight breathing epochs spent oronasal breathing was $29.0 \pm 28.4\%$. The minimum proportion of oronasal breathing epochs seen amongst all subjects was 0%, with the maximum proportion of oronasal breathing epochs observed amongst all subjects being 100%.

When the data were examined for univariate relationships, there was no significant relationship between route of breathing overnight and Δ STUAL, Δ salivary flow, Δ mucosal wetness, RDI, or age (Figures 7.4 to 7.8).

The data were also examined for the percentage of epochs spent oronasal breathing in the final hour of sleep before AM sampling, and univariate analysis was also performed on these data, with no significant relationships identified.

Table 7.1 demonstrates the proportion of subjects (age > 40 years vs age \leq 40 years) who utilised either \leq 50% of total sleep epochs oronasal breathing route or > 50% of total sleep epochs utilising the oronasal breathing route. For those subjects aged >40 years, seven (or 16.3%) of the subjects had increased oronasal breathing (> 50% TSE). However, in light of the small sample size of the study group, this observation should be interpreted with caution and the study may be underpowered for this result to be statistically significant.

RDI vs Oronasal breathing

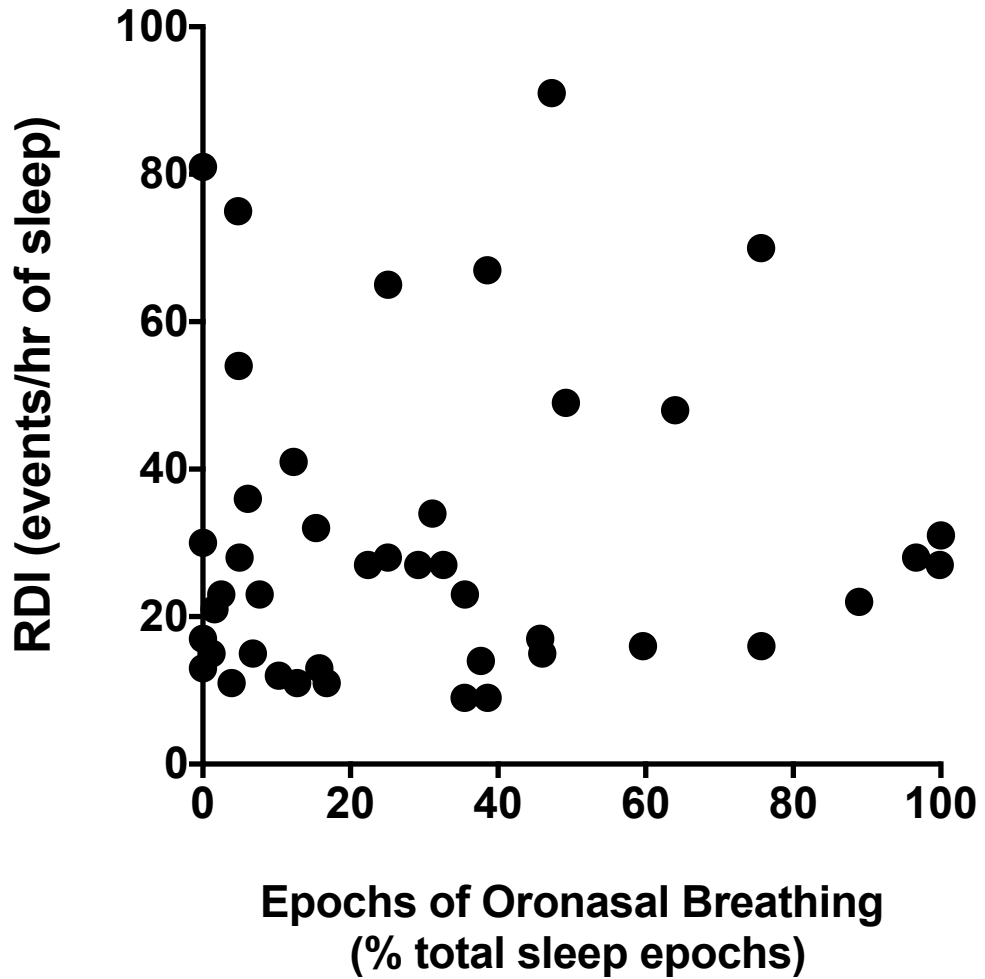


Figure 7.4 Plot of RDI as a function of Epochs of Oronasal Breathing

No significant relationship was identified. RDI = Respiratory Disturbance Index.

Δ STUAL vs Oronasal Breathing

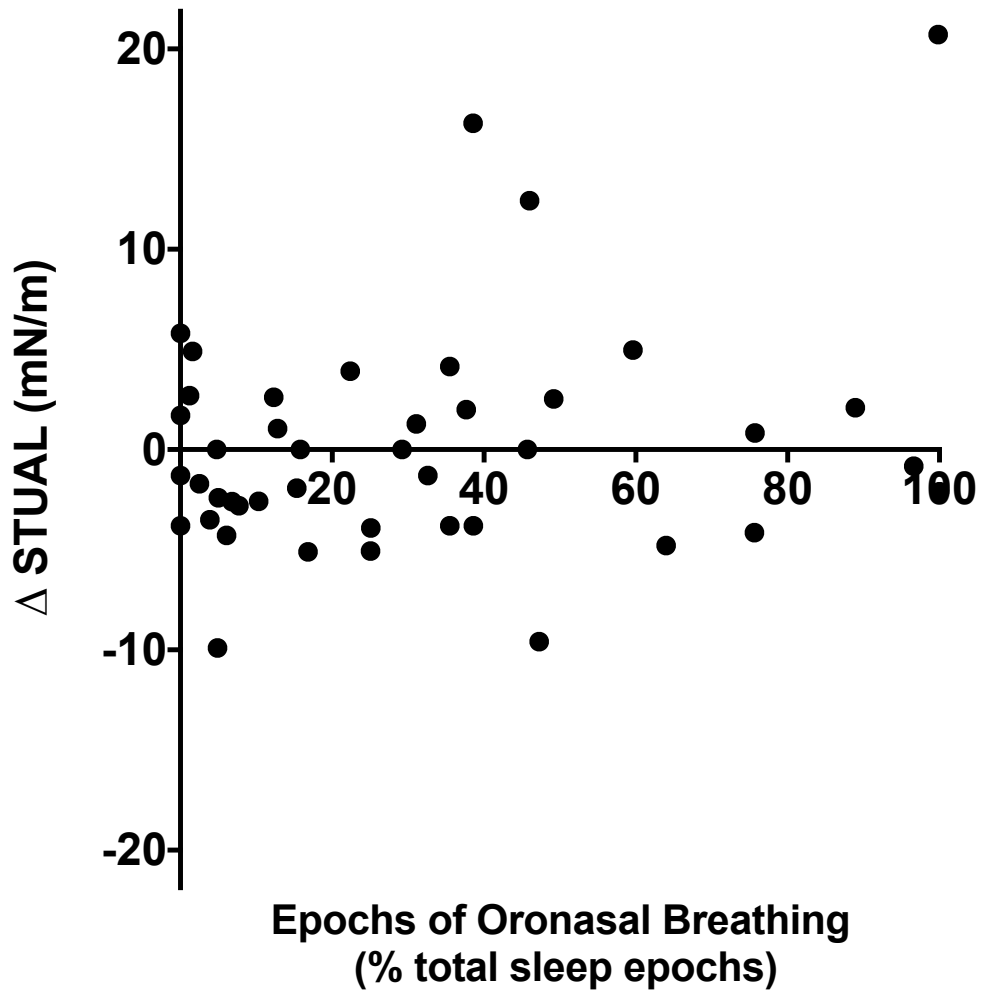


Figure 7.5 Plot of Δ STUAL as a function of Epochs of Oronasal Breathing

No significant relationship was identified. STUAL = Surface Tension Upper Airway Liquid.

Δ Salivary Flow Rate vs Oronasal Breathing

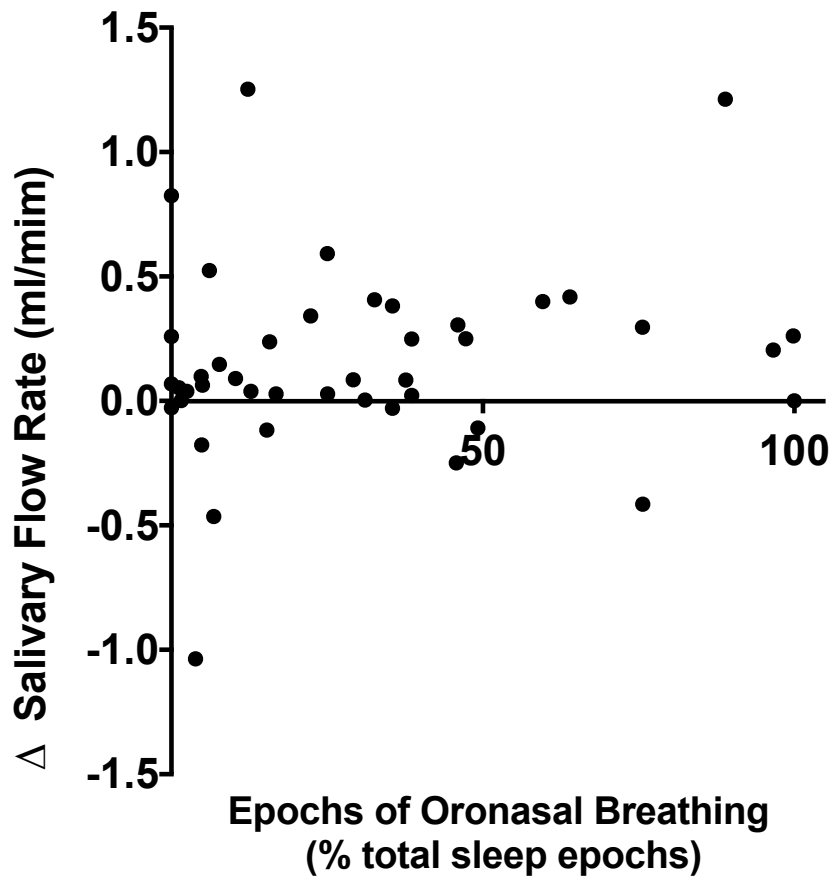


Figure 7.6 Plot of Δ Salivary Flow Rate as a function of Epochs of Oronasal Breathing

No significant relationship was identified.

Δ Mucosal Wetness vs Oronasal Breathing

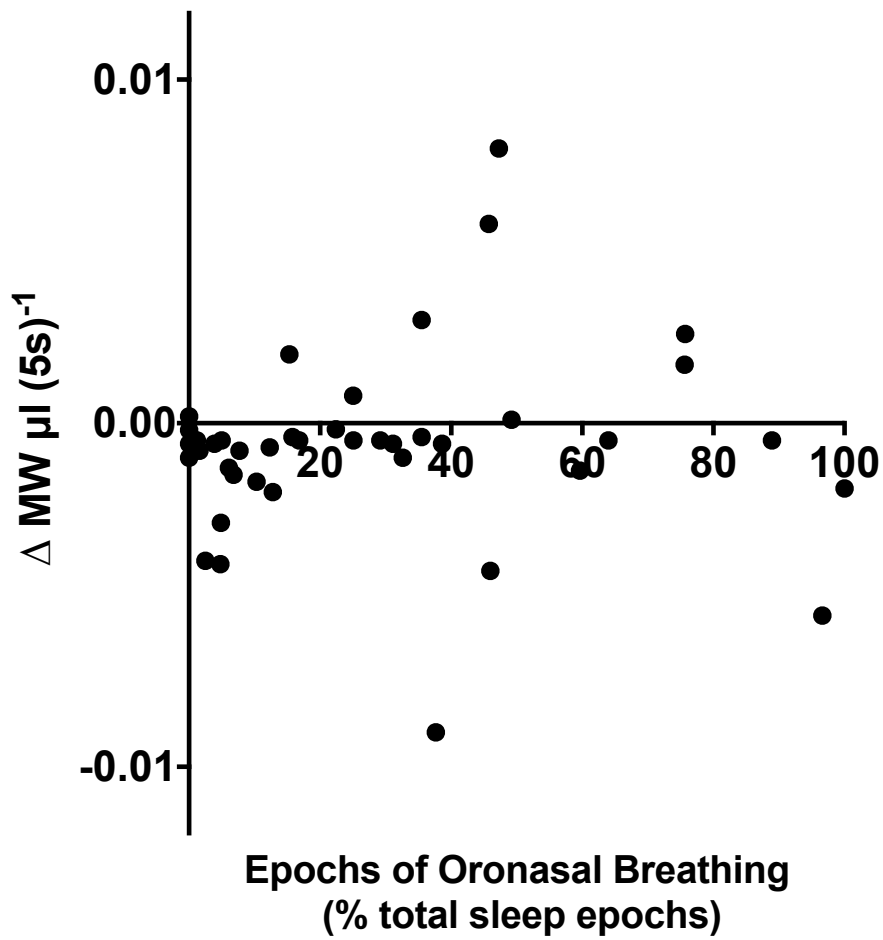


Figure 7.7 Δ Mucosal Wetness (MW) as a function of Epochs of Oronasal Breathing

Plot of Δ Mucosal Wetness (MW) as a function of Epochs of Oronasal Breathing. No significant relationship was identified.

Age vs Oronasal Breathing

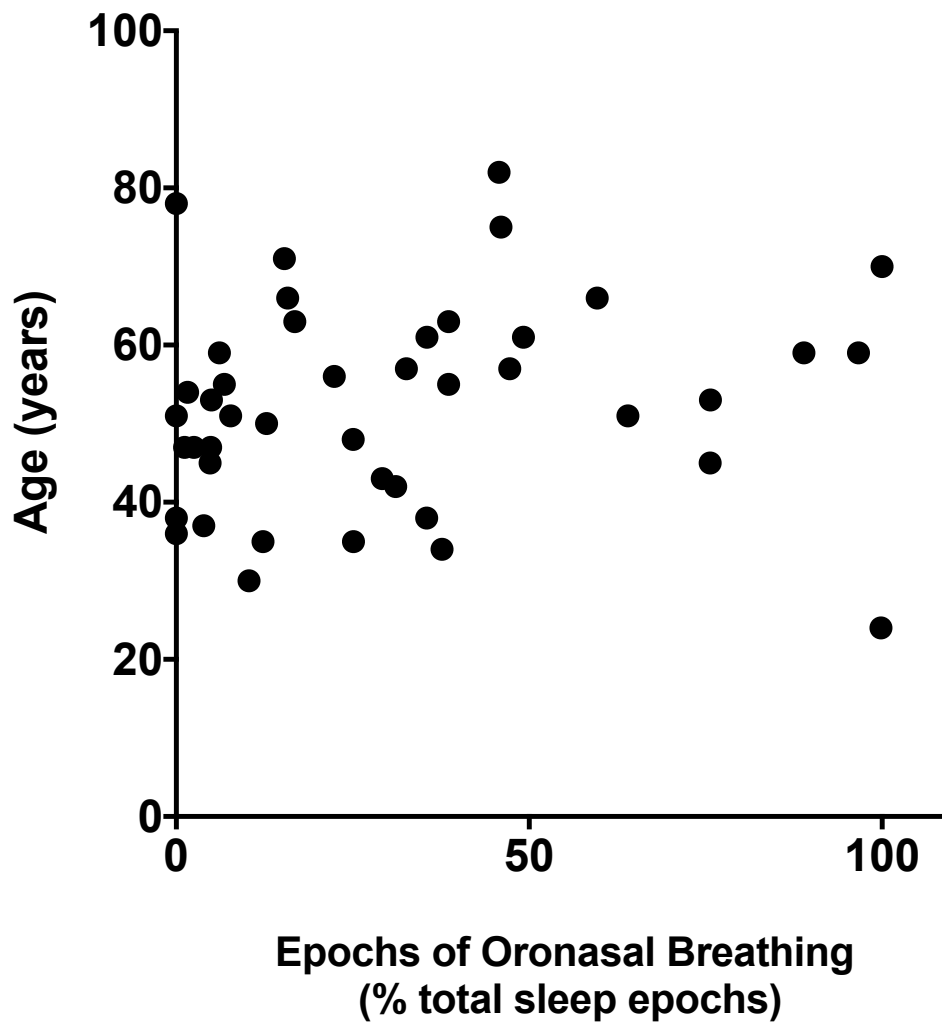


Figure 7.8 Age as a function of Epochs of Oronasal Breathing

Plot of Age as a function of Epochs of Oronasal Breathing. No significant relationship was identified.

Epochs of Oronasal breathing (% total)	Age < 40 years	Age ≥ 40 years
≤ 50%	8 (18.6%)	27 (62.8%)
> 50%	1 (2.3%)	7 (16.3%)
Total (n=43)	9 (20.9%)	34 (79.1%)

Table 7.1 Occurrence of oronasal breathing route during sleep in younger and older subjects.

7.5 Discussion

This study demonstrated that in subjects with OSA, oronasal breathing may be present anywhere between 0% to 100% of TSE. There was no relationship between the % TSE spent oronasal breathing to STUAL, MW, SF and age. There were significant differences in pm vs am MW and SF, with mean values decreasing overnight.

7.5.1 Critique of Methods

The method of analysis for determining route of breathing for each epoch was the same as that previously used in our laboratory (Madronio et al., 2004). In a previous paper published on route of breathing, this method of route of breathing analysis was used to determine nasal, oral, or oro-nasal route of breathing for each epoch (Madronio et al., 2004). Epochs with scored respiratory events (i.e. epochs with obstructive apnoeas or hypopnoeas), were excluded from analysis, and hence it is not possible to determine whether epochs where respiratory events are detected are associated more frequently with nasal, or oro-nasal breathing. Furthermore, a respiratory event and subsequent EEG arousal often resulted in substantial breathing signal artifact, making it impossible to score the route of breathing until stable sleep had resumed.

Route of breathing in this study was determined by use of a dual channel oral and nasal thermocouple device. Despite utility in determining the route of breathing during polysomnography, there were potential methodological problems, which included changes in device positioning during the study as a result of movement or posture changes overnight, or cross contamination of the oro-nasal route with nasal breathing,. These potential issues were identified, and were minimised by careful checking, adjustment and recording of thermistor flow signals separately for the oral, nasal, and oro-nasal breathing route prior to the study, in addition to securing the thermocouple device both to the subject's cheeks and also by looping behind the ears. In addition, the signals from the thermocouple device were carefully monitored by the sleep laboratory technicians overnight for any dislodgement or signal aberration which was addressed as required.

Analysis of route of breathing signals in this manner was primarily to qualitatively detect the route of breathing and there was no quantitative analysis of airflow, since these thermocouple devices are highly sensitive to the presence or absence of airflow (Norman et al., 1997) and are not linearly related to total flow rate. This is a major drawback of this technique as it is unable to quantify the amount of oral airflow during oro-nasal breathing which may be critical to understanding its influence on measures of STUAL and mucosal wetness.

Significant variability was observed between pm and am STUAL values in some patients. The coefficient of variation in the ST measurement

is approximately 5%, however some subjects have changes (increase or decrease in STUAL) which were greater than 10% overnight. This may potentially be due to ingested food or liquid in the hours leading up to the evening sampling, which may have influenced the initial STUAL value despite the nil by mouth period. Alternately, there may have been changes in breathing route overnight in these individuals which may have significantly altered STUAL values from the morning sample.

Furthermore, fluid sampling was only performed at two time points, being the evening prior to the study and the morning after the study, with no sampling points during the middle of the night. Route of breathing exhibited significant variability during the night for individual subjects, with variable periods of nasal and oro-nasal breathing during the night, and the percentage of oro-nasal breathing expressed as a percentage of all scored epochs during the night may not reflect the variability seen with changing breathing route during sleep. The timeline for breathing route to influence STUAL is unknown, and it may not be appropriate to examine whole of night breathing route in the context of an early morning UAL sample. Nevertheless, examination of the breathing route over the final hour of sleep did not demonstrate any better relationship with STUAL or mucosal wetness.

7.5.2 STUAL

In the present study, there was no significant difference in STUAL between evening and morning samples in subjects with non-enforced breathing route. There was no significant relationship between epochs of oronasal breathing overnight and STUAL, MW, Salivary Flow, or RDI . The STUAL values were similar to those seen in previous chapters for both normal subjects and subjects with OSA.

There is evidence that STUAL may be influenced by breathing route. During enforced oral breathing during the day, the STUAL increases significantly from approximately 64 mN/m to 77 mN/m in healthy subjects, with enforced nasal breathing resulting in a decrease from 59 mN/m to 52 mN/m after two hours of enforced breathing route (Verma et al., 2006). In this study, the spontaneous nocturnal breathing route was monitored, but not enforced, and in the setting of significant variability in breathing route seen during the night, the STUAL might also have changed during the course of the night (increased oronasal breathing associated with increased STUAL). However, no such relationship between STUAL and oronasal breathing route was observed. To account for the possibility that STUAL may only be influenced by breathing route in the hour or two prior to sampling (rather than a whole night effect), further analysis was performed for route of breathing in the final hour of sleep prior to morning sampling. There was no significant difference between epochs of oronasal breathing in the final hour of sleep and either the am STUAL or the overnight Δ STUAL. From this present study, there does not appear to be any significant relationship between overall

proportion of oronasal breathing route overnight and STUAL in OSA subjects with non-enforced route of breathing.

7.5.3 Salivary Flow

There was a significant difference seen between pm and am salivary flow. This difference was expected and is consistent with literature describing a reduction in salivary production during sleep. Physiologically, salivary flow rate decreases overnight during sleep (Dawes, 1975), and reduced saliva production is seen in pathology such as in patients with Sjogren's syndrome (Fox et al., 1984). The significance of a reduction in salivary production during sleep is unknown, but it is a recognised phenomenon, and in certain circumstances may contribute to xerostomia overnight during sleep (Thie et al., 2002). There was no significant relationship between Δ salivary flow and proportion of TSE utilizing oronasal breathing route, nor were there any significant relationships identified between epochs of oronasal breathing overnight and (pm and am) salivary flow rates.

7.5.4 Mucosal Wetness

A significant decrease was seen in mucosal wetness overnight in this study which is similar to the decrease seen in salivary flow. It is likely that a combination of both reduced saliva production during sleep, and a degree of

oronasal breathing, results in a “drying out” effect on the tongue and oral mucosa. Verma et al, in addition to describing changes in surface tension with enforced breathing route in awake subjects, also demonstrated a significant reduction in mucosal wetness after enforced oral route of breathing (Verma et al., 2006), with the mucosal wetness at 120 minutes being at the lower limits of detection at $0.1 \mu\text{l. (5s)}^{-1}$. There were no significant relationships identified between mucosal wetness, and the % oronasal breathing. Although both mucosal wetness and salivary flow were significantly reduced at 4 and 8 hours respectively compared to 0 hours, there was no significant correlation between mucosal wetness and salivary flow.

7.5.5 Route of Breathing

Nasal, oral and oronasal breathing occurs during sleep. Oral or oronasal route of breathing may, in turn, affect the STUAL overnight, both due to a “drying effect” of the oral route, such as described by Verma et al in their enforced daytime route of breathing study, or due to reduced salivary production during sleep. Even in the absence of a reduction in salivary production, evaporative water loss from the mucosal surfaces overnight may affect STUAL. However, the lack of an identified relationship between epochs of oronasal breathing and STUAL may possibly be due to the variability of route of breathing overnight, variability in quantity of oral breathing, and variability in oral flow rates, such that the proportion of the night as a whole spent nasal vs oronasal breathing (measured qualitatively) does not reflect

the dynamic and changing influence of switching breathing route overnight and varying the proportions of oral flow quantity and oral flow rate.

In the present study, subjects were observed to either breathe nasally, or oronasally, but there was no exclusive oral route of breathing observed. The predominant route of breathing overall was nasal breathing. This may also have contributed to the absence of any relationship seen in this study. There were no relationships identified between route of breathing and STUAL, MW, salivary flow, or RDI.

In contrast to previous published data which demonstrated a weak correlation between epochs of oronasal breathing and age (Madronio et al., 2004), there was no significant relationship between epochs of oronasal breathing and age in the present study. Previous literature has reported a relationship between age and breathing route, with older subjects having an increased proportion of oronasal route of breathing seen during sleep. This observation, reported by Madronio et al. (2004) which was performed in subjects without OSA, was not seen in the present study in which all subjects had OSA with varying degrees of severity. Furthermore, the route of breathing during a respiratory event i.e. during apnoeas and hypopnoeas, was excluded from analysis, firstly for consistency in analysis since the previous study published by Madronio et al. (2004) was scored for breathing route in an identical manner, and secondly due to limitations in signal interpretation during epochs containing respiratory events. These epochs were often difficult to analyse for breathing route due to signal artifact from

the respiratory disturbance and subsequent arousal. One of the possible reasons for not observing a relationship between route of breathing and age may be the subject population for this study. The subjects in this study had a significant degree of OSA, with the mean RDI being 30.7 ± 21.2 events per hour of sleep, and this, in turn, reduces the number of sleep epochs which may be analysed for breathing route. The route of breathing and age relationship found in the Madronio et al. (2004) study was relatively weak, and the severity of OSA in the subject population in the present study may be a factor in the difference found between the two studies.

7.6 Conclusion

In subjects with OSA, nasal and oronasal breathing routes are observed during sleep. Nasal route of breathing occurs in all subjects and is the predominant breathing route, whilst oronasal route of breathing is also observed in the majority of subjects. There were no exclusive oral breathing epochs observed in any of the subjects, which is consistent with previous literature which has demonstrated that exclusive oral route of breathing epochs occur rarely during sleep.

There was no significant relationship between the route of breathing during sleep and STUAL. There is evidence of enforced route of breathing in awake subjects altering STUAL, with oral breathing increasing STUAL and nasal breathing decreasing STUAL. However in the present study, there was significant variability between subjects seen in breathing route overnight. The overall epochs of oronasal breathing overnight (measured qualitatively) not adequately convey this variability in breathing route overnight, and hence the influence of uncontrolled breathing route on STUAL in OSA subjects during sleep is difficult to accurately quantify. The methodology of the current study may have contributed to the negative findings, and a relationship between route of breathing and STUAL has not been confidently excluded. A study with controlled or enforced breathing route (measured quantitatively) during sleep, may potentially better determine the effect of breathing route upon STUAL, salivary flow, mucosal wetness, and possibly RDI.

The subsequent chapters will investigate the relationship of breathing route, STUAL, and severity of OSA further using enforced oral or nasal overnight breathing routes.

Chapter 8: Influence of Enforced Breathing Route on Surface Tension of Upper Airway Liquid and Severity of Sleep Disordered Breathing in Healthy Subjects

8.1 Introduction

Both nasal and oral breathing occur during sleep. Nasal, oral and oro-nasal breathing route is observed during sleep in subjects without polysomnographic evidence of obstructive sleep apnoea (Madronio, Di Somma et al. 2004). In this study, oral only breathing occurred rarely, while most subjects switched between nasal and oro-nasal breathing.

Oral route of breathing has been associated with increasing upper airway lining liquid surface tension (Verma, Seto-Poon et al. 2006). In a study of awake normal subjects, the STUAL, and mucosal “wetness” were measured after 120 minute periods of enforced oral breathing, and enforced nasal breathing. During enforced oral route of breathing, the STUAL increased from 64.4 mN/m to 77.4 mN/m, while nasal route of breathing resulted in a decrease in STUAL from 59.3 mN/m to 51.8 mN/m. The authors speculated that nasal breathing may contribute to reducing the severity of sleep disordered breathing by reducing STUAL. Kirkness et al have previously shown that changing STUAL may effect the severity of sleep disordered breathing (Kirkness et al., 2005b), and hence breathing route, with its effects on surface tension, may play a role.

Upper airways resistance is also higher with enforced oral breathing route. Fitzpatrick et al investigated the effect of enforcing oral or nasal breathing route on the upper airways resistance and the severity of sleep disordered breathing (Fitzpatrick et al., 2003). Subjects who breathed via the oral route only during sleep had a higher upper airways resistance, and obstructive apnoeas and hypopnoeas were more frequent. The subjects in this study demonstrated no significant obstructive sleep apnoea with nasal only breathing, but demonstrated severe obstructive sleep apnoea in the supine posture (31 ± 8 events per hour of sleep) as well as in the lateral posture (29 ± 9 events per hour) with oral breathing.

Patients with more oral breathing epochs during sleep have higher AHIs and longer duration of obstructive events than snorers without clinically significant OSA (Koutsourelakis, Vagiakis et al. 2006), implying a pathophysiological relationship.

In subjects with severe nasal obstruction and obstructive sleep apnoea, McLean et al demonstrated a modest reduction in the severity of obstructive sleep apnoea following treatment of nasal obstruction in 10 subjects (McLean, Urton et al. 2005). In this group of patients with nasal obstruction, the oral fraction for route of breathing during sleep fell from a mean of 39% to 8% following topical nasal decongestant. However, this did not result in the complete elimination of oral breathing nor bring the severity of sleep disordered breathing back to the normal range. Similarly, studies

looking at treating nasal congestion using nasal steroids, nasal dilators, and nasal decongestants, have shown only modest reductions in the severity of obstructive sleep apnoea (Kohler, Bloch et al. 2007).

8.2 Aims

The aim of this chapter is to study the effect of enforced nasal and oral route of breathing on the surface tension of upper airway lining liquid and any resultant change in the severity of sleep disordered breathing in healthy subjects. Specifically, the aim was to attempt to produce sleep disordered breathing by enforced oral route of breathing, and to examine the influence of enforced oral breathing route at night on STUAL and the severity of any resultant sleep disordered breathing.

8.3 Methods

8.3.1 Subjects

Healthy subjects were screened by questionnaire and clinical history. The subjects were recruited from amongst staff members from Westmead Hospital and from the Ludwig Engel Centre for Respiratory Research. 8 healthy volunteers were recruited for this study (6 male, 2 female; age: 30.3 ± 4.0 years, range 24 – 34 years; BMI: 24.4 ± 3.3 kg / m², range 21.5 – 32.6 kg / m²). Subjects with any nasal obstruction, or history of nasal or sinus pathology were excluded from the study.

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Human Ethics Committee.

8.3.2 Measurements

Surface Tension

Surface Tension of Upper Airway Lining Liquid (STUAL) was measured by the “Pull Off Force Technique” (Kirkness et al., 2005a). This technique is described in previously in more detail in chapter 3 (Figure 3.5). Microliter volumes of upper airway lining liquid are placed between two silica

discs, and the force required to separate the discs with the microliter volume of liquid bridging both disc surfaces, is taken to be the surface tension.

Upper Airway Lining Liquid

Upper airway lining liquid was sampled from the posterior oropharyngeal wall. Samples were aspirated into polyethylene tubing (Tyco Electronics; external diameter 0.8 mm; internal diameter 0.5 mm), using a 23g needle (BD PrecisionGlide 0.6mm x 32mm) attached to a 3ml syringe (Terumo Medical Corporation 3cc / mL).

Samples were then applied onto the surface of a silica disc for measurement of surface tension in the surface force measurement device (Kirkness et al., 2005a).

Salivary flow rate

Non-stimulated salivary flow rate was measured by collecting saliva into a polystyrene cup. The subject sits forward and drools into a collection cup over a five minute period as previously described by Navazesh et al. (1982). The saliva collected was weighed and the flow rate expressed as ml / min (salivary flow rate).

Mucosal Wetness

Mucosal “wetness” of the upper airway was measured using a timed gravimetric contact absorbent paper strip method described by Ciantar et al. in 1998 (Ciantar and Caruana, 1998). The absorbent paper strip (Sialo-Strips for saliva collection, Oraflow Inc, New York, USA) was placed centrally in contact with posterior part of the tongue for 5 seconds. The volume of fluid was calculated by comparing the weight of the paper strip before and after collection and expressed as microlitres / 5 seconds.

Polysomnography

All polysomnographic studies were undertaken at the Westmead Hospital Sleep Laboratory. The variables monitored overnight included EEG (electroencephalogram), ECG (electrocardiogram), submental and diaphragmatic EMG (electromyogram), left and right EOG (electro-oculogram), respiratory inductance plethysmography (for thoraco-abdominal movement), oxygen saturation, sound, and body position. Nasal pressure measurement for airflow was not utilized. All signals were recorded on a Compumedics Profusion 3 software system. Sleep staging was performed on the overnight signals using Rechtschaffen and Kales rules (Rechtschaffen and Kales 1968) and obstructive events were scored using “Chicago” criteria (American Academy of Sleep Medicine task force, 1999). Arousals were scored using the American Sleep Disorders Association criteria (Bonnet M, 1992). All studies were scored by a single sleep technician.

Route of Breathing

Route of breathing was confirmed by using a dual channel oro-nasal thermocouple device (F-ONT2A; Grass, West Warwick, RI, USA). The thermocouple was secured to the patient's face using tape (3M™ Micropore™ Microporous Hypo-Allergenic Surgical Tape), with the thermocouple leads looped behind both ears. The qualitative signals from the thermocouple device were checked recorded for nasal-only, oral-only, and oro-nasal flow at the start of the study. The signals were checked for cross contamination and to confirm route of breathing by having each subject perform periods (up to 10 breaths) of exclusive nasal, exclusive oral breathing, and oro-nasal breathing in the supine posture. Furthermore, to prevent movement or dislodgement during sleep, the thermocouple device was secured to the skin behind the ears and lateral to the cheeks with adhesive tape. Overnight, the signals were checked following any movement or awakening which resulted in dislodgement of the thermocouple device and signals were checked and re-calibrated if necessary.

8.3.3 Protocol

All subjects arrived at the Westmead Sleep laboratory by 6pm on the night of the study and were set up with PSG monitoring after patient identification and PSG documentation was checked by the sleep laboratory technician. The PSG set up included EEG with electrode placement based

on the international 10-20 system (Klem et al., 1999), EMG, EOG, ECG, respiratory inductance plethysmography, finger oximeter probe, nasal pressure cannulae, and dual channel oro-nasal thermocouple device. Subjects remained nil by mouth for at least one hour prior to “lights out” which was approximately 2200 hours. Sampling for STUAL was taken with the subject seated upright following 15 minutes of nasal only breathing, before “lights out”. Immediately following UAL sampling, mucosal wetness was sampled, and the salivary flow was then collected with the subject seated in a forward position, and saliva collected into a cup over a 5 minute period.

The study was conducted over two separate nights. The subject was randomised to either enforced nasal or oral route of breathing for one full night each. Oral route of breathing was enforced with cotton wool inserted into both nares, with tape (3M™ Leukosilk®) external to the nares to seal both nostrils. Nasal breathing route was enforced by using tape (3M™ Leukosilk®) over the mouth. Enforcement of breathing route took place after all samples were taken in the evening and immediately before “lights out”. Sampling for UAL, mucosal wetness, and salivary flow was repeated at 4 hours after lights out, and at 8 hours after lights out at the end of the sleep recording. The patients were woken from sleep for the 4 and 8 hour sampling (Figure 8.1).

8.3.4 Data Analysis

Sleep Parameters

Studies were sleep staged for each 30s epoch using Rechtschaffen and Kales criteria (Rechtschaffen and Kales 1968). The Respiratory Disturbance Index (RDI) was derived using the respiratory inductance plethysmography signal (RIP) as the primary scoring channel, together with oximetry and EEG arousals. The total RDI was an aggregate of the number of apnoeas and the number of hypopnoeas (including “Chicago type” hypopnoeas) divided by the total sleep time during polysomnography. Respiratory Events were identified using American Academy of Sleep Medicine criteria (AASM Task Force; Sleep 1999). An obstructive apnoea was defined as a 10 second or greater absence of airflow (thermistor) with oximetry demonstrating a 3% or greater reduction in oxygen saturation compared to baseline. A hypopnoea was defined as a greater than 30% reduction in respiration (RIP), together with oximetry demonstrating a 3% or greater reduction in oxygen saturation, for a duration of 10 seconds or more. A “Chicago” type hypopnoea was defined as a subjective reduction in respiration (RIP) resulting in an oxygen desaturation of 3% or more, or terminating in an arousal, with the duration of the event being 10 seconds or more (AASM Task Force; Sleep 1999). Additionally, polysomnographic variables were analysed for sleep staging, Sleep Efficiency, Arousal Index, and Respiratory vs Spontaneous Arousals.

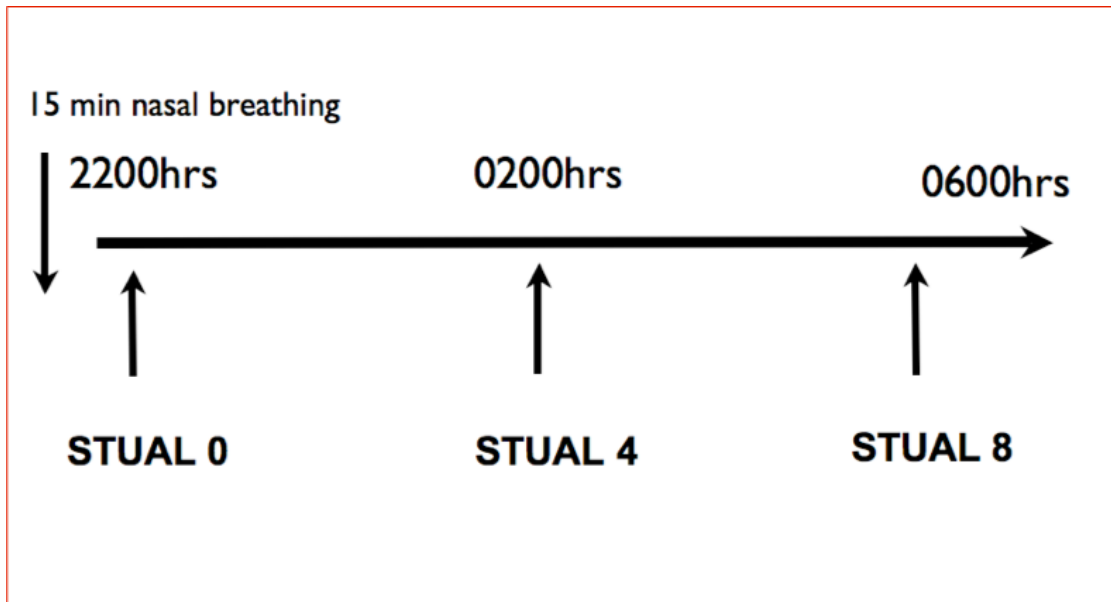


Figure 8.1 Overnight protocol for STUAL sampling

Overnight protocol. Subjects had a 15 minute period of enforced nasal breathing prior to sampling for UAL, Mucosal Wetness, and Salivary flow rate (at 0 hours; STUAL 0). Nasal or Oral Breathing was enforced from “lights out” onwards (2200 hours). Sampling of UAL, Mucosal Wetness, and Salivary flow rate was repeated 4 hours after lights out (STUAL 4), and at the end of the study, 8 hours after lights out (STUAL 8). STUAL = Surface Tension Upper Airway Liquid.

Breathing Route

Route of breathing was confirmed by using a dual channel oro-nasal thermocouple device (F-ONT2A; Grass, West Warwick, RI, USA). Oral route of breathing epochs were defined as nasal or oral based on analysis of phasic signals from the dual channel thermocouple. Nasal-only breathing epochs were defined as epochs containing ≥ 3 consecutive breaths only from the nasal thermocouple signal. Oral-only breathing epochs were defined as epochs containing ≥ 3 consecutive breaths from the oral thermocouple signal. The presence of nasal breathing or oral breathing was expressed as a percentage of the total sleep epochs analysed.

Statistical Analysis

All data were expressed as mean \pm SD. For samples of salivary flow rate, STUAL, and mucosal wetness, individual data were pooled to obtain group mean data for each time period of sampling. Thus, group mean data were obtained for 0 hours, 4 hours and 8 hours. Single comparisons were made using paired t-tests. Differences between the time periods and for the enforced breathing route were analysed using repeated measures ANOVA. $P \leq 0.05$ was considered significant.

8.4 Results

8.4.1 STUAL

On the oral enforced route of breathing night, in comparison to baseline STUAL at 0 hours, there was a significant increase in STUAL at 4 hours and 8 hours (60.4 ± 3.3 mN/m vs 64.9 ± 4.8 mN/m vs 65.9 ± 5.2 mN/m, respectively; ANOVA $p < 0.05$; Figure 8.2). Individual data for STUAL for both nights are demonstrated in Figure 8.3, with individual data for STUAL plotted as dots for each sampling time point, with a line between values for each subject.

For the enforced nasal route of breathing night, there was no significant difference in the STUAL between 0 hours (57.1 ± 3.7 mN/m), 4 hours (57.0 ± 3.7 mN/m), and 8 hours (58.2 ± 3.6 mN/m), respectively (ANOVA, $p = 0.56$). There was a significant difference in the mean STUAL at 0 hours between the enforced oral and enforced nasal route of breathing nights of 3.3 ± 3.9 mN/m (t-test, $p = 0.048$), which is within the limits of variability for the measurement of surface tension using the “pull-off force” technique (Kirkness et al., 2005a).

No relationship was identified between STUAL and RDI. The difference between STUAL on oral vs nasal enforced breathing night (STUAL Oral – Nasal) was compared with the difference in RDI between the two nights (RDI

Oral – Nasal). No significant relationship was found when data were analysed using linear regression ($p = 0.53$)

8.4.2 Salivary Flow Rate

The salivary flow rate exhibited no significant change on the enforced nasal breathing night (ANOVA, $p = 0.12$), although a trend for the salivary flow rate to reduce overnight was noted. The salivary flow rates at 0, 4 and 8 hours were 1.9 ± 0.6 , 1.5 ± 1.3 , and 1.3 ± 0.8 ml/5min respectively. On the enforced oral route of breathing night, there was a significant reduction in the salivary flow rate from 0 hours to 4 hours (2.7 ± 1.8 ml/5min and 1.5 ± 1.4 ml/5min, respectively; ANOVA $p < 0.05$; Figure 8.04) which remained reduced compared with baseline at 8 hours (1.0 ± 0.7 ml/5min $p = 0.007$). (Figure 8.4)

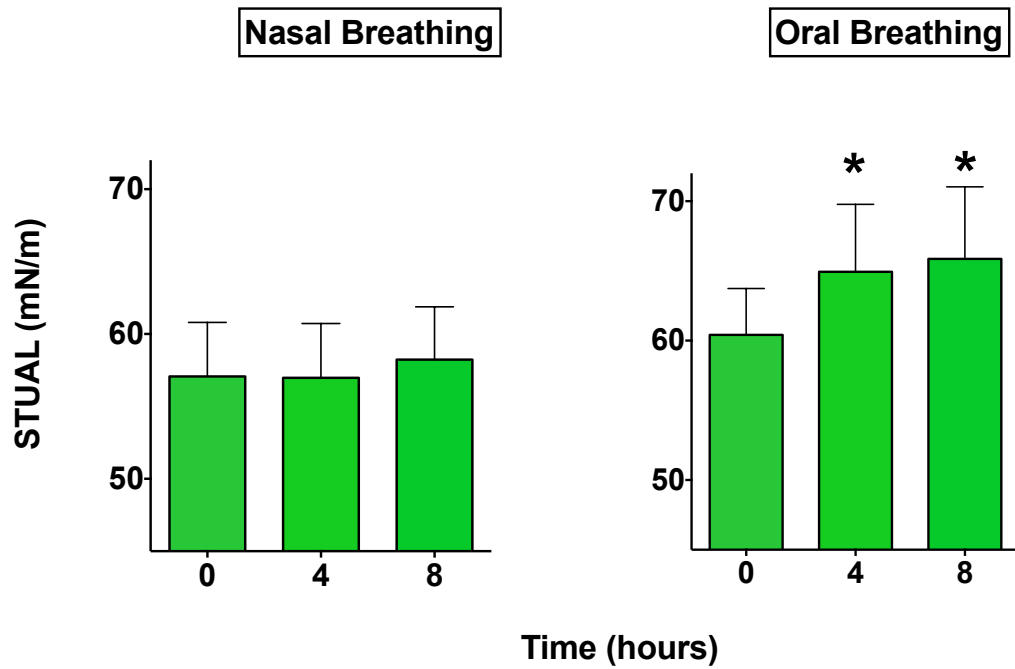


Figure 8.2 Group mean values for STUAL on enforced breathing nights

Group mean values (Bars = +1SD) for STUAL for enforced nasal breathing and enforced oral breathing nights. A significant increase was seen in STUAL at 4 and 8 hours compared to baseline for the oral breathing night but not for the nasal breathing night. * $p < 0.05$ relative to 0 hour value (ANOVA). STUAL = Surface Tension Upper Airway Liquid.

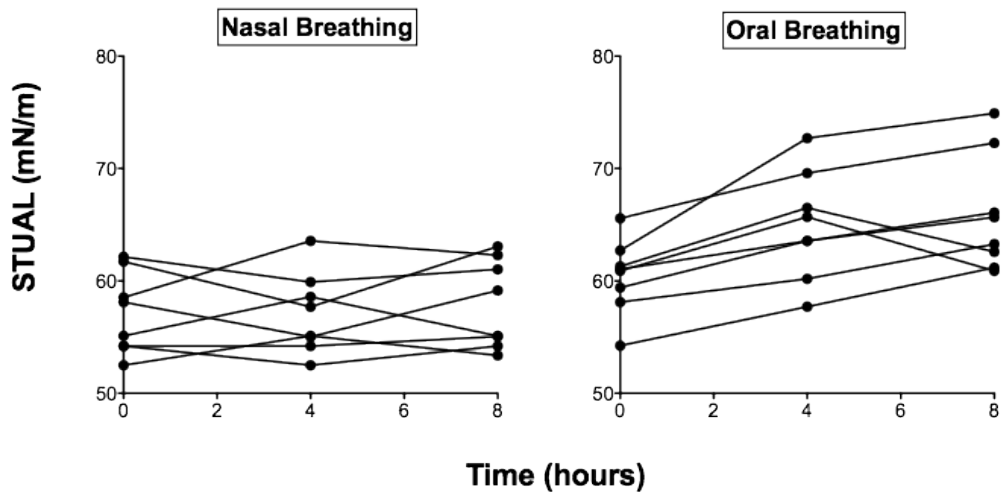


Figure 8.3 Individual data for STUAL on enforced Nasal and Oral breathing nights

Individual data points for STUAL at 0, 4, and 8 hrs for enforced Nasal breathing vs enforced Oral breathing nights. All subjects increased their STUAL after 4 hours of enforced Oral breathing. STUAL = Surface Tension Upper Airway Liquid.

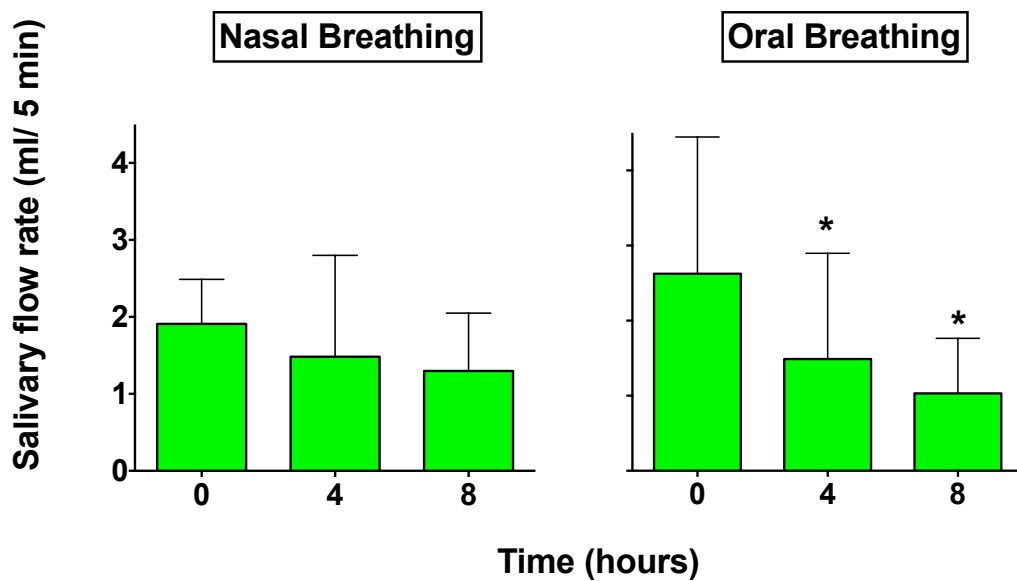


Figure 8.4 Salivary Flow Rate on enforced Nasal and Oral breathing nights

Group mean data (Bars = +1SD) for salivary flow rate on the enforced nasal breathing vs oral breathing nights. No significant changes were seen across the night for enforced nasal breathing, but a significant reduction in salivary flow rate was seen for enforced oral breathing route at 4 hours and 8 hours compared to 0 hours. * $p < 0.05$ relative to 0 hour value (ANOVA).

8.4.3 Upper Airway Mucosal Wetness

The mucosal wetness on the enforced nasal breathing night did not increase in comparison to baseline. Upper airway mucosal wetness was $1.2 \pm 0.3 \mu\text{l}/5\text{s}$ at 0 hours, $0.9 \pm 0.4 \mu\text{l}/5\text{s}$ at 4 hours, and $1.2 \pm 2.7 \mu\text{l}/5\text{s}$ at 8 hours.

On the enforced oral breathing night, the mucosal wetness values decreased significantly from $1.3 \pm 0.6 \mu\text{l}/5\text{s}$ at 0 hours, to $0.3 \pm 0.2 \mu\text{l}/5\text{s}$ at 4 hours, and remained decreased at $0.2 \pm 0.2 \mu\text{l}/5\text{s}$ at 8 hours ($P < 0.05$, ANOVA; Figure 8.5). The values for mucosal wetness at 4 and 8 hours on the oral breathing night were at the lower limits of detection.

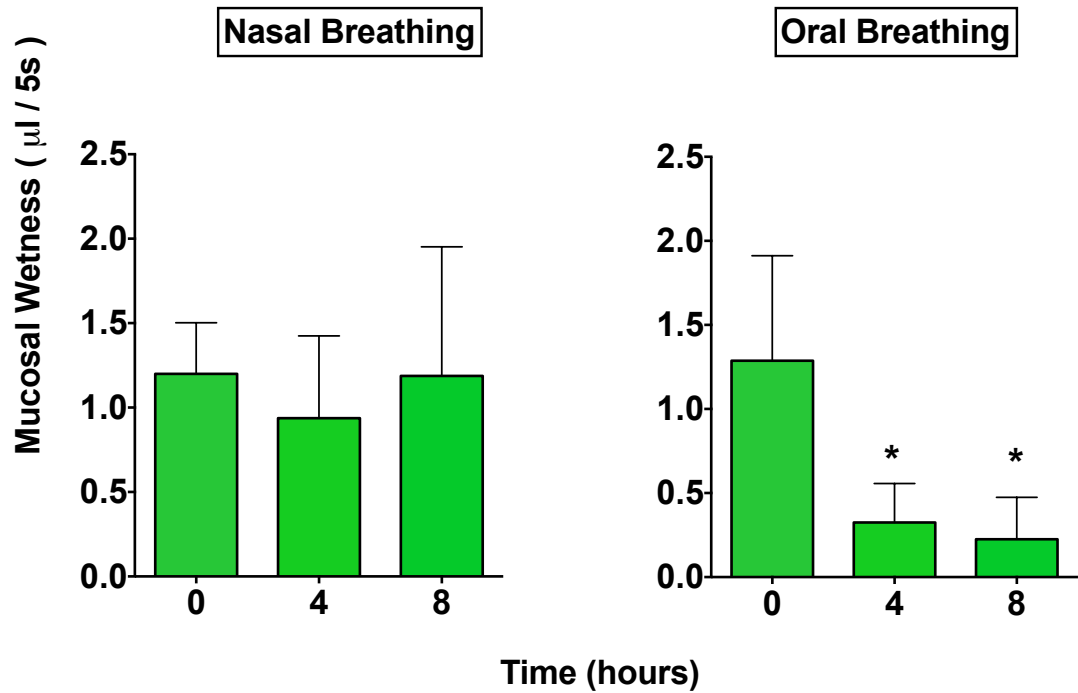


Figure 8.5 Mucosal Wetness on enforced Nasal and Oral breathing nights

Mucosal Wetness group mean data (Bars = +1SD) for enforced nasal breathing and enforced oral breathing route nights. No significant difference across the night for nasal breathing, but a significant reduction in mucosal wetness was seen for enforced oral breathing route at 4 hours and 8 hours compared to 0 hours. * $p < 0.05$ relative to 0 hour value (ANOVA).

8.4.4 Sleep Parameters

There was no significant difference in the sleep staging between the nasal and oral route of breathing nights. There were similar amounts and proportions of Stages 1-4 sleep and REM sleep when the two nights were compared (paired t-test). On the nasal breathing night, the proportion of sleep time in stages 1, 2, slow wave sleep (3 and 4 combined) and REM sleep were $1.5 \pm 1.0\%$, $58.8 \pm 8.4\%$, $19.9 \pm 2.7\%$, and $19.6 \pm 9.1\%$, respectively, and for the oral breathing night, proportions were $1.6 \pm 0.9\%$, $58.6 \pm 6.2\%$, $21.2 \pm 5.5\%$, $18.6 \pm 4.1\%$, respectively (Table 8.1).

Sleep variables including Sleep Efficiency, Arousal Index, the number of Spontaneous vs Respiratory arousals, and the Respiratory Disturbance Index (RDI) were compared for the two nights (paired t-test). There was a significant difference in the Arousal Index and RDI, with both being significantly higher on the enforced oral breathing night vs the nasal breathing night. The arousal index was 9.4 ± 5.2 events per hour of sleep on the nasal breathing night compared with 24.6 ± 12.3 events per hour on the oral breathing night (Table 8.2). The rate of Spontaneous Arousals for both nights was not significantly different, with the increased Arousal Index on the oral breathing vs nasal breathing night being due to an increase in respiratory arousals.

Sleep Stage (%TST)	Nasal	Oral
Stage 1	1.5 ± 1.0	1.6 ± 0.9
Stage 2	58.8 ± 8.4	58.6 ± 6.2
Stage 3/4	19.9 ± 2.7	21.2 ± 5.5
REM	19.6 ± 9.1	18.6 ± 4.1

Table 8.1 Sleep Stages on Nasal vs Oral breathing nights

Comparison of sleep staging between the enforced nasal breathing night vs enforced oral route of breathing night. No differences in sleep stage were found between the nasal breathing vs oral breathing night. TST = Total Sleep Time; REM = Rapid Eye Movement.

	Nasal	Oral
Sleep Efficiency (%)	79.8 ± 8.7	75.5 ± 13.9
Arousal Index (events / hr)	9.4 ± 5.2	24.6 ± 12.3 *
Spontaneous Arousals (events / hr)	8.0 ± 5.7	8.8 ± 8.0
RDI (events / hr)	1.9 ± 1.7	20.8 ± 17.2 *

Table 8.2 Sleep Parameters for Nasal Breathing and Oral Breathing nights

The Arousal Index and RDI were significantly higher for the Oral breathing night compared with the Nasal Breathing night. * $p < 0.05$ (paired t-test). RDI = Respiratory Disturbance Index.

8.4.5 Sleep Disordered Breathing

There was a dramatic difference seen in the severity of sleep disordered breathing between the nasal breathing and oral breathing nights. On the nasal breathing night, the average group RDI was 1.9 ± 1.7 events per hour of sleep, while on the oral breathing night, the RDI was 20.8 ± 17.2 events per hour of sleep (t-test, $p = 0.01$; Figure 8.6). Changing from enforced nasal to enforced oral breathing had a dramatic effect by inducing severe obstructive sleep apnoea (RDI > 30 events per hour of sleep) in 3 subjects. Three more subjects had mild to moderate range OSA (RDI 5-30 events per hour of sleep) on the enforced oral breathing night, while 2 subjects did not have any evidence of OSA on either night. The RDI increased during enforced oral breathing in all subjects except for one (Figure 8.7).

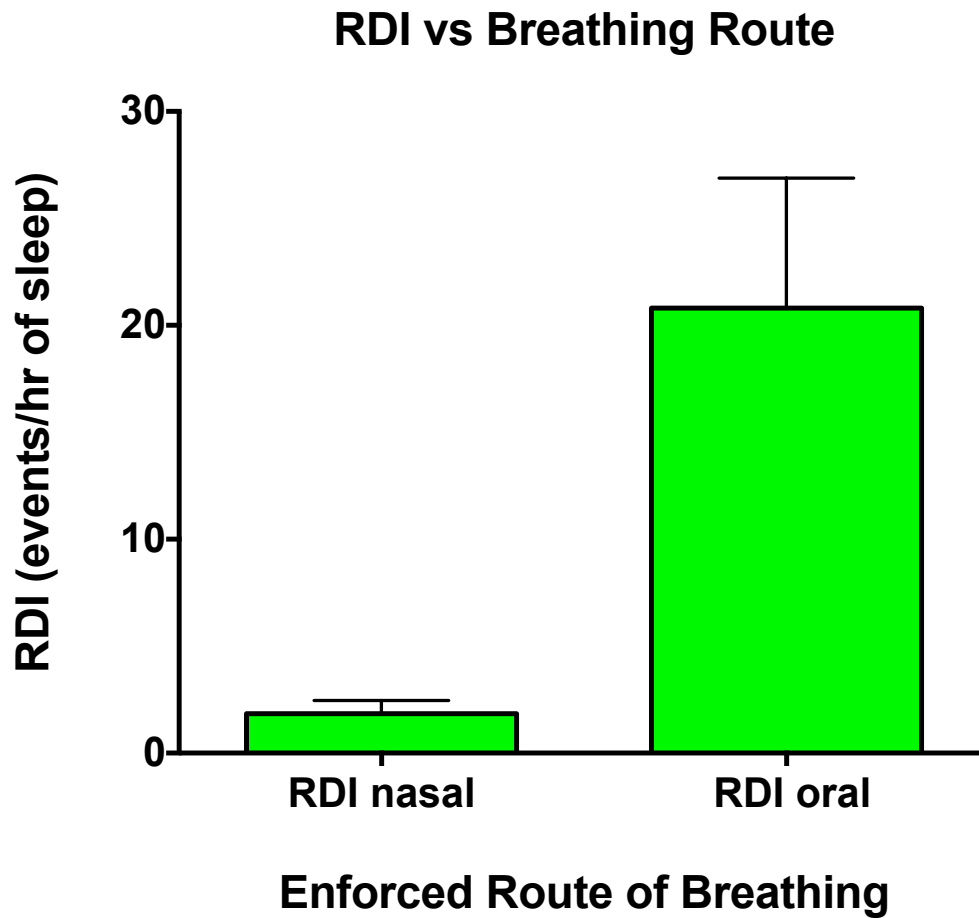


Figure 8.6 Group mean RDI data for enforced Nasal vs Oral breathing nights

Group mean Respiratory Disturbance Index (RDI) data (Bars = +1SD) for enforced nasal vs enforced oral breathing nights. The RDI increased significantly on the enforced oral night, with RDI increasing from 1.9 ± 1.7 events per hour of sleep to 20.8 ± 17.2 events per hour (paired t-test; $p < 0.05$)

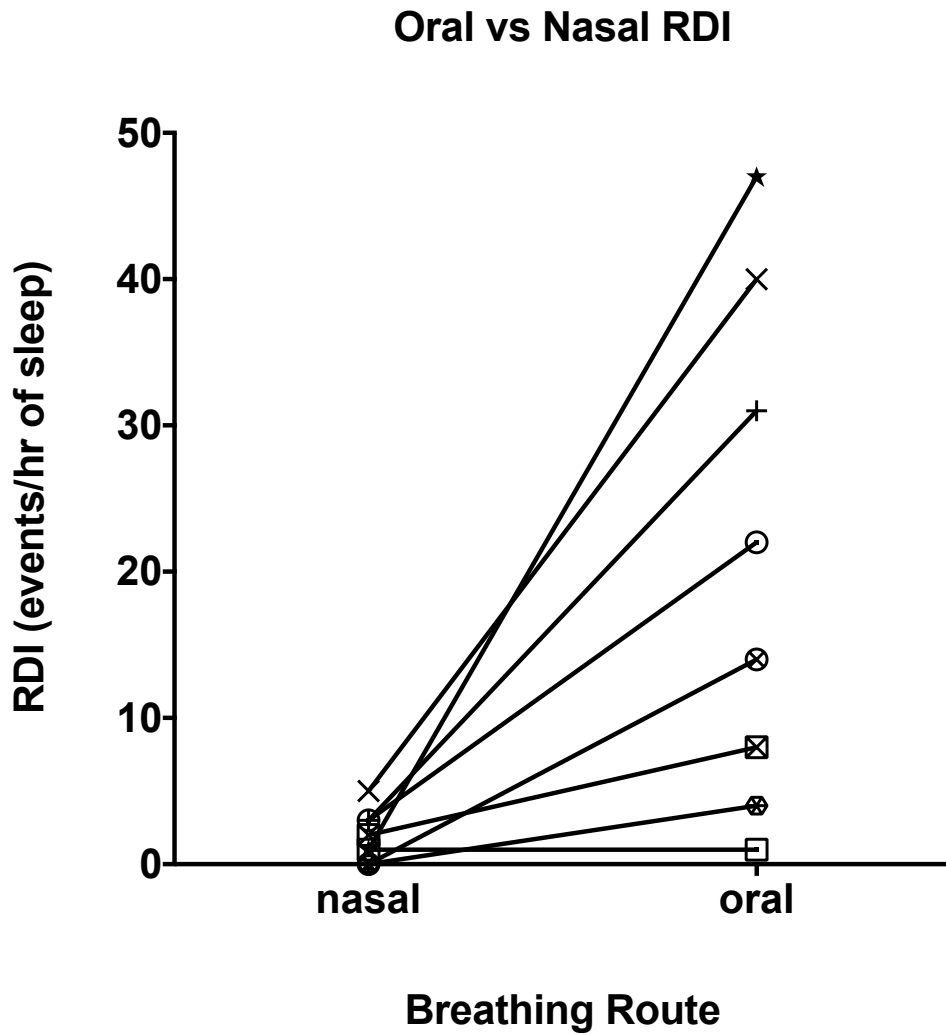


Figure 8.7 Individual data for RDI on Nasal and Oral breathing nights

Individual data for subject's Respiratory Disturbance Index (RDI) for enforced nasal vs oral route of breathing. Different symbols represent individual subjects. The RDI increased on the oral breathing route night in 7 out of 8 subjects.

8.5 Discussion

The findings in this study show that enforced oral vs nasal route of breathing reduces measured salivary flow rate and mucosal wetness, while increasing both the STUAL and the severity of sleep disordered breathing. The major finding is that enforced oral route of breathing induces sleep disordered breathing in all but one subject in an otherwise healthy subject group, free of OSA at baseline.

8.5.1 Critique of Methods

Breathing route was determined by a dual channel thermocouple device as per previous studies investigating route of breathing (Madronio, Di Somma et al. 2004). However, oral airflow could not be accurately quantified, and no nasal pressure cannula or means of quantifying oral or nasal airflow concurrently were used in this study. Other authors have previously measured oral and nasal breathing overnight by using a mask with partitioned independent nasal and oral compartments (Fitzpatrick, McLean et al. 2003). A similar method used in the current study may have influenced the STUAL via an artificial increase in humidification and hence was not considered.

A combination of respiratory inductance plethysmography (thoraco-abdominal bands), a dual channel thermocouple, and oximetry were used to score respiratory events. The lack of nasal pressure cannula may have led to an underscoring of potential respiratory events. However, it has been shown

that thoraco-abdominal bands have a similar sensitivity and specificity to oesophageal pressure for detecting upper airway resistance (ref) and may be able to detect respiratory events that are not detectable by analysis of oro-nasal flow by a thermocouple device (Masa et al).

The baseline values for STUAL between the enforced oral and nasal breathing nights were different (57.1 ± 3.7 mN/m vs 60.4 ± 3.3 mN/m). However, the difference seen in the baseline values for STUAL is within the coefficient of variation of surface tension measured by the “Pull-Off Force Technique” (Kirkness et al., 2005a). This finding is unusual since the subjects were randomized for route of breathing, and the methodology was identical for sampling on both nights. Calibration was performed on the “Pull Off Force” device on a regular basis, and it is unlikely that the difference would be due to slight differences in calibration between study nights.

8.5.2 Surface tension of UAL

STUAL during enforced daytime route of breathing has been previously studied by Verma et al. (2006). In this study, during enforced oral breathing over 120 minutes, the STUAL increased from 64.4 ± 2.7 mN/m to 77.4 ± 1.1 mN/m, while with nasal breathing the STUAL decreased from 59.3 ± 2.2 mN/m to 51.8 mN/m. In the present study, no decrease in STUAL was observed for nasal breathing, but an increase was seen with enforced oral breathing route.

A possible explanation for the lack of decrease in STUAL with nasal breathing may relate to a reduction in salivary flow and production during sleep (Dawes 1975), coupled with a reduction in swallow rate during sleep (Thie, Kato et al. 2002), resulting in less transfer of saliva from the mouth to the posterior pharynx to maintain a lower surface tension (Sonies, Ship et al. 1989). Salivary and nasopharyngeal secretions contain surface active phospholipids (Woodworth, Smythe et al. 2005) and a reduced transfer to the posterior pharyngeal wall due to a combination of reduced production and a decreased swallowing rate in the sleep state, may explain that lack of reduction with enforced nasal breathing during sleep.

The increased surface tension with oral breathing may be possibly due to evaporative losses from the upper airway, resulting in altered surfactant activity of phospholipids and surface active proteins in upper airway lining liquid and saliva.

When compared with the baseline values for STUAL from the Verma et al. (2006) study, the baseline values for STUAL between the enforced oral and nasal breathing nights were also different for the present study (57.1 ± 3.7 mN/m vs 60.4 ± 3.3 mN/m). It is unknown whether anticipation of nasal or oral route of breathing by the subject may influence salivary production, and this may be a possible explanation for the increased baseline STUAL during oral breathing vs nasal breathing. This effect was observed both in the present study with enforced route of breathing during sleep, and in the

Verma et al. (2006) study with enforced route of breathing in awake normal subjects.

8.5.3 Salivary Flow Rate

The flow of unstimulated saliva has a circadian influence, as demonstrated by Dawes (1975). This (non-enforced breathing route study) demonstrated salivary flow was highest at approximately 1700 hours, and lowest at approximately 0500 hours. In the present study, there was a trend for the salivary flow rate to decrease overnight on the nasal breathing night, but the trend did not show statistical significance, possibly since the closed mouth prevented an additional evaporative effect. On the oral breathing night, a combination of an evaporative effect, and a reduction in salivary production may have resulted in the decreased salivary flow rate observed overnight. For both the oral and nasal breathing route nights, the minimum values for salivary flow rate were similar to the values described by Dawes (1975).

8.5.4 Mucosal Wetness

In the enforced oral breathing route group, the mucosal wetness decreased to the limits of detection during sleep, while there was no significant difference in mucosal wetness across the night with the nasal breathing group. This is likely to be due to evaporative loss of mucosal liquid

during enforced oral breathing overnight, combined with the effects of low salivary production and reduced swallowing.

The values obtained for mucosal wetness in this study were less than the daytime values obtained previously (Verma et al., 2006). Daytime values for mucosal wetness in an enforced oral breathing group decreased from $4.5 \pm 0.4 \mu\text{l}/5\text{s}$ at 0 minutes to $0.1 \pm 0.2 \mu\text{l}/5\text{s}$ at 120 minutes (Verma, Seto-Poon et al. 2006), as compared with $1.3 \pm 0.6 \mu\text{l}/5\text{s}$ at 0 hours and $0.2 \pm 0.2 \mu\text{l}/5\text{s}$ at 8 hours on the enforced oral breathing night in this study. The lower baseline mucosal wetness for this study when compared with the daytime study by Verma et al. (2006), may be due to the circadian changes in salivary production and flow, with a lower flow rate seen in the evening and overnight.

Oral route of breathing at night did not produce a lower mucosal wetness when compared with the daytime study, as the values for mucosal wetness were already at the lower limits of detection for the daytime values. An inverse relationship between upper airway mucosal wetness and STUAL was demonstrated in this study (Figure 8.08). This relationship was also previously demonstrated in healthy subjects during daytime enforced oral route of breathing (Verma et al., 2006).

8.5.5 Severity of Sleep Disordered Breathing

There was a significant difference in the severity of sleep disordered breathing when comparing the enforced nasal breathing night to the enforced oral breathing night. Three of the subjects had a normal RDI on the enforced nasal breathing night, but had severe sleep disordered breathing with their RDI > 30 events per hour of sleep on the enforced oral breathing night. Similar increases in the RDI and severity of sleep disordered breathing have been previously reported with enforced oral route of breathing (Fitzpatrick, McLean et al. 2003), but these studies have not measured the changes in salivary flow, mucosal wetness, and STUAL with the enforced breathing route. Although there were dramatic changes in RDI with enforced breathing route, the STUAL demonstrated significant variability and no significant relationship between change in STUAL and with RDI was identified.

Previous studies using instilled surfactant in the upper airway (Jokic, Klimaszewski et al. 1998; Morrell, Arabi et al. 2002; Kirkness, Madronio et al. 2005), have shown that decreasing surface tension with exogenous surfactant can lead to reduced upper airway collapsibility and a reduction in the severity of sleep disordered breathing. In a rabbit model, Kirkness et al. (2003) have also previously demonstrated that the patency of the upper airway is influenced by STUAL. Enforced oral breathing in the present study has been associated with an overnight increase in STUAL, which may promote upper airway collapsibility.

Oral breathing may result in an increase in the closing pressure of the oropharyngeal and velopharyngeal airway, via bite opening and mandibular position change (Ayuse, Inazawa et al. 2004; Isono, Tanaka et al. 2004). This represents a different anatomical mechanism whereby the degree of sleep disordered breathing may be greater with enforced oral breathing, separate to any consideration of the effect of changes in surface forces on upper airway collapsibility.

For enforced oral route of breathing, whether the increase in severity of sleep disordered breathing is due to an increase in surface tension, or a change in mandibular position and degree of bite opening, cannot be determined by the current study.

8.6 Conclusion

This study demonstrates that enforced oral route of breathing induces sleep disordered breathing and increases STUAL.

STUAL is increased with oral breathing most likely due to evaporation of upper airway lining liquid, and reduction of salivary flow rate with sleep onset, consistent with our data showing a reduction in mucosal wetness and salivary flow rate with enforced oral route breathing.

The potential mechanisms whereby enforced oral route of breathing induces sleep disordered breathing include an overnight increase in STUAL, or changes in upper airway patency due to mandibular position change with an open mouth. With the dramatic increase observed in the severity of SDB with enforced oral breathing route demonstrated in this study, it is plausible that varying degrees of nasal obstruction may induce SDB in healthy individuals, with gross nasal obstruction predisposing to a greater severity of SDB

The next chapter will investigate whether the increase in sleep disordered breathing is due to changes in upper airway collapsibility with jaw position, or potentially due to the increased STUAL.

Chapter 9: Effect of Exogenous Surfactant during Enforced Oral Breathing Route on Surface Tension of Upper Airway Liquid and Severity of Sleep Disordered Breathing in Healthy Subjects

9.1 Introduction

Chapter 8 demonstrated that enforced oral route of breathing increased STUAL and induced sleep disordered breathing (SDB) in most normal subjects (severe SDB in some). The study did not determine the pathophysiological mechanisms contributing to the SDB and the postulated mechanisms include an increase in STUAL leading to increased propensity to upper airways collapse , or possibly, changes in mandibular position due to mouth opening with enforced oral breathing route, leading to changes in length/ tension relationships of upper airway muscles and consequent changes in upper airway geometry and anatomical collapsibility (Meurice et al., 1996). No studies to date have examined the effect of lowering STUAL on subjects with oral breathing induced sleep disordered breathing. Previous studies have demonstrated increases in upper airways resistance and severity of SDB with enforced oral breathing (Fitzpatrick et al., 2003), and this study will examine whether or not higher STUAL values seen with enforced oral breathing route may be a factor in determining the severity of SDB.

9.2 Aims

Enforced oral route of breathing is postulated to increase the STUAL, thereby increasing the severity of sleep disordered breathing. In this part of the study, exogenous surfactant was administered to subjects during enforced oral breathing route to reduce the increases in STUAL seen during oral route breathing, and to monitor for any subsequent decrease in the severity of sleep disordered breathing.

9.3 Methods

9.3.1 Subjects

Subjects recruited for this study were from the normal subjects previously recruited for the enforced route of breathing study in Chapter 8. Healthy subjects were screened by questionnaire and clinical history. The subjects were recruited from amongst staff members from Westmead Hospital and from the Ludwig Engel Centre for Respiratory Research.

Of the 8 subjects who participated in the enforced route of breathing study, 6 subjects consented to participate in the present study (5 male, 1 female; age: 30.0 ± 4.6 years, range 24 – 34 years; BMI: 24.2 ± 2.5 kg/m², range 21.5 – 32.6 kg / m²).

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Human Ethics Committee.

9.3.2 Measurements

Surface Tension

Surface Tension of Upper Airway Lining Liquid (STUAL) was measured by the “Pull Off Force Technique”. This technique is described in the methodology of previous chapters (ref). Microliter volumes of upper airway lining liquid are placed between two silica discs, and the force required to separate the discs with the microliter volume of liquid bridging both disc surfaces, is taken to be the surface tension.

Upper Airway Lining Liquid

Upper airway lining liquid was sampled from the posterior oropharyngeal wall. Samples were aspirated into polyethylene tubing (Tyco Electronics; external diameter 0.8 mm; internal diameter 0.5 mm), using a 23g needle (BD PrecisionGlide 0.6mm x 32mm) attached to a 3ml syringe (Terumo Medical Corporation 3cc / mL).

Samples were then applied onto the surface of a silica disc for measurement of surface tension in the surface force measurement device (Kirkness et al., 2005a).

Salivary flow rate

Non-stimulated salivary flow rate was measured by collecting saliva into a polystyrene cup. The subject sits forward and drools into a collection cup over a five minute period as previously described by Navazesh et al. (1982). The saliva collected was weighed and the flow rate expressed as ml/ 5 min (salivary flow rate).

Mucosal Wetness

Mucosal “wetness” of the upper air way was measured using a timed gravimetric contact absorbent paper strip method described by Ciantar and Caruana (Ciantar and Caruana, 1998). The absorbent paper strip (Sialo-Strips for saliva collection, Oraflow Inc, New York, USA) was placed centrally in contact with posterior part of the tongue for 5 seconds. The volume of fluid was calculated by comparing the weight of the paper strip before and after collection and expressed as microlitres / 5 seconds.

Polysomnography

All polysomnographic studies were undertaken at the Westmead Hospital Sleep Laboratory. The variables monitored overnight included EEG (electroencephalogram), ECG (electrocardiogram), submental and diaphragmatic EMG (electromyogram), left and right EOG (electro-oculogram), respiratory inductance plethysmography (for thoraco-abdominal movement), oxygen saturation (pulse oximetry), sound, and body position. Nasal pressure measurement for airflow was not utilized. All signals were recorded on a Compumedics Profusion 3 software system. Sleep staging

was performed on the overnight signals using Rechtschaffen and Kales rules (Rechtschaffen and Kales, 1968) and obstructive events were scored using “Chicago” criteria (American Academy of Sleep Medicine task force, 1999). Arousals were scored using the American Sleep Disorders Association criteria (Bonnet M, 1992). All studies were scored by a single sleep technician.

Route of Breathing

Route of Breathing was confirmed by using a dual channel oro-nasal thermocouple device (F-ONT2A; Grass, West Warwick, RI, USA). The thermocouple was secured to the patient’s face using tape (3M™ Micropore™ Microporous Hypo-Allergenic Surgical Tape), with the thermocouple leads looped behind both ears. The qualitative signals from the thermocouple device were checked for oral-only flow at the start and during the study, to confirm oral-only breathing during the entire sleep period.

9.3.3 Protocol

All subjects arrived at the Westmead Sleep laboratory by 6pm on the night of the study. They remained nil by mouth for at least one hour prior to “lights out” which was approximately 2200 hours. PSG preparation and set up as per Chapter 8. Sampling for STUAL was taken with the subject seated upright following 15 minutes of nasal-only breathing, before “lights out”. Immediately following UAL sampling, mucosal wetness was sampled, and

the salivary flow was then collected with the subject seated in a forward position, and saliva collected into a cup over a 5 minute period.

The study was conducted over one night. Oral route of breathing was enforced for the whole night with cotton wool inserted into both nares, with tape (3M™ Leukosilk®) external to the nares to seal both nostrils. A sample of STUAL was obtained prior to collection of samples for mucosal wetness and salivary flow. Exogenous surfactant (2.5ml Beractant, Abbott, USA) was then administered by atomiser spray into the nose and oropharynx. Half of the dose (1.25ml) was sprayed by atomizer onto the posterior pharyngeal wall, whilst the other 1.25ml of the dose was sprayed towards the posterior nasopharynx. An extension tube attached to the atomizer was used for delivery of surfactant and was passed through the nares to direct surfactant to the posterior nasopharynx.

Enforcement of oral breathing route took place after all samples were taken in the evening and immediately before “lights out”. The patient was woken, and sampling for UAL, mucosal wetness, and salivary flow were repeated 4 hours after lights out. A further dose of 2.5ml exogenous surfactant was administered using the same methodology after the 4 hour sampling was performed. The subjects were then allowed to return to sleep for another 4 hour period before final sampling for STUAL, mucosal wetness, and salivary flow at 8 hours after lights out at the end of the sleep recording.

2.5 ml exogenous surfactant was administered via atomiser spray with the patient in the seated position. The dose was split with 1.25ml delivered into the nasopharynx via a nostril and the remaining 1.25ml was delivered into the posterior oropharynx.

9.3.4 Data Analysis

Data from the matching six subjects from Chapter 8 were used to compare to the data obtained in this study.

Sleep Parameters

Studies were sleep staged for each 30s epoch using Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968). Respiratory Events were identified using the 1999 American Academy of Sleep Medicine task force criteria (AASM, 1999). An obstructive apnoea was defined as a 10 second or greater absence of airflow (thermistor) with oximetry demonstrating a 3% or greater reduction in oxygen saturation compared with baseline. A hypopnoea was defined as a greater than 30% reduction in respiration (RIP), together with oximetry demonstrating a 3% or greater reduction in oxygen saturation, for a duration of 10 seconds or more. A “Chicago” type hypopnoea was defined as a subjective reduction in respiration (RIP) resulting in an oxygen desaturation of 3% or more, or terminating in an arousal, with the duration of the event being 10 seconds or more (American Academy of Sleep Medicine task force, 1999).

The Respiratory Disturbance Index (RDI) was derived from the respiratory inductance plethysmography signal (RIP) as the primary scoring channel, together with oximetry and EEG arousals. The total RDI was an aggregate of the number of apnoeas and the number of hypopnoeas (including “Chicago type” hypopnoeas) per hour of sleep.

Breathing Route

The absence of nasal breathing and the presence of oral only breathing was confirmed by using the dual channel oro-nasal thermocouple device (F-ONT2A; Grass, West Warwick, RI, USA).

Statistical Analysis

All data were expressed as mean \pm SD. For samples of salivary flow rate, STUAL, and mucosal wetness, individual data were pooled to obtain group mean data for each time period of sampling. Thus, group mean data were obtained for 0 hours, 4 hours and 8 hours. Single comparisons were made using paired t-tests with Bonferroni correction. Differences between the time periods for the enforced breathing route were analysed using repeated measures ANOVA. $P \leq 0.05$ was considered significant.

9.4 Results

9.4.1 Surface Tension of UAL

Following administration of exogenous surfactant at “lights out”, STUAL decreased from 62.6 ± 1.6 mN/m at baseline to 57.6 ± 3.6 mN/m (ANOVA, $p < 0.05$).

At 4 hours after lights out, STUAL remained decreased at 57.4 ± 3.6 mN/m, and following a further 2.5ml of exogenous surfactant, this decreased to 55.5 ± 1.8 mN/m (ANOVA, $p < 0.05$). Final sampling of STUAL at 8 hours demonstrated a STUAL of 60.4 ± 1.3 mN/m (Figure 9.1).

Enforced Oral Breathing with Exogenous Surfactant

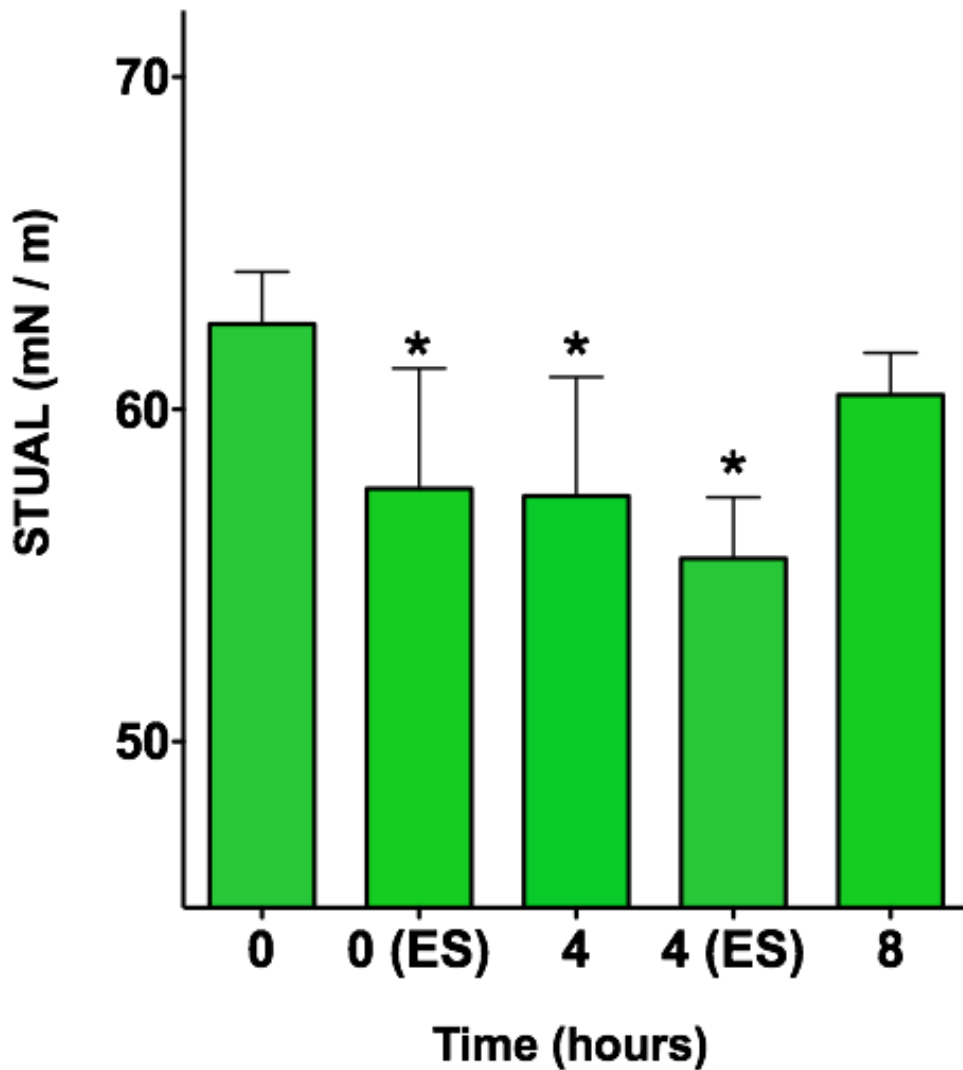


Figure 9.1 Group mean values for STUAL

Group mean values (Bars = +1SD) for STUAL during enforced oral breathing with exogenous surfactant. ES = Post Exogenous Surfactant. Note significant reduction in STUAL with ES at 0 hours which persists for 4 hours. STUAL increases from 4 to 8 hours (despite ES), but only to levels typical for Nasal Breathing. * $p < 0.05$ relative to 0 hour value (ANOVA). STUAL = Surface Tension Upper Airway Liquid.

9.4.2 Salivary Flow Rate

The results for salivary flow rate during nasal breathing (from Chapter 8), oral breathing (from Chapter 8), and oral breathing with exogenous surfactant are shown in Figure 9.02.

On the enforced oral breathing with exogenous surfactant night, there was a significant reduction in the salivary flow rate between 0 to 4 hours, from 2.3 ± 1.1 g/5min to 1.1 ± 0.6 g/5min. The salivary flow remained reduced when sampled at 8hrs at 0.9 ± 0.7 g/5min. There was no significant difference in the salivary flow rate at 0 hours, 4 hours or 8 hours compared across the three different nights (ANOVA, all $p > 0.05$).

For both the enforced oral route of breathing and the enforced oral breathing route with exogenous surfactant nights, there was a decrease in salivary flow rate at 4 hours and at 8 hours relative to 0 hours, but this decrease was not evident on the nasal breathing night (Figure 9.2).

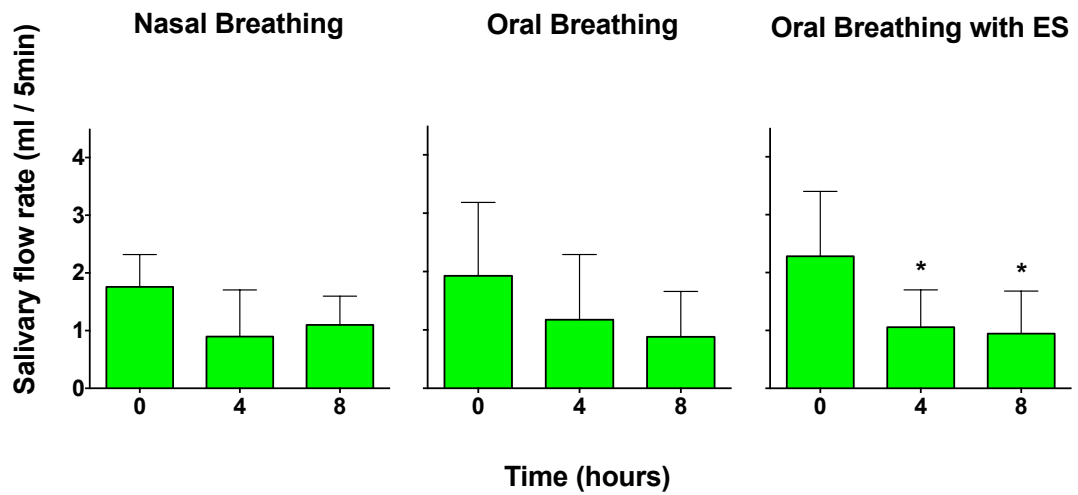


Figure 9.2 Salivary Flow Rate across the 3 study nights

Salivary flow rate at 0, 4, and 8 hours on the nasal breathing, oral breathing, and oral breathing with exogenous surfactant nights. There is a decrease in salivary flow rate at 4 and 8 hours on the oral breathing with ES night. Data are mean; bars = 1SD (n = 6).

* p < 0.05 relative to 0 hour value (ANOVA). ES = Exogenous Surfactant.

9.4.3 Mucosal Wetness

The mucosal wetness on the oral breathing with exogenous surfactant night demonstrated a significant reduction at 4 and 8 hours compared with baseline. On the surfactant study night, the mucosal wetness values decreased significantly from $1.6 \pm 0.4 \mu\text{l}/5\text{s}$ at 0 hours, to $0.6 \pm 0.2 \mu\text{l}/5\text{s}$ at 4 hours, and remained decreased at $0.4 \pm 0.2 \mu\text{l}/5\text{s}$ at 8 hours ($P < 0.05$, ANOVA; Figure 9.3). The measured values for mucosal wetness at 4 and 8 hours were reduced by a similar amount to the enforced oral breathing night without exogenous surfactant (Figure 9.3).

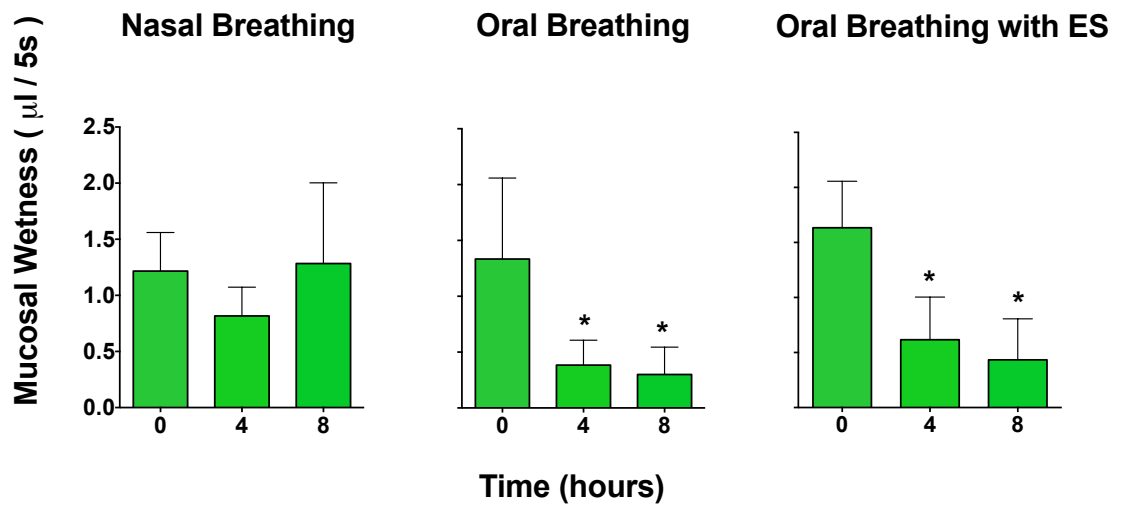


Figure 9.3 Mucosal Wetness across the 3 study nights

Mucosal wetness at 0, 4, and 8 hours on the nasal breathing, oral breathing, and oral breathing with exogenous surfactant nights. Note the decrease in mucosal wetness at 4 and 8 hours on both oral breathing nights. Data are mean; bars = 1SD (n = 6). * p<0.05 relative to 0 hour (ANOVA).

9.4.4 Sleep Parameters

As described in Chapter 8, there was no significant difference in Sleep Efficiency between the enforced nasal and oral breathing nights. There was a significant increase in the Arousal Index when comparing the enforced oral and nasal breathing nights. On the enforced oral route of breathing with exogenous surfactant night, the Arousal Index was increased to a similar degree to the oral breathing night at 22.8 ± 10.6 events per hour of sleep. On further review of arousal type, the majority of these were respiratory arousals. Spontaneous arousals were 9.5 ± 3.8 events per hour of sleep, which was similar to that seen on both the nasal and oral breathing nights (table 9.1).

	Nasal	Oral	Oral + ES
Sleep Efficiency (%)	79.5 ± 9.7	71.45 ± 14.4	70.7 ± 8.6
Arousal Index (events / hour)	9.3 ± 6.0	26.45 ± 13.6*	22.8 ± 10.6*
Spontaneous Arousals (events / hour)	7.8 ± 6.7	6.0 ± 5.8	9.5 ± 3.8

Table 9.1 Sleep Parameters for Nasal, Oral, and Oral + ES nights

Sleep parameters on the nasal breathing, oral breathing, and oral breathing with exogenous surfactant (Oral + ES) nights. Overall sleep efficiency was similar between the three nights. However the Arousal Index was increased on the oral breathing and oral breathing + ES nights, due to an increase in respiratory arousals. Data are mean ± SD; n = 6. * p<0.05 relative to nasal (ANOVA).

9.4.5 Sleep Disordered Breathing

There was a significant and dramatic increase in the RDI from the nasal route of breathing night to the oral route of breathing night. The RDI increased from 2.1 ± 1.9 events per hour of sleep to 26.4 ± 14.1 events per hour of sleep ($n = 6$). On the oral breathing with exogenous surfactant night, the RDI was reduced at 17.8 ± 13.6 events per hour of sleep, which was a significant decrease when compared to the enforced oral breathing night (Figure 9.4). Respiratory events were analysed for the first vs second part of the night, for nasal and oral breathing route, and no significant differences were observed. All of the subjects demonstrated a reduction in RDI on the enforced oral breathing night with exogenous surfactant, although sleep disordered breathing was still present (Figure 9.5).

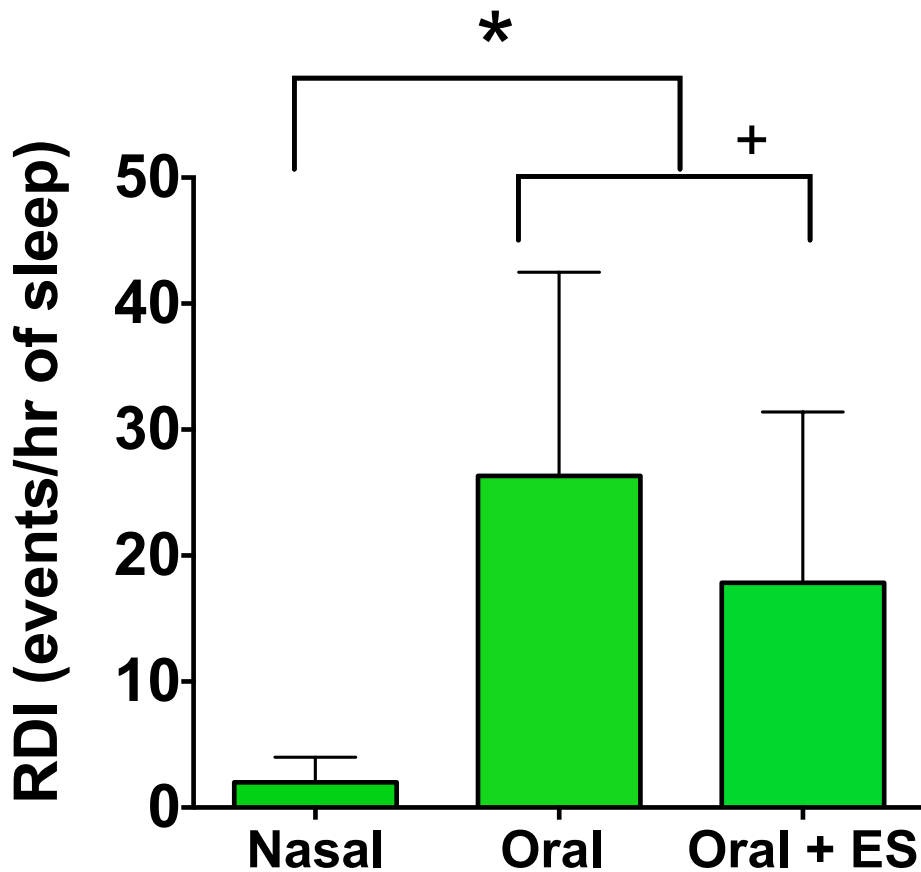


Figure 9.4 Mean RDI values for 3 study nights.

Respiratory Disturbance Index (RDI) on the nasal breathing, oral breathing, and oral breathing with exogenous surfactant nights. Data are mean; bars = 1SD (n = 6). For the oral breathing route, there was moderate to severe sleep disordered breathing, which was significantly reduced, although not eliminated, with exogenous surfactant. * = p < 0.01 relative to nasal; + = p < 0.01 relative to oral route (paired t-tests with Bonferroni correction). ES = Exogenous Surfactant.

RDI: Nasal vs Oral vs Oral + Surfactant

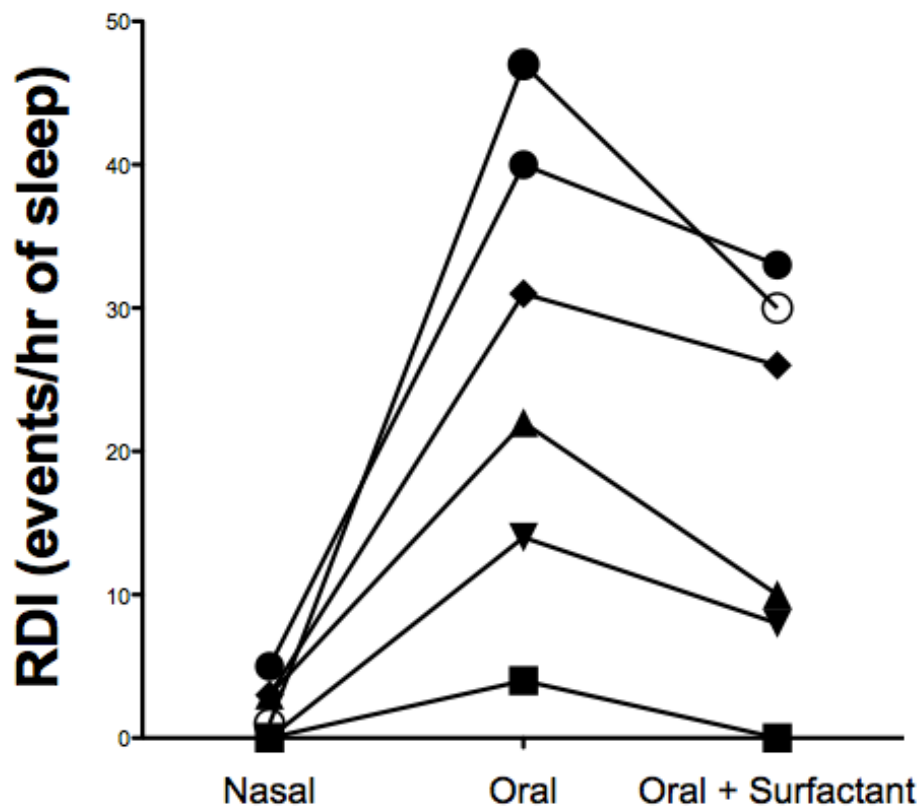


Figure 9.5 Individual Subject Data for RDI

Individual data for Respiratory Disturbance Index (RDI) for each subject ($n = 6$; different symbols) during the enforced nasal, oral, and oral with exogenous surfactant nights. With enforced oral breathing, exogenous surfactant reduced the RDI in all subjects.

9.5 Discussion

The findings in this study show that exogenous surfactant reduces STUAL and the severity of sleep disordered breathing during enforced oral route of breathing. All subjects had a reduction in their RDI, with a drop in STUAL similar to values seen in the enforced nasal route of breathing night (Chapter 8). Similar to the enforced oral breathing night in the previous Chapter, there were significant decreases in salivary flow rate and mucosal wetness on the enforced oral breathing with surfactant night.

9.5.1 Critique of Methods

The limitations of this study are similar to those encountered in Chapter 8, namely the limitations of quantifying breathing route, and scoring of respiratory events without nasal pressure cannula signals (respiratory events were scored from dual channel nasal thermocouple device, respiratory inductance plethysmography (thoraco-abdominal bands), and oximetry).

The reduction in STUAL seen in this study was not as low as previously reported with exogenous surfactant (Kirkness, et al, JAP 2003, Morrell et al, ERJ, 2002). Previous studies utilising exogenous surfactant on subjects with OSA performed in our laboratory (Kirkness et al., 2003c) have used Exosurf Neonatal (Exosurf Neonatal, Glaxo Smith Kline, Greenville, NC), but the current study uses 2.5ml Beractant (Abbott, USA). It is unclear whether Beractant and Exosurf are equivalent in terms of surface tension

lowering effect or if the mucosal spreading qualities are the same for the same dose.

This study demonstrates a reduction in both STUAL and RDI with exogenous surfactant in patients who have enforced oral route of breathing, but does not eliminate SDB or return it to nasal route of breathing values. Other variables such as differences in mandibular position and muscle length tension relationships may account for some the difference seen in severity of SDB with different breathing routes, but this study does not address these other factors.

9.5.2 *Surface tension of UAL*

The STUAL seen with exogenous surfactant and enforced oral route of breathing is similar to the STUAL seen on the enforced nasal route of breathing night. Exogenous surfactant did not reduce STUAL to that seen in previously published studies (Morrell et al., 2002, Kirkness et al., 2003c). In previous studies investigating the effect of exogenous surfactant, Exosurf (Exosurf neonatal GSK) was instilled into the nasopharynx, while in this study, Beractant (Abbott, USA) was used as exogenous surfactant. Exosurf is a protein free synthetic surfactant, while Beractant is a modified bovine surfactant extract. It is not known whether Beractant has the same dose - effect for surface tension lowering as Exosurf, and the mucosal spreading qualities may be different for the two agents. Despite the instillation of Beractant into the naso- and oro-pharynx in this study, the values observed

for STUAL remained higher than in previous studies. The lowest values for surface tension in this study were similar to the STUAL observed on the nasal breathing night.

The STUAL with exogenous surfactant and oral breathing reached a nadir value of 55.5 ± 1.8 mN/m which is not as low as in previous work by Kirkness et al. (2003). This may be due to the enforced oral route of breathing keeping the ST higher, but possibly also due to the different type of surfactant used in the two studies.

Additionally, the STUAL demonstrated a return to baseline values at 8 hours. It is possible that upon waking, subjects may swallow more frequently, thereby clearing any remaining surfactant from the upper airways and increasing the surface tension back to baseline values for the oral breathing route.

9.5.3 Salivary Flow Rate

The salivary flow rate values on the enforced oral breathing with exogenous surfactant night demonstrated a reduction across the night. It is likely that the evaporative effects of oral route of breathing resulted in a reduction in measured salivary flow rate overnight. However when the data for the 6 subjects were analysed for salivary flow rate on the enforced oral

breathing night, there was no longer a significant reduction seen across the night. This may possibly due to a type II error.

9.5.4 Mucosal Wetness

Mucosal wetness values on the enforced oral breathing with exogenous surfactant night were similar to the values obtained on the enforced oral route of breathing night. There was no difference in mucosal wetness between oral route of breathing and oral route of breathing with surfactant nights, and the 4 and 8 hour values were reduced to the limits of detection. Despite the administration of exogenous surfactant, there was a reduction in mucosal wetness that was similar to that seen on the enforced oral breathing night. It should be noted that the exogenous surfactant was not directly applied to the mucosa where mucosal wetness testing was undertaken

9.5.5 Severity of Sleep Disordered Breathing

This study demonstrates that despite enforced oral route of breathing, there was a reduction in RDI in all subjects when exogenous surfactant was administered to the upper airway. The RDI was significantly reduced from 26.3 ± 16.1 to 17.8 ± 13.6 events per hour of sleep, but exogenous surfactant did not eliminate the presence of moderate obstructive sleep apnoea seen with oral route of breathing. Despite exogenous surfactant, the RDI did not

return to the values seen on the enforced nasal breathing route night from the first part of this study (Chapter 8). This suggests that although surface tension forces represent part of the pathophysiology leading to sleep disordered breathing during enforced oral route breathing, there are other pathophysiological factors which also play a significant role in upper airway collapse during oral route breathing while asleep. This study does not address the question of what other factors may be responsible for the dramatic difference seen in sleep disordered breathing during nasal and oral route breathing. Although oral route of breathing changes surface forces in a direction which increases the propensity to upper airways collapse, the mechanical effect of an open mouth on upper airway anatomy, the change in length/ tension relationships of upper airway muscles, and any posterior movement of upper airways structures with the mouth open may potentially have a greater impact.

9.6 Conclusion

Oral breathing route induced sleep disordered breathing appears to be partially mediated by the increases in STUAL. In Chapter 8, the study demonstrated that enforced oral route of breathing induces sleep disordered breathing and increases STUAL. The hypothesis for this part of the study was that oral route breathing induced sleep disordered breathing can be reduced or eliminated by applying exogenous surfactant to prevent oral breathing associated increases in STUAL. The MW was dramatically reduced on the oral compared to the nasal breathing night, and the “drying” effect oral breathing was not normalised by the administration of exogenous surfactant. While the STUAL may have been reduced with administration of exogenous surfactant, there may have been little liquid present at the upper airway mucosal surface to provide a therapeutic effect. Hence, despite reducing STUAL to levels seen on nasal breathing nights, the RDI on the enforced oral breathing with surfactant night was not reduced to the RDI seen on the nasal breathing night, but nonetheless did decrease in all subjects.

In conclusion, for healthy humans during sleep, enforced oral route of breathing is associated with induction of sleep disordered breathing and is associated with increased STUAL. Lowering STUAL with exogenous surfactant to nasal breathing levels reduces, but does not eliminate oral breathing induced sleep disordered breathing. Therefore, we conclude that oral breathing induced sleep disordered breathing in healthy subjects is only partially mediated by the associated increases in STUAL.

Chapter 10: Effect of Exogenous Surfactant in Subjects with Mild to Moderate Obstructive Sleep Apnoea

10.1 Introduction

Surface tension of upper airways lining liquid (STUAL) influences upper airway patency. Previous animal studies have demonstrated the effects of surfactants in reducing upper airways resistance and snoring in anaesthetised dogs (Widdicombe and Davies, 1988). Studies in anaesthetised rabbits demonstrated the influence of exogenous surfactant on upper airways collapse, with a reduction of STUAL leading to reduced upper airways collapsibility, lower opening pressures, and increased upper airway patency (Kirkness et al., 2003a). Further studies in anaesthetised rabbits demonstrated re-opening and closing pressures increased when salivary production decreased and STUAL was increased using atropine, demonstrating that altering surface tension affects upper airways collapsibility and re-opening (Lam et al., 2008).

In human subjects, instillation of exogenous surfactant reduces STUAL, and subsequently leads to a reduction in the intraluminal pressure required to re-open the closed pharyngeal airway (Kirkness et al., 2003b).

Several studies have demonstrated that lowering of STUAL may reduce the severity of sleep disordered breathing in subjects with OSA, and

lead to a reduction in pharyngeal resistance (Jokic et al., 1998, Morrell et al., 2002, Kirkness et al., 2003c).

In a single night observational study performed by Kirkness et al. (2003) on 9 subjects with severe OSA, 2.5 mL of exogenous surfactant (Exosurf neonatal, GSK) was instilled into the posterior pharyngeal airway via a nasopharyngeal catheter. The study was divided into two halves, with the first half of the study spent determining the effects of exogenous surfactant on Pcrit changes. Exogenous surfactant was re-administered during the second half of the study and the effects on AHI and RDI were measured. The study demonstrated a reduction in STUAL by approximately 25% in sleeping OSA subjects following instillation of exogenous surfactant. This was associated with a significant group mean reduction in the RDI by approximately 31%. Interestingly, no significant relationships were identified between the reduction in STUAL and measures for upper airway mechanics including Pcrit and upstream upper airway resistance (RUS), but the lack of a relationship may have potentially reflected the small number of subjects in the study.

Instillation of exogenous surfactant in this study reduced STUAL and served to generate the hypothesis that exogenous surfactant can reduce the severity of sleep disordered breathing.

10.2 Aims

The aims of the studies in this chapter are: 1) to develop a pilot randomised controlled trial of exogenous surfactant (ES) in subjects with mild to moderate severity OSA; 2) to pilot a new, less invasive delivery method for ES; and 3) to pilot a different surfactant agent (Beractant, Abbott, USA) and investigate its potential use as a therapeutic agent in OSA.

10.3 Methods

10.3.1 Subjects

Study subjects were recruited from the Westmead Hospital Sleep laboratory between June 2009 and June 2012. These subjects were recruited from patients referred into the Westmead Hospital Sleep Laboratory for investigation of sleep disordered breathing with diagnostic polysomnography. Patients who had mild to moderate obstructive sleep apnoea as defined by an RDI of 5 to 29 events per hour of sleep were eligible for recruitment into the study.

12 subjects were recruited into the study (8 male, 4 female). The mean age for the group was 51.5 ± 10.8 years (range 37 to 78 years), and average BMI was 30.2 ± 6.1 kg/m² (range 25-35 kg/m²).

Exclusion criteria for the study included current smoking status, current respiratory tract infection on history, any inflammatory condition or medical illness affecting the mouth or upper aerodigestive tract, previous head and neck irradiation, and history of airways disease requiring inhaled steroid medication or inhaled anticholinergic agent. Subjects who could not ingest products containing bovine derived protein for medical, cultural, or religious reasons were also excluded as Beractant is a surfactant agent derived from a bovine source.

Informed written consent was obtained from each subject and ethics approval was obtained from the Western Sydney Area Health Service Ethics Committee.

10.3.2: Measurements

Surface Tension

UAL samples were aspirated from the posterior pharyngeal wall using fine bore polyethylene tubing (Tyco electronics; external diameter 0.8mm, internal diameter 0.5mm), attached to a 23g needle (BD Precisionglide 0.6mm x 32mm) and a 3 ml syringe (Terumo Medical Corporation 3cc / mL).

Small microlitre volumes were aspirated into the tubing and subsequently transferred with a 1 ml syringe (7500.5N, Hamilton Company, Reno, NV, USA) to the surface of a silica disc for measurement of Surface Tension using the “Pull off force” technique as described in chapter 3 (figure 3.5) (Kirkness et al., 2005a).

This method measures the force required to separate two silica discs bridged by a droplet of the liquid, with the force required for disc separation taken as the liquid’s surface tension.

Polysomnography

All subjects underwent overnight polysomnography for investigation of sleep disordered breathing. Studies were all undertaken at the Westmead Hospital Sleep Laboratory.

Variables monitored during the overnight polysomnography included nasal pressure (1600 Nasal Cannula; Salter Labs Inc., Arvin, CA, USA), EEG (electroencephalogram), ECG (electrocardiogram), submental and diaphragmatic EMG (electromyogram), left and right EOG (electro-oculogram), respiratory inductance plethysmography (for thoraco-abdominal movement), oxygen saturation, sound, and body position. Monitored signals were recorded on a Compumedics Profusion 3 software system for further sleep stage and respiratory event scoring and analysis. Sleep staging was performed on the overnight EEG using Rechtschaffen and Kales scoring criteria (Rechtschaffen and Kales 1968).

Obstructive events and cortical arousals from sleep were identified by using recommended American Academy of Sleep Medicine (AASM) criteria (AASM Manual for the Scoring of Sleep and Associated Events, Iber et al., 2007). The Respiratory Disturbance Index (RDI) was calculated by adding Respiratory Effort Related Arousals (RERAs) to the Apnoea Hypopnoea Index (AHI).

10.3.3 Protocol

The study was conducted over three nights. The first night was a standard diagnostic polysomnography night. Subsequently, subjects were randomised to a Normal Saline (NS) night or Exogenous Surfactant (ES) treatment night for the second and third nights of the study.

Subjects arrived at the Westmead Hospital Sleep Laboratory by 6pm on the study night. Nil by mouth was maintained for a minimum time of one hour prior to “lights out” time at approximately 2200 hours. UAL samples were taken from the posterior oropharynx with the subject in the seated position immediately following 15 minutes of nasal only breathing, at 2100 hours. Following sampling, the subject was allowed to retire to sleep at approximately 2200 hours.

On the second and third study nights, either 2.5mls of normal saline or Beractant was administered via an atomiser into the nasopharynx (1.25mls) and also the oropharynx (1.25mls) immediately before “lights out”. UAL was sampled following either ES or NS administration at 2200 hours, with a period of 5 minutes between administration of ES or NS, and sampling of UAL. Overnight, at approximately 0200 hours, subjects were woken from sleep for sampling and for administration of ES or NS. Sampling of UAL was performed 5 minutes before and 5 minutes after administration of either ES or NS at ~0200 hours. Final sampling of UAL was performed at 0600 hours the following morning.

10.3.4 Data Analysis

Sleep Parameters

Studies were sleep staged for each 30 second epoch using Rechtschaffen and Kales scoring criteria (ref). Respiratory events were scored according to American Academy of Sleep Medicine criteria (AASM Manual for the Scoring of Sleep and Associated Events, Iber et al., 2007) with the respiratory disturbance index (RDI) calculated as the total number of both apnoeas, hypopnoeas, and RERAs divided by the total sleep time during overnight polysomnography. An apnoea was defined as a complete cessation of airflow on the nasal pressure cannula signal, for ≥ 10 s, with continued respiratory effort evident on thoracic and abdominal movement bands, associated with oxygen desaturation or arousal from sleep. Hypopnoeas were defined by a $\geq 50\%$ airflow reduction and a $\geq 3\%$ desaturation on oximetry or and EEG arousal. Increased respiratory efforts and arousals related to these efforts were scored as RERAs (Respiratory Effort Related Arousals).

Statistical Analysis

All data were expressed as mean \pm SD. Data were compared using ANOVA to determine differences between time points. Single comparisons were made using paired t-tests. Statistical data were graphically represented and analysed using Graphpad Prism. $P < 0.05$ was considered significant.

10.4 Results

10.4.1 STUAL values for 3 nights

Diagnostic Night

For the diagnostic polysomnography night, the mean pm STUAL for the group was 63.3 ± 3.1 mN/m while the mean am STUAL was 62.7 ± 2.7 mN/m. Overall, there was no significant difference (t-test, $p = 0.62$) in STUAL between pm and am values (Figure 10.1). The STUAL values observed on the diagnostic polysomnography night were similar to the values described in earlier chapters.

Normal Saline Night

For the NS night, the sampling times were 2100, 2200, 0155, 0205 and 0600 hours. The mean STUAL values for the group for these sampling times were 62.5 ± 2.4 mN/m, 63.5 ± 3.0 mN/m, 62.4 ± 3.8 mN/m, 61.7 ± 4.1 mN/m, and 61.8 ± 2.6 mN/m, respectively (Figure 10.2). There were no significant differences identified between these sampling times during the NS night (ANOVA, $p = 0.37$). There was no significant difference between baseline pm STUAL values on the diagnostic night and 2100 hour values on the NS night (t-test, $p = 0.57$). Similarly, there were no differences in 0600 STUAL values between the NS night and diagnostic PSG night (t-test, $p = 0.39$).

STUAL Diagnostic Night

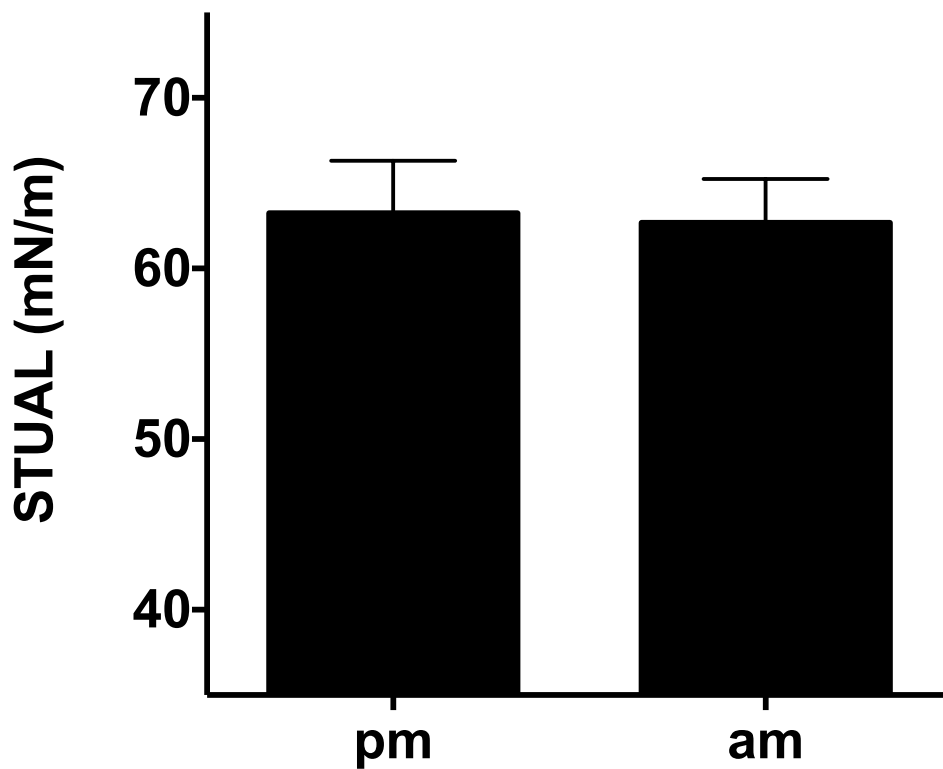


Figure 10.1 Group Mean Values for STUAL (Diagnostic night)

Group mean values (error bars = +1SD) for evening (pm) and morning (am) STUAL on the diagnostic polysomnography night. No significant difference between PM and AM values (t-test, $p = 0.62$). STUAL = Surface Tension Upper Airway Liquid.

STUAL Saline night

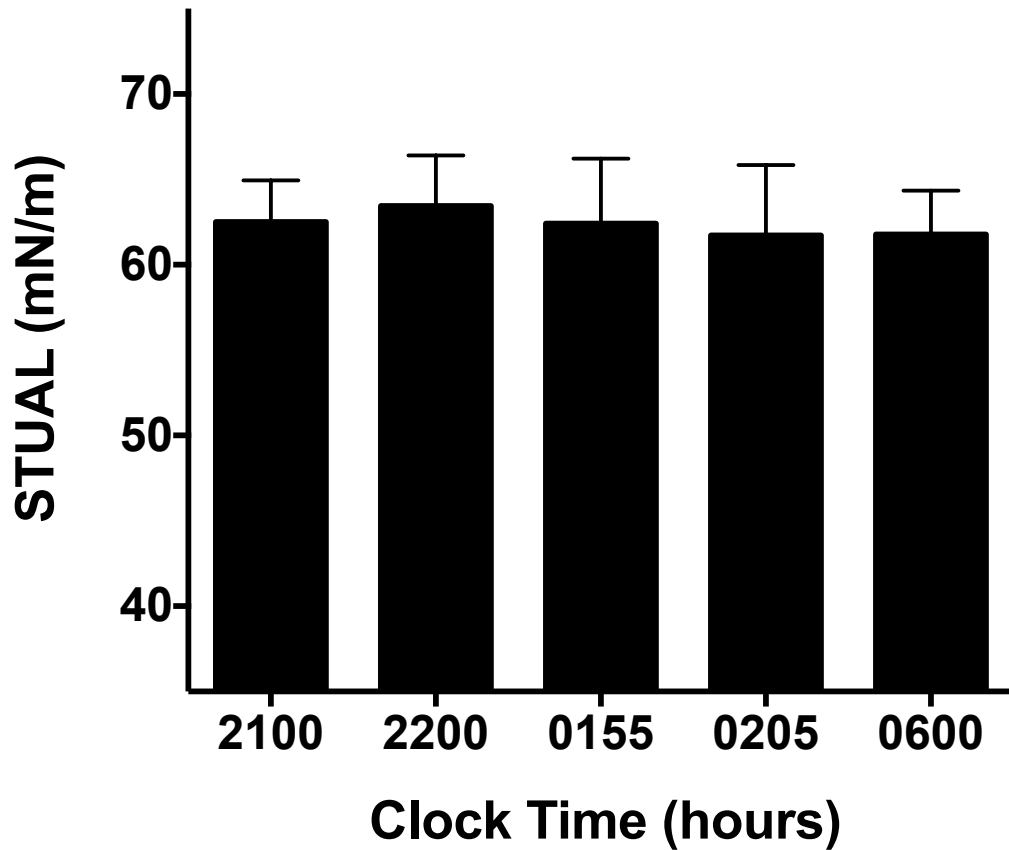


Figure 10.2 Group Mean STUAL values (NS night)

Group mean values (error bars = +1SD) for STUAL on the NS night at the five time points sampled. No significant difference in STUAL values were identified across the sampling times (ANOVA, $p = 0.37$). STUAL = Surface Tension Upper Airway Liquid.

Exogenous Surfactant Night

The sampling times on the ES night were the same as for the NS night (2100, 2200, 0155, 0205 and 0600 hours), with the group mean STUAL for these times being 63.8 ± 2.3 mN/m, 61.4 ± 2.6 mN/m, 57.2 ± 3.1 mN/m, 55.5 ± 3.2 mN/m, and 61.8 ± 1.2 mN/m, respectively. In comparison to the STUAL value at 2100 hours, there was a significant decrease (ANOVA, $p < 0.05$) in STUAL at 0155 hours and 0205 hours.

There was no significant difference in STUAL at 2100 hours vs 2200 hours, whilst at 0155 hour vs 0205 hour sampling times (done approximately 5 minutes pre and post administration of ES), and there was also no significant difference seen in STUAL (ANOVA, $p = 0.29$). By 0600 hours, the STUAL values had returned to the baseline STUAL seen at 2100 hours (Figure 10.3). The group mean values for STUAL on the ES night are demonstrated in Figure 10.3. Individual data for STUAL on both ES and NS nights are demonstrated in Figure 10.4

STUAL Surfactant night

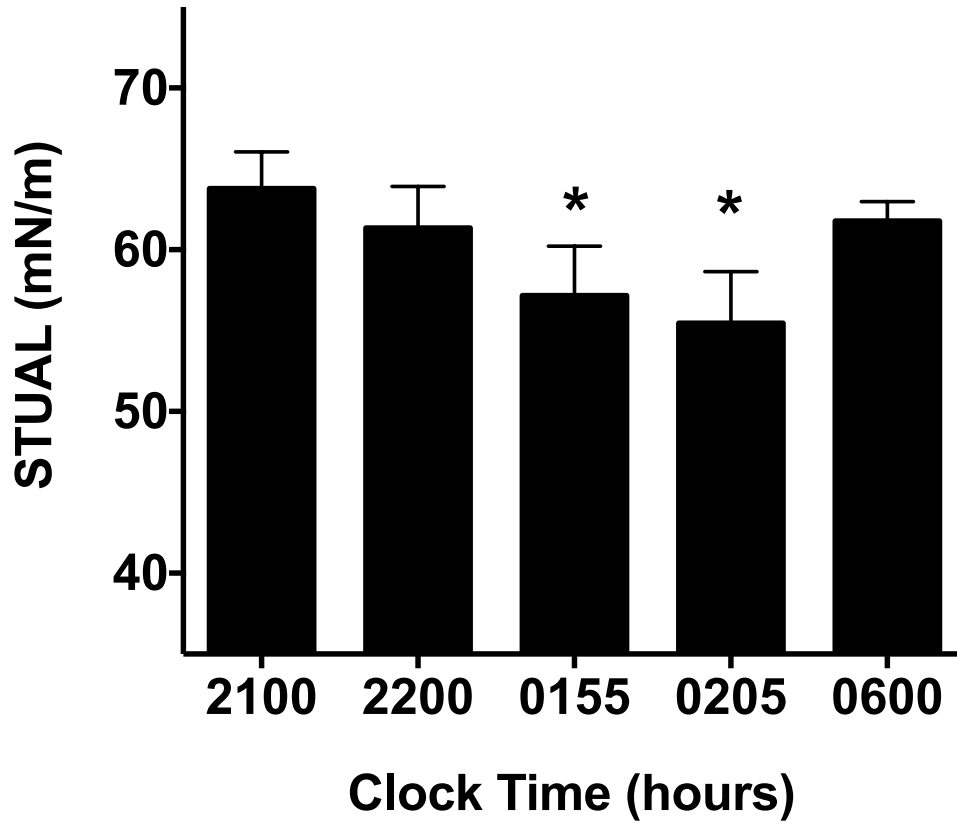


Figure 10.3 Group Mean STUAL values (ES night)

Group mean values (error bars = +1SD) for STUAL on the ES night at the five time points sampled. In comparison to 2100 hours, STUAL values at 0155 hours and 0205 hours were significantly reduced. STUAL returned to baseline values by 0600 hours. * ANOVA, $p < 0.05$ relative to 2100 hour value. STUAL = Surface Tension Upper Airway Liquid.

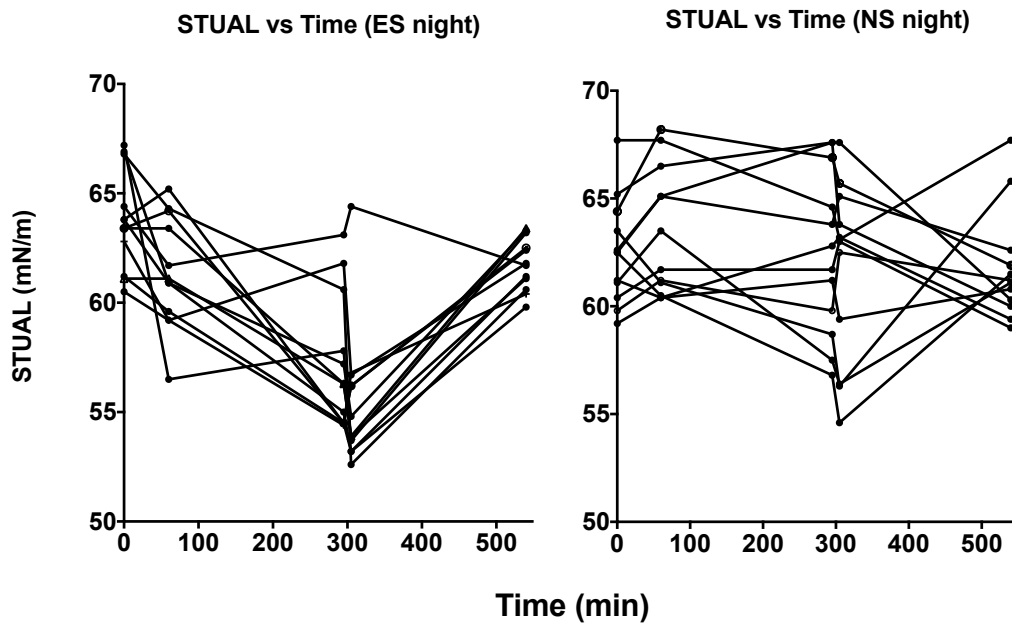


Figure 10.4 Individual subject data for STUAL on ES and NS nights

Plot demonstrating the STUAL values across the nights in individual subjects ($n = 12$) with mild to moderate OSA on both the ES night and NS night. Individual data for STUAL are plotted as dots for each sampling time point, with a line connecting values for each subject. Sampling times were at 0, 60, 295, 305, and 540 minutes which represented sampling at 2100, 2200, 0155, 0205, and 0600 hours respectively. STUAL = Surface Tension Upper Airway Liquid; ES = Exogenous Surfactant; NS = Normal Saline.

10.4.2 Polysomnography Results

Sleep Parameters

Sleep architecture was observed across the three study nights, and no significant differences were seen in the proportion of sleep spent in Stages 1,2, slow wave sleep (SWS) and REM sleep (ANOVA, all $p > 0.07$).

There was no significant difference seen in the group mean arousal index between the diagnostic night, NS night and ES night with the arousal indices for these 3 nights being 21.6 ± 12.8 , 21.6 ± 12.0 , and 20.6 ± 8.0 arousals / hour, respectively (ANOVA, $p = 0.95$). When the proportion of respiratory arousals to spontaneous arousals was analysed, there were no differences seen between the three study nights. Other parameters which were analysed are demonstrated in Tables 10.1 and 10.2, and did not demonstrate a significant difference between the three nights included sleep efficiency, and oxygen desaturation index (ANOVA, all $p > 0.45$)

Sleep Disordered Breathing

The AHI (apnoeas + hypopnoeas) and RDI (AHI + RERAs) were analysed separately. The AHIs for the diagnostic, NS, and ES nights were 10.2 ± 9.4 , 6.6 ± 6.7 , and 7.1 ± 7.5 events / hour, respectively. The RDIs for the three study nights were 16.5 ± 7.4 , 18.3 ± 11.9 , and 19.9 ± 13.1 events / hour, respectively. There were no significant differences seen for either AHI or RDI across the three study nights (ANOVA, all $p > 0.45$). Individual data for AHI and RDI for the three study nights demonstrated significant variability,

and no consistent pattern was apparent for individual subjects over the three study nights (Figures 10.5 and 10.6).

The RDI and ST values for the NS night and ES night were analysed further for any relationship. The difference in RDI between the NS and ES nights was expressed as Δ RDI (RDI on ES night – RDI on NS night) whilst the difference between individual mean STUAL values on the two nights was expressed as Δ STUAL (mean STUAL on ES night – mean STUAL on NS night). There was no relationship identified between Δ RDI and Δ STUAL (linear regression, $p = 0.33$).

	Diagnostic	NS	ES
Sleep Efficiency (%)	78.1 ± 8.7	79.3 ± 10.0	79.3 ± 9.4
Arousal Index (events/hr)	21.6 ± 12.8	21.6 ± 14.0	20.6 ± 8.0
Spontaneous Arousals (events/hr)	8.1 ± 7.4	6.8 ± 3.5	6.3 ± 3.9
Respiratory Arousals (events/hr)	10.7 ± 5.9	13.4 ± 14.2	13.6 ± 7.5
RDI (events/hr)	16.5 ± 7.4	18.3 ± 11.9	19.9 ± 13.1
AHI (events/hr)	10.2 ± 9.4	6.6 ± 6.7	7.1 ± 7.5
RERA (event/hr)	8.1 ± 7.4	6.8 ± 3.5	6.3 ± 3.9

Table 10.1 Sleep Parameters and Severity of OSA across 3 study nights

Sleep Efficiency, arousal indices, RDI, AHI, and RERAs for the three nights of the study. The severity of sleep disordered breathing was similar for all 3 nights of the study (ANOVA, all $p > 0.45$). NS = Normal Saline night; ES = Exogenous Surfactant night; RDI = Respiratory Disturbance Index; AHI = Apnoea Hypopnoea Index; RERA = Respiratory Effort Related Arousals.

	Diagnostic	NS	ES
Stage 1 (%TST)	8.5 ± 8.9	2.6 ± 1.9	3.1 ± 4.2
Stage 2 (%TST)	49.0 ± 10.2	54.7 ± 10.6	57.2 ± 8.0
Slow Wave Sleep (%TST)	22 ± 6.8	23.0 ± 9.2	20.3 ± 8.3
REM (%TST)	20 ± 6.9	19.6 ± 5.4	17.9 ± 5.6

Table 10.2: Sleep Architecture across 3 study nights

Sleep Architecture across the 3 nights of the study (Diagnostic PSG night, Normal Saline (NS) night, and Exogenous Surfactant (ES) night). There were no significant differences seen in sleep architecture for the group across the 3 nights of the study (ANOVA, all $p > 0.07$). REM = Rapid Eye Movement; TST = Total Sleep Time.

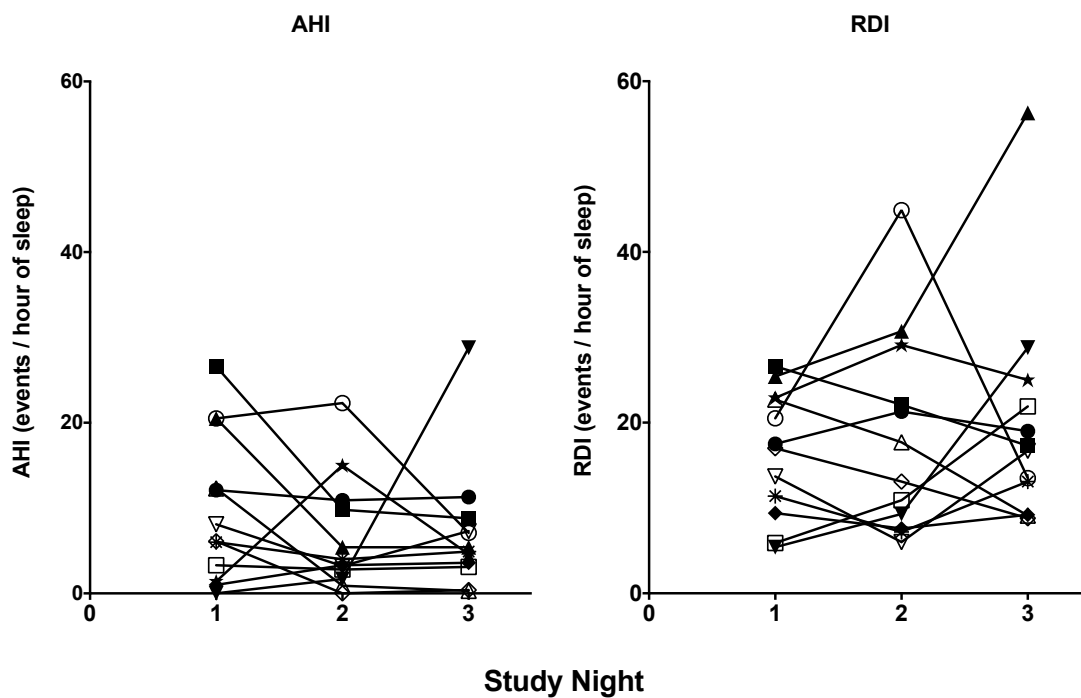


Figure 10.5 Individual subject data for AHI and RDI

Individual subject data for AHI and RDI across the three study nights. Study night 1 = Diagnostic sleep study; study night 2 = normal saline night ; study night 3 = exogenous surfactant night. Each symbol represents an individual subject. Note the variability seen in AHI and RDI for individual subjects, with no consistent pattern. AHI = Apnoea Hypopnea Index; RDI = Respiratory Disturbance Index.

Δ RDI vs Δ STUAL (ES-NS)

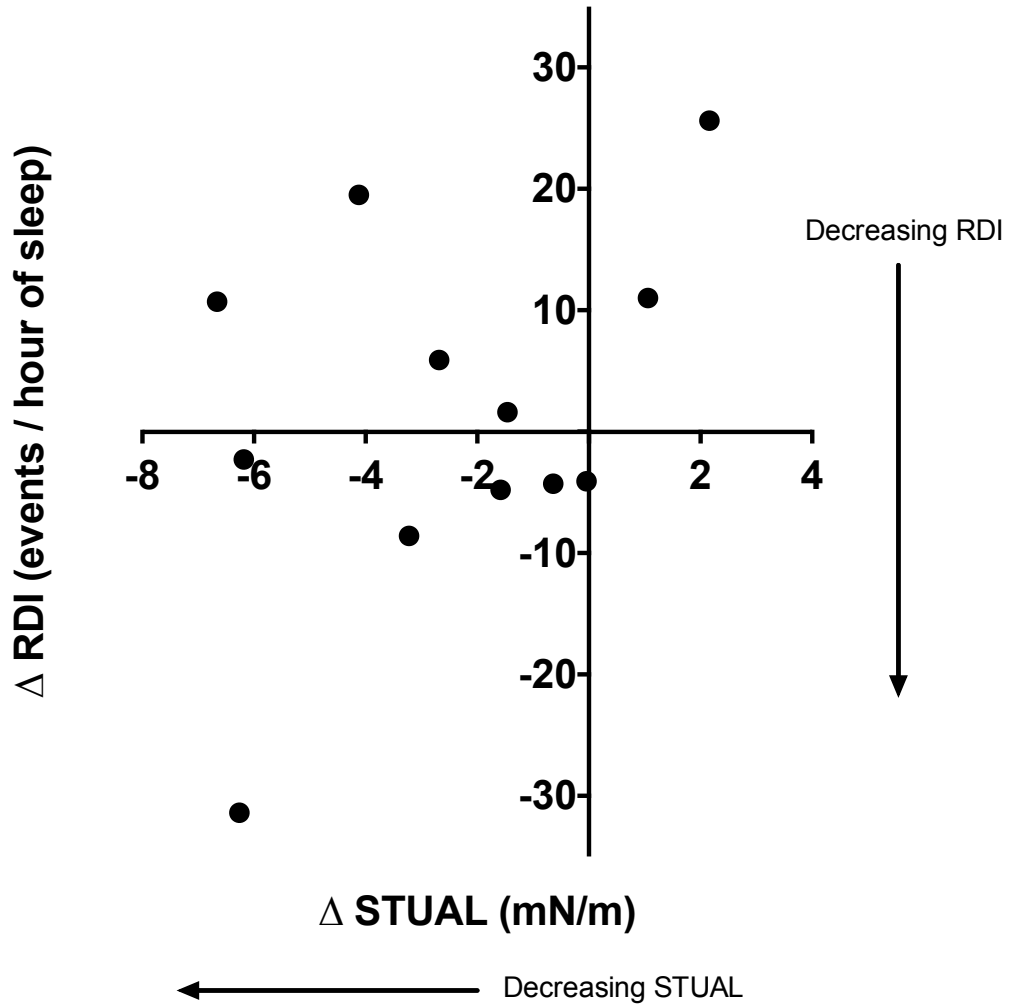


Figure 10.6 Change in RDI vs change in STUAL

The change in RDI (between Surfactant and Normal Saline nights) plotted against the change in STUAL (mean STUAL on Surfactant night – mean STUAL on Normal Saline night) for individual subjects. Decreasing STUAL did not have any significant effect on RDI (linear regression, $p = 0.33$). RDI = Respiratory Disturbance Index; STUAL = Surface Tension Upper Airway Liquid; ES = Exogenous Surfactant night; NS = Normal Saline night.

10.5 Discussion

This study demonstrates that exogenous surfactant delivered via atomizer spray reduces the STUAL in subjects with OSA. However, there is individual variability seen in the overnight change in RDI following administration of exogenous surfactant or normal saline. There is no overall reduction in the severity of OSA seen with an exogenous surfactant (Beractant) administered to a group of subjects with mild to moderate OSA.

10.5.1 Critique of Methods

In comparison to previous studies performed by our laboratory, the methods used in this study differ with regard to the type of surfactant used and the delivery method. This may partially explain the reason for the lack of a significant effect of surfactant on RDI between the NS night and ES nights.

In previous studies performed through our laboratory (Kirkness et al., 2003c)(Kirkness et al., 2003b) the exogenous surfactant used was Exosurf Neonatal (Exosurf Neonatal; GSK, Greenville, NC, USA). 2.5ml of Exosurf was instilled into the posterior wall of the nasopharynx in human subjects over a period of one minute. However, in the present study, the exogenous surfactant used was Beractant (Abbott, USA), due to Exosurf no longer being available at the time of this study.

The surface tension lowering effect of Beractant was similar to that seen for Exosurf. However, Exosurf is a protein - free surfactant and utilises spreading agents, whereas Beractant is derived from a bovine extract, and does not contain spreading agents.

The delivery method utilised in this study also differs from the previous study by Kirkness et al investigating the relationship of STUAL and upper airway collapsibility in OSA (Kirkness et al., 2005b). In the current study, surfactant was delivered by atomiser, with 1.25 mls sprayed into the nasopharynx, and 1.25 mls sprayed into the oropharynx. For the current study, there are potentially two issues regarding this administration. Firstly, since Beractant does not contain additional spreading agents, its spreading properties may not be the same as those found in Exosurf, and the distribution and time taken for the delivered dose to spread through the upper airways may be affected. Secondly, the delivery of surfactant via atomiser spray may have different upper airway deposition patterns in comparison to the instilled method. In this study, half the dose of Beractant was delivered into the oropharynx (aimed at the posterior oropharyngeal wall) whilst the other half of the dose was delivered into the nasopharynx, with the potential for increased time taken for the dose to spread to the posterior nasopharyngeal region, and for some of this dose to be retained at the level of the turbinates and sinuses.

The route of breathing in this study was not enforced. Previous chapters have demonstrated the effect of enforced route of breathing upon STUAL values and also upon severity of OSA, and non-enforced route of breathing in this study may have potentially affected the efficacy of any surfactant dosing depending upon the amount of any oro-nasal breathing in any individual subject overnight. The subjects were not controlled for sleep posture, however, there were no significant differences observed in sleep posture between nights for individual subjects. Similarly, sleep architecture did not demonstrate any significant variations for individual subjects between the three study nights.

Subjects were woken prior to sampling and surfactant administration overnight in the present study. Any swallowing or increase in oro-nasal breathing during the awake period may have potentially affected the STUAL, or the clearance of any surfactant from the upper airways at the time of overnight sampling prior to repeat Beractant administration.

10.5.2 STUAL

Both the diagnostic and NS nights in this study did not demonstrate any significant change in STUAL overnight. The STUAL values measured on the diagnostic and NS nights are similar to those seen in previous chapters. Interestingly, although normal saline has a higher surface tension of approximately 72 mN/m, there was no significant increase in STUAL on the

NS night, either after initial administration at 2200 hours or before and after sampling at approximately 0200 hours. Delivery of normal saline into the oro-pharynx and nasopharynx may have potentially stimulated salivary production (Neyraud et al., 2003) which may have reduced the concentration of the atomised normal saline and potentially mitigated the surface tension increasing effect of saline upon STUAL. Alternately, normal saline may not have the same spreading or binding effects on the local airway mucosa, and its effect and presence in the upper airway may be short-lived.

On the ES night, the STUAL demonstrated a significant decrease overnight. However, this decrease was not apparent until the STUAL sample taken prior to the 0200 dose of surfactant. Only a small, but insignificant decrease was seen immediately after the first ES administration. The STUAL values from 0155 hours and 0205 hours were not significantly differently from each other, although a trend to a small reduction in STUAL was observed immediately following 0200 dosing. Both values were significantly decreased when compared to the 2100 hour sample. STUAL samples taken just after the 2200 hour dose of ES did not demonstrate a significant reduction compared to the baseline samples, consistent with the lack of change seen at the middle of the night sample. This suggests the possibility of inadequate time for the delivered dose to spread through the oro-pharynx and nasopharynx. However, these data do demonstrate that the delivered dose of surfactant used in this protocol does have a surface tension lowering effect that persists for up to four hours.

Although the sampling at approximately 0200 hours indicates a persistent reduction in STUAL compared with baseline, this persistent reduction is not repeated following the administration of surfactant at 0200 hours (i.e. between 0200 and 0600 hours). By 0600 hours, the STUAL returns to baseline in all of the subjects on the ES night. Human saliva is known to contain surfactant, and also exhibits a circadian rhythm, whereby production is at its nadir in the early hours of the morning (Dawes, 1975). This has also been demonstrated in previous chapters, with lower salivary flow and mucosal wetness in the morning. It is possible that the surface active effects of ES may require the normal physiological production of saliva to have a significant effect in lowering STUAL. Another possibility may include an increase in oronasal breathing upon waking which increases STUAL. However, this is unlikely as even in the presence of enforced oral breathing the STUAL takes 1-2 hours to increase significantly, and most subjects were actually woken from sleep for the 0600 sampling, which minimised the time available for awake oronasal breathing to occur.

The minimum STUAL values that were obtained in the present study using Beractant were not as low as those seen in the Kirkness et al. (2003) study using Exosurf. The differences in both delivery of surfactant and the surface active and spreading properties in the two surfactant agents may have made a significant difference to the SDB outcomes seen in this study. The reductions achieved in STUAL may not have been of sufficient magnitude to have made a significant reduction in sleep disordered breathing

10.5.3 Sleep Disordered Breathing

The overall group values for AHI and RDI in this study were fairly similar across the three nights, with no significant differences found in the severity of sleep disordered breathing between the three nights. Previous studies in our laboratory (Kirkness et al., 2003c) have demonstrated a decrease in the RDI which correlated to a decrease in the surface tension of liquid lining the upper airway, with 8 of 9 subjects demonstrating a significant reduction in RDI from 51 events / hour of sleep to 35 events / hour of sleep. The sleep disordered breathing was severe on the diagnostic night, and although significantly reduced with surfactant, still remained severe on the surfactant night in the Kirkness et al. (2003) study. The subject group in the present study only had mild to moderate OSA. The reduction in RDI was not replicated with the current study using a different surfactant, a new method of dose delivery, and a less severe target patient group. It is possible that not all OSA patients will respond to the surfactant therapy, and it may be that a particular subgroup of patients (or phenotypic group) will need to be defined to further test this type of intervention, as it appears to be more effective in severe patients than in mild to moderate OSA patients.

The previous chapter demonstrated a significant reduction in RDI when normal subjects with oral breathing induced OSA were given ES. It remains possible that, for this combination of surfactant and method of delivery, a reduction in the severity of sleep disordered breathing may not be achieved without enforcing breathing route (i.e., combination of enforced nasal breathing route, together with exogenous surfactant delivered via

atomiser oronasally). It is possible that a combination of enforced nasal breathing route and exogenous surfactant may still be useful in the mild to moderate OSA group, but this study was not designed to test this hypothesis.

10.6 Conclusion

Although a reduction was seen in the STUAL on the ES night, there was no associated reduction in the severity of OSA on the ES night. There was a small (but not statistically significant) reduction in STUAL immediately after ES administration, with a further sustained decrease at 4 hours. There was a further (but not statistically significant) decrease after the second ES dose, which was not sustained at a further 4 hours.

Individual subjects demonstrated substantial variability in RDI when comparing diagnostic vs NS vs ES nights, with large changes seen in both directions. However, there was no overall reduction in the severity of OSA with exogenous surfactant administered to a group of patients with mild to moderate OSA. Future studies investigating a surfactant intervention to improve SDB may need to better define the suitable patient phenotype who will respond to this therapy and also to control for overnight breathing route.

Chapter 11: General Discussion

This thesis has examined the role of surface tension and breathing route on the severity of sleep disordered breathing (SDB). Our laboratory has previously examined the influence of breathing route and surface tension on obstructive sleep apnoea (OSA) separately. The series of experiments contained within this thesis examines the influence of both breathing route and surface tension, by firstly enforcing breathing route, secondly by manipulating surface tension with exogenous surfactant, and finally, by enforcing oral breathing route and lowering surface tension of upper airway liquid concurrently.

The range of surface tension values across the day for upper airway liquid in healthy subjects was defined in Chapter 5, which demonstrated that STUAL values remained relatively stable across a 24 hour period. This has not been previously described, with other studies demonstrating changes between evening and morning STUAL values only (Kirkness et al., 2005b). The range of STUAL values in subjects with OSA was examined in Chapter 6, and demonstrated surface tension values as low as that seen with exogenous surfactant administered to the upper airway (approximately 45-60 mN/m), and as high as that seen with normal saline (72 mN/m). This represents a wide range of STUAL values in the largest cohort of OSA subjects tested to date.

A positive association was found between OSA subjects ≥ 40 years of age and increased STUAL. Interestingly, there is increased oro-nasal breathing route in “older” healthy subjects ≥ 40 years of age (Madronio et al., 2004), which raises the question of whether this phenomenon of increased oro-nasal breathing route is influencing STUAL in “older” OSA subjects.

The role of breathing route was further examined in Chapter 7 in OSA subjects. Breathing route was not enforced in this study, but quantified by a method of scoring breathing route previously used in our laboratory (Madronio et al., 2004). There was no association found between breathing route and STUAL, and there were no significant differences found in pm and am STUAL. However, there was a decrease in salivary flow rate and mucosal wetness overnight, which are likely to reflect circadian changes, with salivary production reduced overnight during sleep (Dawes, 1975).

To determine the effect of enforced breathing route on STUAL and severity of sleep disordered breathing, a three night study was performed. Subjects had one night each utilising enforced nasal breathing route and enforced oral breathing route, with the subjects on the final study night utilising oral breathing route and given exogenous surfactant (administered both nasally and orally). Compared with the nasal breathing night, enforced oral breathing route induced a moderate degree of SDB in normal subjects.

Similar results have previously been reported with oral enforced breathing route inducing sleep apnoea (Fitzpatrick et al., 2003), however a causal mechanism between route of breathing and OSA has not been elucidated.

Exogenous surfactant (Beractant) administered on the enforced oral breathing route night to healthy subjects was shown to reduce STUAL in Chapter 9. In comparison to the enforced oral route of breathing night without exogenous surfactant, the RDI on the exogenous surfactant night decreased in all of the subjects. Additionally, although exogenous surfactant was able to reduce the STUAL to values seen on the nasal breathing night, this did not eliminate oral breathing induced SDB. It is likely that surface tension forces partially mediate the severity of SDB due to enforced oral breathing route, but other mechanisms such as anatomical factors with conformational changes in upper airway i.e. jaw position, soft palate and tongue position (Meurice et al., 1996), may also play an important role in upper airway closure.

Although exogenous surfactant was shown to decrease the RDI in healthy subjects with oral breathing route induced SDB, this was not evident when OSA subjects were administered exogenous surfactant. In the final study of this thesis (Chapter 10), exogenous surfactant was administered to subjects with mild to moderate severity of OSA. Breathing route was not enforced in this study, and the subjects underwent a diagnostic polysomnography night, and two intervention nights, one with exogenous

surfactant (Beractant) administered and one with Normal Saline. Although exogenous surfactant reduced STUAL, there was no associated reduction in the severity of OSA. The possible causes for this may include the new delivery method used in this study or, more likely, the type of surfactant used. Our laboratory has previously used a different type of surfactant (Exosurf neonatal, Burroughs Wellcome Australia) with the surfactant used in this study being Beractant, which may not have the same surface tension characteristics, duration of action, or spreading and mucosal adherence qualities as Exosurf.

In summary, surface tension forces have been recognised to influence upper airway patency (Morrell et al., 2002, Van der Touw et al., 1997), with a reduction in STUAL resulting in decreased upper airway collapsibility (Lam et al., 2008, Kirkness et al., 2003a) and decreased severity of OSA (Kirkness et al., 2003c). Both nasal and oronasal route of breathing occur in healthy subjects (Madronio et al., 2004) and in subjects with OSA, with enforced oral breathing increasing the severity of OSA (Fitzpatrick et al., 2003). The link between enforced oral route of breathing and increased surface tension has been reported in awake subjects (Verma et al., 2006), while in healthy, “older” subjects, there is a greater proportion of oronasal breathing during sleep (Madronio et al., 2004).

The work in this thesis has 1) established a wide range of surface tension values in healthy subjects and subjects with OSA; 2) explored the

route of breathing and its influence on surface tension in healthy and OSA subjects; 3) examined the effect of enforced route of breathing on STUAL and sleep disordered in healthy subjects; 4) induced a moderate degree of SDB in healthy subjects with enforced oral breathing route; 5) demonstrated a reduction in the severity of this oral breathing induced SDB with exogenous surfactant; 6) piloted a trial of a different exogenous surfactant agent and a new, less invasive delivery method to reduce STUAL in subjects with mild to moderate OSA.

Although we did not find an effect of exogenous surfactant in decreasing the severity of OSA in Chapter 10 of this thesis, it remains possible that exogenous surfactant therapy may have a role in a certain patient phenotype with OSA. Future trials may need to better define the subject population who may respond to this intervention, and perhaps subjects with mild OSA and predominantly oral route of breathing may be an appropriate group to study. Alternately, enforced nasal breathing route may be utilized together with exogenous surfactant in this patient group to determine if the combination of lowering STUAL and nasal breathing route has any influence on the severity of sleep disordered breathing.

It is becoming apparent that there is an increasing focus not only in Sleep Medicine, but with medical management of diseases in general, on moving towards an era of personalized medicine, where identifying the combination of disease characteristics and patient risk factors and traits in defining the patient's phenotype may maximize the efficacy of medical

intervention, and also help identify novel therapeutic targets. With regards to OSA, the phenotypic traits may include the patient's upper airway anatomy and craniofacial shape, breathing route during sleep, their upper airway neuromuscular responses, ventilatory control manifested by loop gain, and cortical arousal thresholds.

Perhaps it is now time to better define the measurement of the phenotypic traits of breathing route and surface tension to potentially identify phenotypic subgroups of OSA patients, and to consider the development of new targeted therapies for these novel phenotypic traits.

Chapter 12: Summary of Results

12.1 Surface Tension of Upper Airway Liquid in Healthy Subjects

Sampling UAL and Saliva across a 24 hour period in healthy subjects demonstrated:

- 1) A wide range of STUAL with a range from 56.6 to 70.4 mN/m.
- 2) A wide range of ST of Saliva with a range from 56.0 to 71/4 mN/m.
- 3) ST of Saliva was positively correlated with STUAL across all time periods.
- 4) STUAL values appeared unaffected by the time of day.

12.2 Surface Tension of Upper Airway Liquid in Obstructive Sleep Apnoea Subjects

UAL and Saliva samples were collected prior to sleep from subjects with OSA and the surface tension of these sample was measured. This study demonstrated:

- 1) A strong correlation between ST Saliva and STUAL ($r = 0.78$, $p < 0.0001$).
- 2) A range of STUAL values, from 45.1 to 73.7 mN/m, and a mean STUAL of 61.4 ± 5.2 mN/m.
- 3) A range of ST Saliva values, from 49.5 to 77.2 mN/m, and a mean ST Saliva of 61.3 ± 5.1 mN/m.

- 4) In older subjects with severe OSA, increasing age was weakly correlated with increasing STUAL

12.3 Non-enforced Breathing Route and Surface Tension of Upper Airway Liquid in Subjects with Obstructive Sleep Apnoea

Breathing Route and STUAL, MW, and Salivary Flow Rate were analysed in subjects with Obstructive Sleep Apnoea. The results are as follows:

- 1) No difference between evening and morning values for STUAL.
- 2) A significant decrease in MW overnight.
- 3) A significant decrease in Salivary Flow Rate overnight.
- 4) A range of nasal and oronasal breathing route. All subjects had nasal breathing , with the range of oronasal breathing ranging from 0 – 100% of total sleep epochs.
- 5) The mean percentage of total sleep epochs spent oronasal breathing was 29%.

12.4 Influence of Enforced Breathing Route on Surface Tension of Upper Airway Liquid and Sleep Disordered Breathing in Healthy Subjects

Healthy subjects were randomized to enforced nasal or oral route of breathing. The severity of OSA was determined by polysomnography and samples were obtained for STUAL, MW, and Salivary Flow Rate at 0,4, and 8 hours from the start of the study. This demonstrated:

- 1) A significant increase in the severity of SDB was associated with increases in STUAL on the enforced oral route of breathing night.
- 2) MW and Salivary Flow Rate decreased overnight on the enforced oral breathing night, but remained unchanged on the enforced nasal breathing night.
- 3) Enforced oral breathing route induces sleep disordered breathing in normal subjects.

12.5 Effect of Exogenous Surfactant during Enforced Oral Breathing Route on Surface Tension of Upper Airway Liquid and Severity of Sleep Disordered Breathing in Healthy Subjects

Following on from the previous study examining the role of enforced oral breathing route on STUAL and severity of SDB in healthy subjects, a subsequent study was performed using exogenous surfactant (ES) to lower the STUAL during enforced oral breathing route. The results are as follows:

- 1) ES significantly reduced the severity of oral breathing induced SDB in healthy subjects, but did not eliminate SDB.
- 2) All of the subjects demonstrated a reduction in RDI when administered ES.
- 3) ES administration resulted in STUAL values that were reduced to values seen with enforced nasal breathing route.

12.6 Effect of Exogenous Surfactant in Subjects with Mild to Moderate Obstructive Sleep Apnoea

A pilot study was conducted to 1) develop a randomized controlled trial of exogenous surfactant (ES) in subjects with mild to moderate OSA; 2) to pilot a new, less invasive delivery method for ES; and 3) to pilot a different surfactant agent (Beractant, Abbott, USA) and investigate its potential use as a therapeutic agent in OSA. The study was conducted over 3 nights and subjects underwent a diagnostic polysomnography night, and two intervention nights receiving either normal saline (NS) or exogenous surfactant (ES) via atomizer spray. The results are as follows:

- 1) ES delivered via atomizer spray reduces STUAL in OSA subjects.
- 2) There is no overall reduction in the severity of OSA with ES.
- 3) STUAL values decreased to a nadir at 4 hours and returned to baseline values by 8 hours.
- 4) Individual subjects demonstrated substantial variability in RDI when comparing diagnostic vs NS vs ES nights, with large changes in both directions.

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