UPPER AIRWAY SIZE DURING INHALATION FOLLOWING MANDIBULAR ADVANCEMENT WITH A STEPPED MOUTHPIECE: EVALUATION WITH ACOUSTIC PHARYNGOMETRY

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Abstract

The size of the upper airway is critical during oral inhalation of drugs. Mandibular advancement through oral appliances has been introduced in the treatment of subjects with obstructive sleep apnoea (OSA) as a method to increase the size of the upper airway but has not been extended to subjects using inhalers.
The main objectives of the 4 studies were to correlate upper airway cross-sectional areas (CSA) and volumes measured with acoustic pharyngometry with oropharyngeal and lung depositions, to evaluate the impact of mandibular advancement and incisor opening achieved with stepped mouthpieces on the upper airways, and to investigate *in vitro* the impact of an open velum on the acoustic pharyngogram.
Statistically significant correlations between oropharyngeal and lung depositions, and upper airway CSA at glottis and volume between epiglottis and glottis, were shown in 9 healthy subjects.
Four healthy subjects were included in a proof-of-concept study of a new stepped mouthpiece (without tongue depressor) with which different mandibular advancements (-3 to +6 mm) and incisal openings (10, 15 and 20 mm) were achieved. The upper airway CSA and volume was shown to increase in all 4 subjects.
Sixty subjects (30 healthy and 30 with OSA) were included in a study of the impact of mandibular advancement (0 to 5 mm) and incisal opening (18 mm) achieved with a stepped mouthpiece (with tongue depressor) on the size of the upper airways. Statistically significant effects were shown following both incisal opening and mandibular advancement, and the effects were larger for the healthy subjects. In the *in vitro* study the effect of an open velum on the acoustic pharyngogram was investigated through a cast of a human upper airway. The results showed that during acoustic pharyngometry an open velum would pass acoustic impulses into the nasal airways which would create an overestimation of the volume of the upper airways from the pharynx to the glottis.
The thesis highlights the possibility to increase the size of the upper airways during inhalation of drugs.

Keys words: acoustic pharyngometry, mandibular advancement, incisal opening, stepped mouthpiece, tongue depressor, and nebuliser.

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Dedications and acknowledgements

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List of abbreviations

AAD	Adaptive Aerosol Delivery
ABPI	Association of the British Pharmaceutical Industry
AE	Adverse Event
AHI	Apnoea/Hypopnoea Index (Apnea/Hypopnea Index)
AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
AR	Acoustic Reflection
ARP	Acoustic Reflection Pharyngometer
ATM	Atmospheric pressure
ATS	American Thoracic Society
AUC	Area under the Curve
BFL	B = Baseline, M = mid tidal inhalation and I = Inhalation
BMI0	B = Baseline, F = FRC and L = Landmarks
BMI	Body Mass Index
CAP	Continuous Airway Pressure
CBCT	Cone Beam Computed Tomography
CF	Cystic Fibrosis
CNAP	Continuous Negative Airway Pressure
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CSA	Cross-Sectional Area
CT	Computed Tomography

CV	Coefficient of Variation
DPI	Dry Powder Inhaler
^{99m}Tc-DTPA	^{99m}Tc-Diethylene Triamine Penta-acetic Acid
EG	Epiglottis
ERV	Expiratory Reserve Volume
FEV_1	Forced Expiratory Volume exhaled in 1 second
FDA	Food and Drug Administration
FPF	Fine Particle Fraction
FRC	Functional Residual Capacity
GI tract	Gastro-intestinal tract
GCP	Good Clinical Practice
GL	Glottis
GOF	Goodness of Fit
H_2O	Water
IC	Inspiratory Capacity
ICH	International Conference on Harmonisation
INF-γ	Interferon –γ
IPF	Idiopathic Pulmonary Fibrosis
IRV	Inspiratory Reserve Volume
kHz	Kilohertz
L/s	Liter per second
mBq	Millibecquerel
MMD	Mass Median Diameter
MMAD	Mass Median Aerodynamic Diameters

MRI	Magnetic Resonance Imaging
NA	Not Applicable
OPJ	Oropharyngeal Junction
OSA	Obstructive Sleep Apnoea
PC	Personal Computer
PCT	Patent Cooperation Treaty
PP	Polypropylene
PVC	Polyvinyl Chloride
pMDI	Pressurised Metered Dose Inhaler
RV	Residual Volume
SAS	Statistical Analysis System
SEM	Standard Error of Mean
SMI	S = Stepped mouthpiece, M = measurement at mid tidal inhalation, I = Inhalation and 0 = 0 mm, no advancement with stepped mouthpiece
SD	Standard Deviation
SOP	Standard Operating Procedure
SSI	S = Stepped mouthpiece, S = measurement during slow and deep inhalation, I = Inhalation and 0 = 0 mm, no advancement with stepped mouthpiece
TBM	Tidal breathing Mode
TIM	Target Inhalation Mode
TV	Tidal Volume
TLC	Total Lung Capacity
VC	Vital Capacity
VHC	Valved Holding Chamber

Publications and patents

Parts of this PhD thesis have been already published as follows:

1. Nikander, K., Prince, I., Coughlin, S., Warren, S. & Taylor, G. (2010) Mode of breathing - tidal or slow and deep - through the I-neb Adaptive Aerosol Delivery (AAD) System affects lung deposition of ^{99m}Tc-DTPA. *J Aerosol Med Pulm Drug Deliv*. 23(Suppl 1), S37–S44.
2. Nikander, K., Petherbridge, I., Scarberry, E., Von Hollen, D., Viviano, J. & Chrystyn, H. (2010) Mandibular advancement achieved through a stepped mouthpiece design can change the size of the upper airways. In: *Respiratory Drug Delivery*. Dalby, R.N., Byron, P.R., Peart, J., Suman, J.D., Farr, S.J. & Young, P.M., editors. Davis Healthcare Int. Publishing LLC, River Grove, IL; pp. 747-51.
3. Nikander, K., Petherbridge, I., Scarberry, E., Von Hollen, D., Viviano, J. & Chrystyn, H. (2010) Manipulation of upper airway volume using a stepped mouthpiece. *Eur Resp J.* 36 (Suppl 54), S350.

Inventions/patents:

1. US Patent 2011/0240015 A1: Method and apparatus comprising stepped mouthpiece for aerosol drug delivery.
2. US Patent 2012/0240922 A1: Apparatus and method comprising adjustable stepped mouthpiece for aerosol drug delivery.

Chapter 1 Introduction

1.1 Background

There are several advantages of the delivery of locally acting drugs through the pulmonary route for treatment of diseases of the lungs. The inhaled drugs are targeted directly to the airway surfaces, avoid inactivation through hepatic first pass metabolism, relatively small amounts are required, and onset of action is relatively rapid in comparison with swallowed drug (Newman et al., 2009). The mouth, the pharynx and the larynx are, however, potential sites of aerosol deposition in the upper airways during oral inhalation.

The right angle bend of the lumen at the back of the mouth, the variable position of the tongue during inhalation, the variable size and shape of the lumen in the pharynx and larynx, a number of diseases of the upper airways, and the breathing pattern could – in addition to aerosol characteristics - promote upper airway deposition and restrict lung deposition (Kumazawa et al., 1997; Borgström et al., 2006; Newman et al., 2009; Nikander et al., 2010c; Scheuch et al., 2010; Diaz et al., 2012; van Velzen et al., 2015). The part of the pharynx (oropharynx) located behind the tongue, and mainly between the oropharyngeal junction (OPJ) and the epiglottis (EG), seems to present the narrowest part of the upper airways (Fajdiga, 2005). Mandibular advancement has in the past been practiced as a means to open up the upper airway behind the tongue during inhalation (Tissier, 1903).

Tissier discusses the opposition of the EG through its “oblique position over the entrance to the larynx” to the penetration of atomised liquids into the larynx. He also describes an interesting “general method” of practicing inhalation of atomised liquid, gas or vapour, as follows:

"In the more general method, patients are first instructed to project the tongue as far as possible. It is then grasped with a cloth held in the fingers, preferably between the thumb and forefinger of the patient's right hand, and pulled downward as far as possible. Lazarus recommends that the organ be rolled, as it were, around the lower lip. In this way is prevented the arching of the base of the tongue that often causes a narrowing of the ostium of the pharynx, while the lingual traction causes the epiglottis to be lifted up and well forward. The patient throws his head slightly forward, at the same time tilting it a trifle backward and upward, bringing his lower jaw as far as possible. These manoeuvres have for their object the greatest possible widening out of the angle between the axes of the buccal and laryngeal cavities. In this position the medication may be made to reach the vestibule of the larynx, even in the most difficult cases."

Mandibular advancement was already used during the late 1800s in cases of mandibular retrusion and is still used as a means to prevent collapse up the upper airway during sleep in subjects with obstructive sleep apnoea (OSA) (Bailey, 2005; Fleetham et al., 2010; Wee, 2012; Friedman et al., 2014). There are presently a number of different oral appliances available for the treatment of OSA, which are used to increase the size - and prevent a collapse - of the upper airway by either advancing the mandible or the tongue (Fleetham et al., 2010). There is a wide variety among the oral appliances in terms of design, material, location of coupling mechanism, and amount of possible horizontal (advancement or protrusion) and vertical jaw movement (Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Chan et al., 2007; Hoffstein, 2007; Fleetham et al., 2010; Wee, 2012; Friedman et al., 2014; Sutherland et al., 2014). The terminology regarding the oral appliances is somewhat variable and some of the labels in English include: oral appliances, functional appliances, mandibular advancement devices, mandibular advancement splints, mandibular repositioning devices, anterior mandibular positioners, oral airway dilators and airway orthotic devices (Viviano, 2002a; Bailey, 2005; Horchover, 2007; Fleetham et al., 2010; Friedman et al., 2014). Two of the main proposed mechanisms of action during sleep for these devices are increasing the size of the upper airway (Ryan et al., 1999), and decreasing the collapsibility of the upper airway (Ng et al., 2003; Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Hoffstein, 2007; Fleetham et al., 2010).

A number of airway-imaging studies have been performed in both healthy subjects and in patients with OSA using oral appliances. The imaging techniques used included cephalometry, CT, MRI and videoendoscopy (Fleetham et al., 2010). Mandibular and tongue advancement have been shown to increase the size of the upper airway and alter the shape of the upper airways – particularly in the velopharynx in healthy subjects and in subjects with OSA (Ferguson et al., 1997a). The use of oral appliances have in other studies been shown to increase the anteroposterior diameter of the upper airway (Ng et al., 2003), to increase the total volume of the upper airway and CSAs of the retropalatal and retroglossal regions (Sam et al., 2006; Kyung et al., 2005) and to increase the lateral dimensions of the velopharynx (Zhao et al., 2008; Chan et al., 2010a). The Tissier described method and the results achieved with oral appliances in subjects with OSA indicate that mandibular advancement might expand the upper airway during inhalation.

During the analysis of the study presented in Chapter 3 the question regarding the size of the upper airways and the impact of the anatomy of the upper airways on lung deposition was discussed. The possibility to enlarge the upper airway through mandibular advancement was suggested, and a new stepped mouthpiece was developed as a tool in order to achieve mandibular advancement. The newly developed stepped mouthpieces without (patent US 2011/0240015 A1) and with a tongue depressor (patent US 2012/0240922 A1) are shown in Figure 1.1 (without a tongue depressor) and in Figure 1.2 (with tongue depressor).

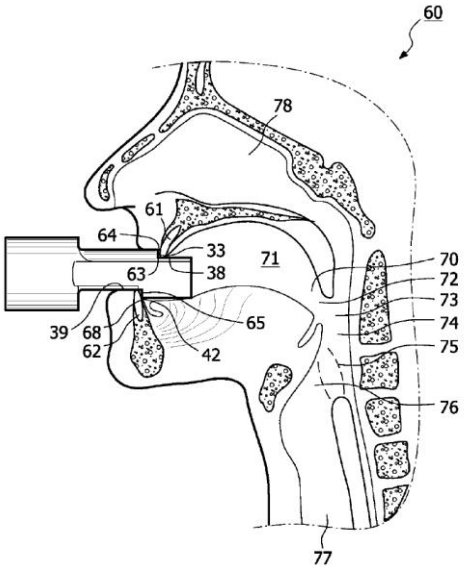


Figure 1.1: Schematic presentation of the new stepped mouthpiece without tongue depressor and the mandibular protrusion achieved with it. The numbers in the schematic presentation refer to the "Method and apparatus comprising stepped mouthpiece for aerosol drug delivery" section in the patent. From patent US 2011/0240015 A1.

The stepped mouthpiece without a tongue depressor was tested in the proof-of-concept study presented in Chapter 4. It was designed in several configurations with front ends with 10 mm, 15 mm and 20 mm orifices (vertical diameters). These front orifices were also designed with a single protrusion on the upper side for the upper incisors and 4 protrusions on the lower side at different distances (-3 mm, ±0 mm, +3 mm and +6 mm) in relation to the protrusion on the upper side for horizontal movement of the mandible (Figure 1.1). The horizontal offsets were -3 mm (lower jaw moved back from an incisal edge-to-edge position), ±0 (incisal edge-to-edge position), + 3 mm and +6 mm (mandible moved forward from an incisal edge-to-edge position). The stepped mouthpiece was 40 mm long.

The stepped mouthpiece with a tongue depressor was developed based on the experience from the proof-of-concept study presented in Chapter 4. The new stepped mouthpiece was 81 mm long fully extended including tongue depressor, and the external horizontal and vertical diameters were 34

mm and 24 mm, respectively. The tongue depressor and the related part of the mouthpiece to be held in the mouth were 33 mm long, and the external horizontal and vertical diameters 34 mm and 18 mm, respectively.

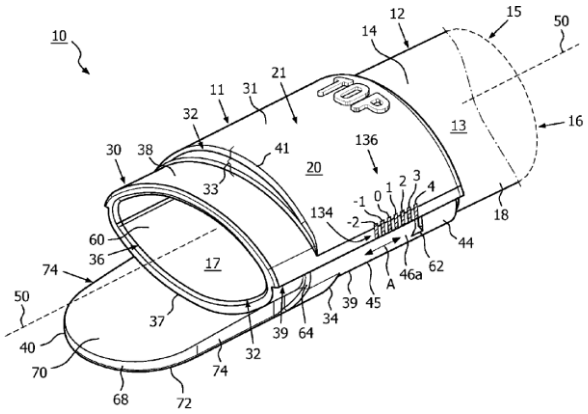


Figure 1.2: Schematic presentation of the stepped mouthpiece with tongue depressor (left end). The numbers in the schematic presentation refer to the "Apparatus and method comprising adjustable stepped mouthpiece for aerosol drug delivery" section in the patent. From patent US 2012/0240922 A1.

The 18 mm vertical external diameter was chosen partly based on the results of the previous proof-of-concept study in which the largest mouthpiece had an external vertical mouthpiece diameter of 20 mm, and partly as this is a common vertical size of a jet nebuliser mouthpiece. The length of the stepped mouthpiece from the round end to the position for the upper incisors was 52 mm (Figure 1.2).

1.2 Aim and objectives

1.2.1 Aim

The aim of this research work was to:

- Investigate through acoustic pharyngometry the effects of mandibular advancement and incisal opening, achieved with a novel stepped mouthpiece, on the upper airways during inhalation.

- Investigate the open velum (soft palate) effect on the pharyngogram in an *in vitro* study design using a cast of the human upper airways and a surrogate open velum.

1.2.2 Objectives

1. To develop and evaluate a new stepped mouthpiece without and with tongue depressor using acoustic pharyngometry.
2. To evaluate the impact of the mandibular advancement achieved with the stepped mouthpiece on the upper airways in healthy subjects, and in subjects with OSA, using acoustic pharyngometry.
3. To evaluate the impact of the incisal opening achieved with the stepped mouthpiece on the upper airways in healthy subjects, and in subjects with OSA, using acoustic pharyngometry.
4. To develop an automatic procedure for analysis of large amounts of pharyngograms in order to identify deviating pharyngograms within each measurement consisting of 4 pharyngograms. A measure of "Goodness of Fit" (GOF) was required for the process, and each of the 4 pharyngograms was compared to the median pharyngogram and those deviating too much were removed. GOF was calculated as the square root of the average squared vertical distance between the median curve and the curve under study. The region over which the GOF-calculation was performed was limited to the region from the start of the pharyngogram to the glottis (GL).
5. To develop a method for the analysis of pharyngograms in terms of cross-sectional areas (CSAs) at the landmarks (OPJ, EG, and GL), and volume (area under the curve, AUC) between the incisors and the OPJ, between the OPJ and the EG, and between the EG and the GL.
6. To evaluate through an *in vitro* study design the impact of leakage through an open velum (soft palate) on the pharyngogram.

1.3 Thesis structure

The work in this thesis is as follows:

Chapter 1: a general introduction with a brief summary of work.

Chapter 2: an overview of literature related to the areas of study.

Chapter 3: describes the measurement of the upper airways of 9 healthy subjects by means of acoustic reflection (AR) using an acoustic pharyngometer (Kamal, 2001; Kamal, 2002; Jung et al., 2004; Kamal, 2004a; Kamal, 2004b; Monahan et al., 2005; Gelardi et al., 2007; Shiota et al., 2007; Kumar et al., 2015). The subjects had been included in a previous lung deposition study (Nikander et al., 2010c). The measurements were performed with the subjects seated in the same position as when they were inhaling through an I-neb nebuliser in the previous lung deposition study. The subjects were also instructed to inhale with the same inspiratory flow as in the previous study.

Chapter 4: describes a proof-of-concept study in 4 healthy subjects. The study was designed to evaluate the impact of mandibular advancement and incisal opening, achieved with a newly invented stepped mouthpiece, on the size of the upper airways of the subjects. The measurements of the upper airways were performed by means of AR using an acoustic pharyngometer. The upper airway included the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx, and the GL. These were analysed in terms of CSAs and the AUCs.

Chapter 5: describes a clinical study in 60 subjects without (30 subjects) and with OSA (30 subjects), in which the primary objective was to measure through acoustic pharyngometry the impact of different horizontal mandibular advancements - achieved with a new stepped mouthpiece with a tongue depressor - on the size of the upper airways. The upper airways included the area from the incisors to the GL. The measurements were performed while the subjects were seated in a chair and inhaled room air during tidal breathing through the stepped mouthpiece. The secondary objectives included assessment of the most protrusive and most retrusive positions of

the mandible, measurement of the upper airways through acoustic pharyngometry during slow and deep breathing while the subjects used a stepped mouthpiece, and assessment of the most comfortable mandibular advancement position for the subjects when using the stepped mouthpiece during tidal breathing and during slow and deep breathing,

Chapter 6: describes an *in vitro* study, in which the primary objective was to measure through acoustic pharyngometry the impact of leakage through an open velum (soft palate) on the pharyngogram. The *in vitro* study was designed to investigate the possible artefact found in Chapter 3, which was related to the use of nose clips during the acoustic pharyngometer measurements. Based on published data on the open velum effect, this was a plausible reason for the observed increases in the CSAs and AUCs (Molfino et al., 1990; Marshall et al., 1993). An *in vitro* study design was chosen as it would allow controlled acoustic pharyngometer measurements to be made through a cast of the human upper airways with a surrogate for a closed or an open velum (Cheng et al., 1990).

Chapter 7: describes a general conclusion from these studies and suggestions for future work.

Chapter 2 Literature review

2.1 The human respiratory system

Ventilation of the lungs is the major function of the respiratory system as the normal cellular metabolism requires a continuous supply of oxygen and disposal of carbon dioxide. The respiratory system can be divided into two main parts: the upper respiratory (nasal airways, pharynx and larynx) and the lower respiratory tracts (trachea, primary bronchi and lungs) (Figure 2.1). From a functional perspective the lower respiratory tract can be divided into three distinct zones: the conducting, the transitional and the respiratory zones (Forrest, 1993). The conducting zone is involved in the movement of air and includes bronchi with cartilage and bronchioles without cartilage, but no alveoli. The transitional zone is a transition between the conducting and the respiratory parts of the airway – from bronchioles to the gas exchanging tissue - and includes occasional alveoli.

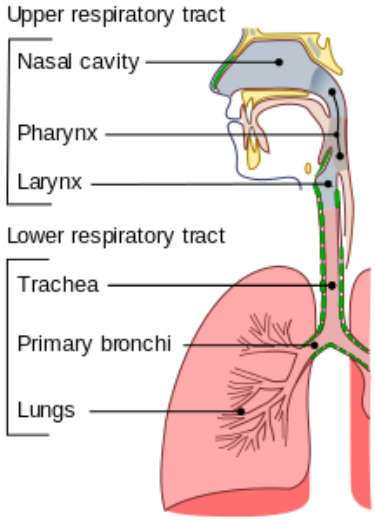


Figure 2.1: A sagittal view of the respiratory system with upper and lower respiratory tracts (Respiratory System Upper Tracts at www.yahoo.com).

Gas exchange occurs in the respiratory zone (Forrest, 1993). In adults the CSA of the trachea is 2-3 cm^2, the diameter of the alveoli ~200 µm and the area of the gas exchange 40-100 m^2 (Merkus, 1993). Inspiration is based on contractions of the major inspiratory muscles (the diaphragm and the intercostal muscles) which expand the chest and inflate the lungs, whereas expiration occurs passively through an elastic recoil of the lungs and the chest wall (Berne et al., 1988).

2.2 The upper airways

Multiple terms have been used for the description of the upper airways between the nasal airways and the larynx which tend to cause confusion. For this thesis the terminology presented by Fogel et al (2004) and Tung (2007), and highlighted in Figure 2.2 (Fogel et al., 2004), will be used.

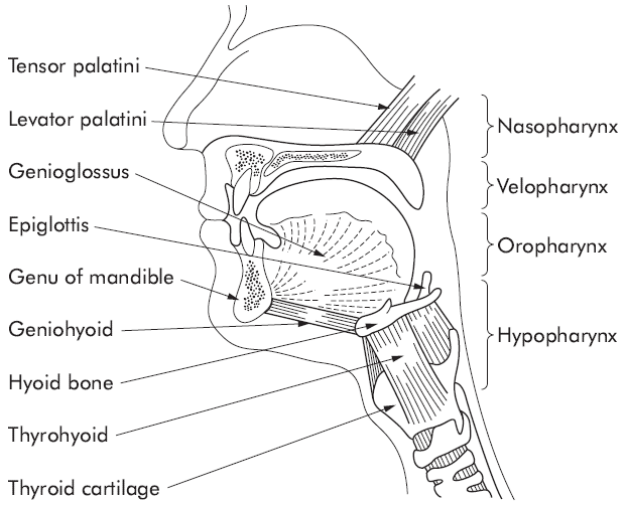


Figure 2.2: A sagittal view of the upper respiratory tract including the airway from the nose to the trachea. The pharynx has been divided into the nasopharynx, the velopharynx, the oropharynx and the hypopharynx (Fogel et al., 2004). An almost identical figure has been published by Ayappa et al (2003), with reference to Kuna et al in "Anatomy and Physiology of Upper Airway Obstruction" (MH Kryger, T Roth and WC Dement (Eds). Principles and Practice of Sleep Medicine, 3rd Edn. W.B. Saunders Company).

Fogel et al (2004) and Tung (2007) divided the upper airway between the nasal airways and the larynx into the nasopharynx, the velopharynx, the oropharynx, and the hypopharynx (Figure 2.2; Fogel et al., 2004; Tung, 2007). Tung (2007) defined the upper airway as the passage for gas and

food with mouth and nose as starting points, and EG and vocal cords as end points. The anatomy could be described with an "X" shape as there are two distinct entry points (mouth and nose), a common middle part (pharynx) and two exit points (larynx and esophagus). The functions of the upper airway covers breathing, mastication, communication, swallowing, taste and smell (Tung, 2007).

The swallowing reflex has been divided into an oral or voluntary phase, a pharyngeal phase and an esophageal phase. During the oral phase a bolus of food is moved by the tongue upward and backward in the mouth, forcing the bolus into the pharynx stimulating tactile receptors that initiate the swallowing reflex. The pharyngeal phase consists of a series of events: the nasopharynx is closed by the soft palate which is moved upward, the trachea is closed by the EG which covers the larynx, and the vocal cords are pulled together, the upper esophageal sphincter is relaxed to receive the bolus of food. A peristaltic wave is finally activated with contraction of the pharyngeal constrictor muscles which forces the bolus through the esophageal sphincter (Berne et al., 1988).

2.2.1 The nose

In the nose the airway is both double and convoluted, whereas there is a single airway from the nasopharynx to the trachea. The nasal cavity is located directly above the oral cavity and the hard palate separates the two cavities. Data on the CSA of the nasal valve indicate that it would be ~0.4 cm^2 and the CSA of the nasal cavity ~1.5 cm^2 (Sahin-Yilmaz et al., 2011). The inspired air is warmed, moistened and filtered during the passage through the nose and the relative humidity is close to 100% in the nasopharynx. The heat and moisture is recovered during expiration. The velocity of the inspired air is high past the middle turbinate (18 m/s) and slows down through the main part of the nasal cavity (2-3 m/s). Inspiratory airflows up to 20-30 L/min can be nasal,

whereas higher flows during for example exercise are oral (Chang et al., 1993; Tung, 2007, Sahin-Yilmaz et al., 2011)

2.2.2 The nasopharynx

The nasopharynx is located below the nasal cavity and at this junction the hard palate changes to form the soft palate and the upper airway lumen makes a 90° turn downwards behind the posterior part of the soft palate. The soft palate is a muscular flap that hangs almost vertically and terminates in the uvula. The length of the soft palate in 8 healthy subjects was shown to be 30.5 mm (range 28-34 mm), whereas the area of the soft palate was 3.2 cm^2 (range 3.0-3.6 cm^2) (Ciscar et al., 2001). The nasopharynx is "closed" by the soft palate when it is moved upward and thus changes position. The soft palate directs the flow or air to pass either through the nose or the mouth (Tung, 2007).

2.2.3 The oral cavity

In the oral cavity the hard and soft palates form the "roof", the lingual mucosa the "floor" and the buccal mucosa the "walls". The anterior palatine tonsils mark the junction between the oral cavity and the oropharynx, the OPJ. The pharynx is a 12-15 cm long muscular tube stretching vertically downward from the level of the soft palate to the cricoid cartilage. In healthy subjects the pharyngeal tube is oval in cross section with the long dimension oriented from medial to lateral (Chang et al., 1993; Tung, 2007, Sahin-Yilmaz et al., 2011).

2.2.4 The velopharynx and the oropharynx

The terms retropalatal region of the oropharynx and velopharynx both refer to the same area behind the soft palate, below the nasopharynx and posterior to the oral cavity. The velopharynx forms the part of the "X" where the oral and nasal cavities meet (Tung, 2007). The retroglossal region of the oropharynx extends from the tip of the soft palate superiorly to the base of the EG inferiorly. The

CSA of the oropharynx is generally larger than the CSA of the velopharynx. Any reduction of CSA in the velopharynx and the oropharynx seems to mainly occur through a thickening of the lateral pharyngeal walls, and the shape of the lumen seems to change from an oval with the long axis oriented laterally to a more circular shape with the long axis oriented anteroposteriorly (Tung, 2007).

2.2.5 The hypopharynx

The term hypopharynx is often used for the lower boundary of the retroglossal region of the oropharynx, and the area is bounded anteriorly by the base of the tongue and the EG, and posteriorly/laterally by the inferior pharyngeal constrictor muscle. Below the EG the hypopharynx runs parallel with the esophagus, and the larynx splits off anteriorly with the vocal cords below the EG (Tung, 2007).

2.2.6 The larynx and the glottis (GL)

The upper airway branches into the trachea (anteriorly) and the esophagus (posteriorly) below the EG. The larynx covers the area bounded superiorly by the EG, inferiorly by the vocal cords, and laterally by the aryepiglottic folds (Tung, 2007). The GL is located within the larynx in the space between the vocal folds with an elliptical and triangular shape (Brouns et al., 2007; Scheinherr et al., 2015, Boiron et al., 2015; Figure 2.3).

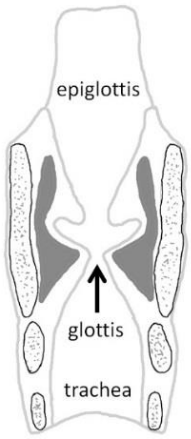


Figure 2.3: A frontal view of the GL between the vocal folds. (Boiron et al., 2015; from poster presented at the ISAM congress 2015).

2.2.7 The shape of the lumen of the upper airways

The pharyngeal lumen is deformable whereas the nose, the larynx and the trachea have a framework of cartilage. The narrowest section of the pharynx is located behind the soft palate at the level of the velopharynx (Suratt et al., 1983). There are more than 20 muscles surrounding the upper airway which either constrict or dilate the upper airway lumen. The muscles that interact to determine the patency of the upper airway can be divided into four groups: muscles regulating the position of the soft palate, the tongue, the hyoid apparatus and the posterolateral pharyngeal walls. The tonsils, the soft palate, the uvula, the tongue and the lateral pharyngeal walls are all soft tissues that form the walls of the upper airway. The mandible and the hyoid bone are the main craniofacial bony structures that determine the upper airway size and presumably provide anchoring for muscles and soft tissue (Ayappa et al., 2003). The shape of the lumen of the upper airway is quite variable from the nasal valve to the trachea (Figure 2.4).

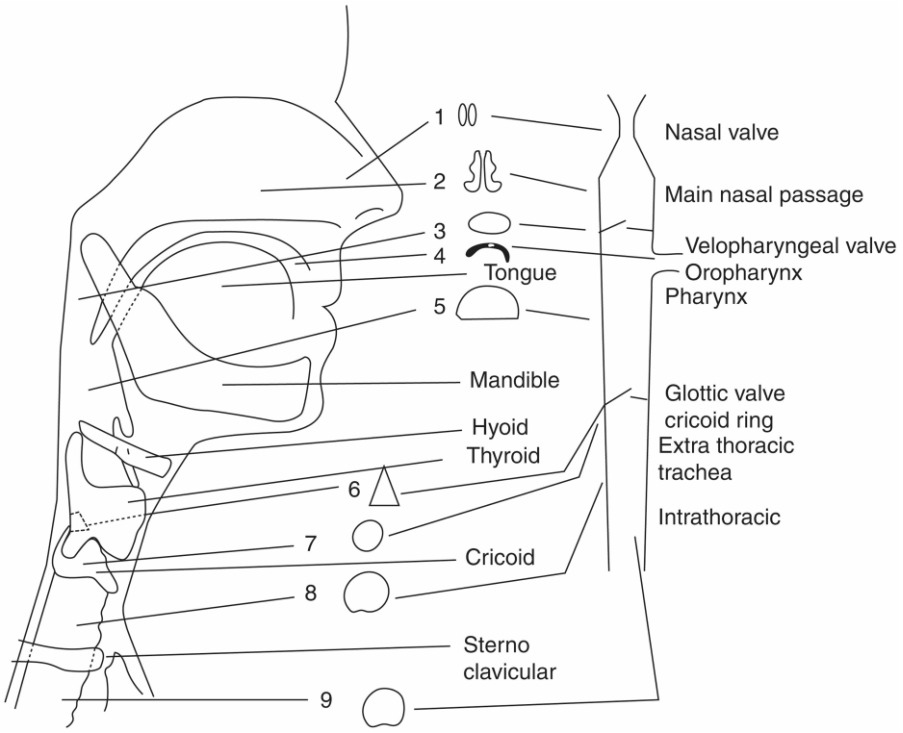


Figure 2.4: A sagittal view of the upper airway which highlights the shape and comparative size of the airway lumen and the location of the nasal, velopharyngeal, glottal or laryngeal valves (Proctor, 1983; Strohl et al., 2012).

The nostrils, the lips, the palate and the larynx are narrow parts of the upper airways that limit the lumen calibre and contributes to the overall airflow resistance (Proctor, 1983; Strohl et al., 2012). A fall in pressure in the pharynx tends to collapse the pharyngeal airway but can be prevented by the contraction of pharyngeal muscles (Suratt et al., 1983).

2.2.8 Impact of breathing on the size of the upper airways

Inhalation of an aerosol is the preferred mode of administration of a number of drugs in the treatment of different respiratory disorders. The size of the lumen in the upper airways is therefore of interest, especially since breathing related motion of soft tissue in the pharynx (the retropalatal and retroglossal regions), and related bony structures has been shown to create dimensional changes in this part of the upper airways (Figure 2.5; Schwab et al., 1993a; Schwab et al., 1993b;

Schwab, 1998). Schwab highlighted 4 distinct phases during breathing which affected the size of the pharynx (Figure 2.5):

1) At the beginning of inspiration an increase in pharynx from resting position.
2) During the rest of inspiration the size of the pharynx is relatively constant.
3) At the beginning of expiration the pharynx is enlarged.
4) During end of expiration the size of the pharynx returns to the resting position.

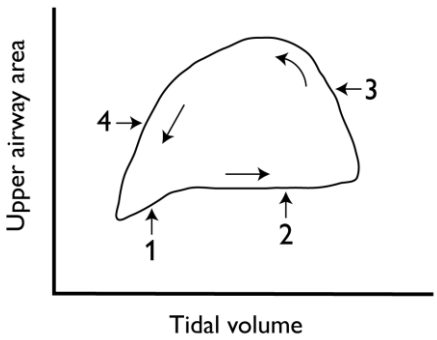


Figure 2.5: The figure illustrates changes in the pharynx (the retropalatal and retroglossal regions) as a function of tidal volume during breathing in an apnoeic subject. 1 = early inspiration; 2 = inspiration; 3 = early expiration; 4 = late expiration (Schwab, 1998).

Schwab et al concluded that most imaging studies of the upper airways have indicated that airway narrowing was greatest in the velopharyngeal region and that the changes occurred mainly in the lateral dimension (Schwab et al., 1993a; Schwab et al., 1993b; Schwab, 1998). Interestingly, a breathing dependent movement of the glottal area has also been shown to occur through studies with fibreoptic bronchoscope and (Brancatisano et al., 1983) nasofibroscope (Scheinherr et al., 2015). In contrast to the results of the Schwab et al studies on pharyngeal movement, Brancatisano and colleagues (1983) showed in healthy subjects that both GL width and area increased during inspiration, and decreased during expiration. These results have been supported by the late Scheinherr et al study (2015). Thus different parts of the upper airways seem to move in opposite directions during the breathing cycle.

2.2.9 The cross-sectional area (CSA) of the upper airways

The size of the upper airways has been shown to be larger in men than in women, and to decrease with increasing age (Martin et al., 1997). A number of authors have published data on the CSAs (centimetre or millimetre square; cm^2 or mm^2) of the velopharynx, the pharynx, the OPJ, the EG, the GL, and the larynx measured with an acoustic pharyngometer (Eccovision; Table 2.1) (in alphabetical order: Allen et al., 2014, Busetto et al., 2009; Jung et al., 2004, Kamal., 2001, Kamal., 2002; Kamal, 2004b; Monahan et al., 2005; Shiota et al., 2007).

Table 2.1: The size of different parts of the upper airway lumen in adult healthy subjects expressed as mean CSA and/or range (maximum to minimum) in cm^2. Results from studies using the Eccovision ARP have been included. The measurements were performed during (end) expiration while the subjects were seated - if supine, this has been highlighted specifically.

1st author, year published	**Subjects (male)**	**CSA (cm^2, mean and range)**
Allen et al., 2014	80 (no data), 20/ethnic group	***Mouth to larynx:*** Caucasian = 2.7 cm^2 ; Chinese = 2.9 cm^2 ; Japanese = 2.6 cm^2 ; Korean = 2.9 cm^2
Busetto et al., 2009	145 (no male)	Seated: ***Pharynx*** = 2.6 cm^2 (0.7-5.8); ***OPJ*** = 1.6 cm^2 (0.3-4.0); ***GL*** = 2.2 cm^2 (0.5-4.4) Supine: ***Pharynx*** = 2.2 cm^2 (0.7-4.4); ***OPJ*** = 1.2 cm^2 (0.5-2.0); ***GL*** = 2.0 cm^2 (0.5-3.9)
Jung et al., 2004	16 (14)	Seated: ***Pharynx*** = 2.5 cm^2; ***OPJ*** = 1.6 cm^2; ***GL*** = 1.8 cm^2 Supine: ***Pharynx*** = 1.9 cm^2; ***OPJ*** = 1.3 cm^2; ***GL*** = 1.4 cm^2
Kamal, 2001	350 (271)	***Pharynx***: Men = 2.7 - 3.8 cm^2; Women = 2.1 - 3.4 cm^2 ***GL***: Men = 0.9 - 1.2 cm^2; Women = 0.8 - 1.1 cm^2
Kamal, 2002	40 (29)	***Pharynx***: Men = 3.2 cm^2, Women = 2.8 cm^2
Kamal, 2004b	20 (16)	***Pharynx***: Test 1 = 3.2 cm^2, Test 2 = 3.2 cm^2, Test 3 = 3.2 cm^2
Monahan et al., 2005	75 (36) white 62 (23) black	***Oropharynx***: White = 2.7 cm^2, (1.9 - 3.8); Black = 2.4 cm^2 , (1.7 - 3.3) ***OPJ***: White = 2.4 cm^2; Black = 2.0 cm^2 ***EG*** : White = 2.2 cm^2; Black = 2.6 cm^2
Shiota et al., 2007	27 (16)	Supine: ***Velum to GL*** = 2.7 cm^2 (baseline)

The CSAs of the upper airways were somewhat larger in men than in women, and also larger when measured in a seated position in comparison with in a supine position. When seated the OPJ CSAs

ranged from 0.3 to 4.0 cm^2, the EG CSAs from 2.2 to 2.6 cm^2, the GL CSAs from 0.5 to 4.4 cm^2, the oropharynx CSAs from 1.7 to 3.8 cm^2, and the pharynx CSAs from 0.7 to 5.8 cm^2 (Table 2.1). Data on the upper airway CSAs are available in studies using other measurement techniques. These include MRI, CT, and fibreoptic bronchoscopy/nasofibroscopy. A number of these studies are included in Table 2.2 presenting data of adult healthy subjects while in supine and/or seated positions during wakefulness.

Table 2.2: The size of different parts of the upper airway lumen in adult healthy subjects expressed as mean CSA in cm^2. Results from studies using MRI, CT and fibreoptic bronchoscopy/nasofibroscopy (F/N) techniques are included.

1st author, year published	**Subjects (male), technique**	**CSA (cm^2, mean)**
Ehtezazi et al., 2004	10 (6) MRI supine	***Oropharynx*** pMDI = 1.5 cm^2 spacer = 2.1 cm^2 DPI = 2.8 cm^2
Ciscar et al., 2001	8 (2) MRI, supine	***Velopharynx*** 1.2 cm^2, range 1.0 – 1.2 cm^2
Schwab et al., 1993b	15 (10) CT supine	***Nasopharynx***: 2.2 cm^2 (maximal expiration); 2.0 cm^2 (maximal inspiration) ***Velopharynx*** (high***)***: 1.5 cm^2 (maximal expiration); 1.4 cm^2 (maximal inspiration) ***Velopharynx*** (low): 2.0 cm^2 (maximal expiration); 1.9 cm^2 (maximal inspiration) ***Hypopharynx***: 2.6 cm^2 (maximal expiration); 2.5 cm^2 (maximal inspiration)
Brancatisano et al., 1983	12 (10) F/N seated	***GL*** Group = 1.3 cm^2 (max, inspiration) Group = 0.7 cm^2 (min, expiration)
Scheinherr et al., 2015	20 (10) F/N seated	***GL, slow breathing*** Men = 2.2 cm^2 (max, inspiration; range 1.9-3.5) Men = 1.8 cm^2 (max, expiration) Women = 1.9 cm^2 (max, inspiration; range 1.7-2.7) Women = 1.7 cm^2 (max, expiration)

The CSAs of the upper airways were as with acoustic pharyngometer derived data somewhat larger in men than in women. The nasopharynx CSAs ranged from ~2.0 to 2.2 cm^2, the velopharynx CSAs ranged from 1.0 to 2.0 cm^2, the oropharynx CSAs from 1.5 to 2.8 cm^2, the hypopharynx

ranged from ~2.5 to 2.6 cm^2 and the GL CSAs ranged from 1.7 to 3.5 cm^2 (Table 2.2). Overall, the inter-subject variability both with acoustic pharyngometry, MRI and fibreoptic techniques seems to be relatively large even in these small populations.

2.3 Lung volumes and capacities

Inspiratory and expiratory airflow and lung volumes are of importance as these can be used to differentiate and characterize pulmonary disorders (obstructive or restrictive) and to evaluate responses to treatment (Jonson et al., 1998). Measurements with an acoustic pharyngometer have also been shown to be lung volume dependent (Kamal, 2002). The lung volumes and capacities are presented in Figure 2.6.

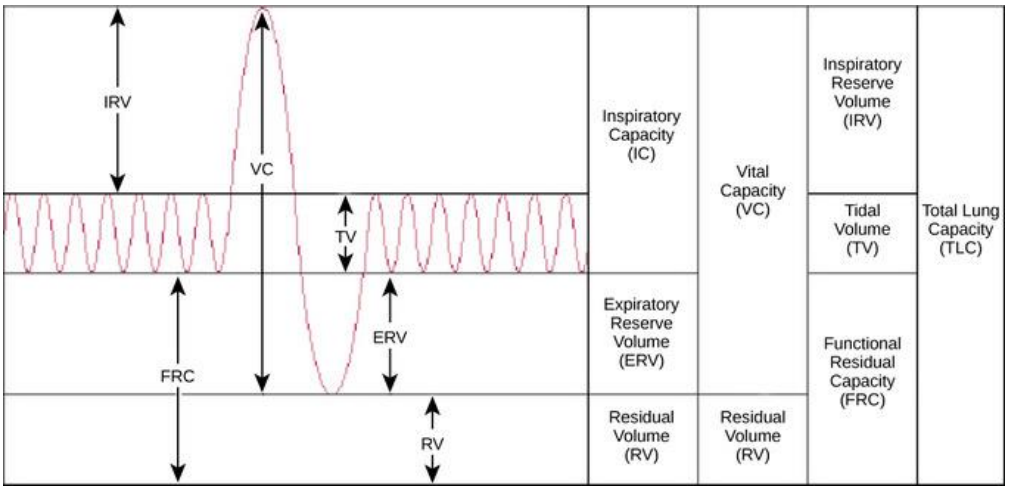


Figure 2.6: Lung volumes and capacities. Available at and accessed October 2015: https://www.boundless.com/biology/textbooks/boundless-biology-textbook/the-respiratory-system-39/gas-exchange-across-respiratory-surfaces-220/lung-volumes-and-capacities-834-12079/.

The TV consists of a dead space volume and an alveolar volume. The IRV and ERV represent the maximal volume of air that can be voluntarily inspired or expired. The FRC represents the volume of air in the lungs at the end of a tidal breath. The VC represents the maximal volume of air that can be inhaled and exhaled, whereas the TLC represents the total volume (~6-7 L) of the lung

(Jonson et al., 1998). The lung volumes are usually presented as either absolute volumes or as percentages of predicted values based on ethnicity, gender, age, and height, and at the same height the male values tend to be ~25% higher than the female values (Berne et al., 1988; Jonson et al., 1998).

2.4 The aerosol and deposition mechanisms

2.4.1 The aerosol

An aerosol has been defined as a dispersion or suspension of solid particles or liquid droplets in a gaseous medium (Newman et al., 2009). The size of the aerosol particle (or droplet) that could be deposited in the human lungs range between 0.5 and 10 µm (Newman et al., 2009). The general relationship between particle size and deposition in the upper airway and the lung of healthy subjects is shown in Figure 2.7 (Boe et al., 2001).

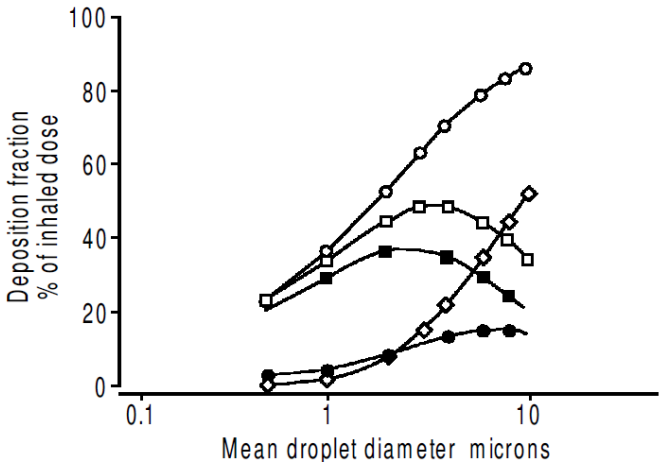


Figure 2.7: Relationship between aerosol aerodynamic diameter and deposition in the lung based on *in vitro* models: ○ total body, □ total lung, ◊ oropharyngeal, ● central airways, and ■ peripheral airways (Boe et al., 2001).

A particle of for example 1 µm, will more likely deposit in the peripheral airways than in the upper airway, whereas a particle of for example 10 µm will more likely deposit in the oropharynx.

2.4.2 Deposition mechanisms

Findeisen published in 1935 the first attempt regarding deposition patterns of inhaled particles, and identified 4 deposition mechanisms: impaction, sedimentation, Brownian movement, and the "rim-effect" (Findeisen, 1935; Zanen, 2003). Presently 3 main mechanisms for the deposition of an aerosol in the human upper airway and lungs tend to be acknowledged; inertial impaction, gravitational sedimentation, and Brownian diffusion (Figure 2.8; Newman et al., 2009; Carvalho et al., 2011).

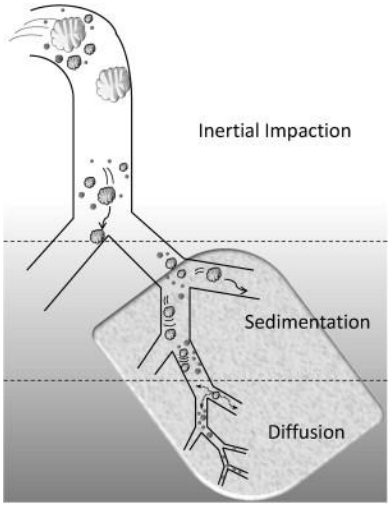


Figure 2.8: Schematic presentation of the deposition of particles in the upper airway and the lungs through inertial impaction, sedimentation and diffusion (Carvalho et al., 2011).

Deposition by inertial impaction occurs in the upper airways - during both inhalation and exhalation - at bifurcations between the central airways within the lungs. When the airway or the airstream change direction the larger particles or droplets have too much inertia to change direction and will therefore impact on the airway wall (Figure 2.8, top section).

Deposition by gravitational sedimentation occurs in the small conducting airways during slow inhalation or during breath holding when aerosol particles or droplets sediment under gravity onto an airway surface (Figure 2.8, middle section). Finally, deposition by Brownian movement or

diffusion is the most likely deposition mechanism mainly in the alveoli for particles or droplets <1 µm in diameter, as these particles or droplets have insufficient inertia for impaction and too low settling velocity for gravitational sedimentation (Figure 2.8, bottom section). In the Brownian diffusion mechanism the particle or droplet is deflected by molecular bombardment, and therefore moved towards the airway surface (Newman et al., 2009).

The "rim-effect" was defined by Findeisen as a situation when a particle by pure chance in close vicinity of the airway surface touches it and is deposited (Zanen, 2003).

2.5 Aerosol delivery to and through the upper airways

The mouth, the pharynx and the larynx are potential sites of aerosol deposition in the upper airways during oral inhalation. The right angle bend of the lumen at the back of the mouth, the variable position of the tongue during inhalation, the variable size and shape of the lumen in the pharynx and larynx, and the breathing pattern can – in addition to aerosol characteristics - promote upper airway deposition and restrict lung deposition (Kumazawa et al., 1997; Newman et al, 2009; Nikander et al., 2010c; Scheuch et al., 2010; Diaz et al., 2012; van Velzen et al., 2015). Aerosol delivery can be directed to the upper airways (Kumazawa et al., 1997), and obviously through the upper airways to the lungs (Newman et al., 2009).

2.5.1 Aerosol delivery to the upper airways

Delivery of aerosol to the upper airways may be indicated for treatment of inflammation in the upper airways (pharyngitis, laryngitis) and rhinitis, for anesthesia, and for delivery of aerosol for systemic effects (Nilsestuen et al., 1994; Kumazawa et al., 1997). A high upper airway (pharynx and larynx) deposition was the target in the scintigraphy study by Kumazawa et al (1997). The study was designed to compare the upper airway and lung deposition of a nebulised saline solution into which 40 mBq of ^{99m}Tc-DTPA had been mixed. Six healthy subjects inhaled the aerosol from

an ultrasonic nebuliser using 3 different breathing patterns and vocalisation as follows: deep and slow inhalation during 12 breaths/min, fast inhalation during 36 breaths/min, and fast inhalation during 36 breaths/min with intermittent vocalisation. No information on droplet size was given. The results showed that lung deposition decreased and deposition in larynx increased statistically significantly when the subjects changed from deep and slow inhalation to fast inhalation with vocalisation (Figure 2.9). The authors did not report any data on inspiratory flows, but the results indicate that a deep and slow inhalation breathing pattern maximised lung deposition, whereas a fast inhalation breathing pattern decreased lung deposition and increased larynx deposition with no changes in the pharynx deposition.

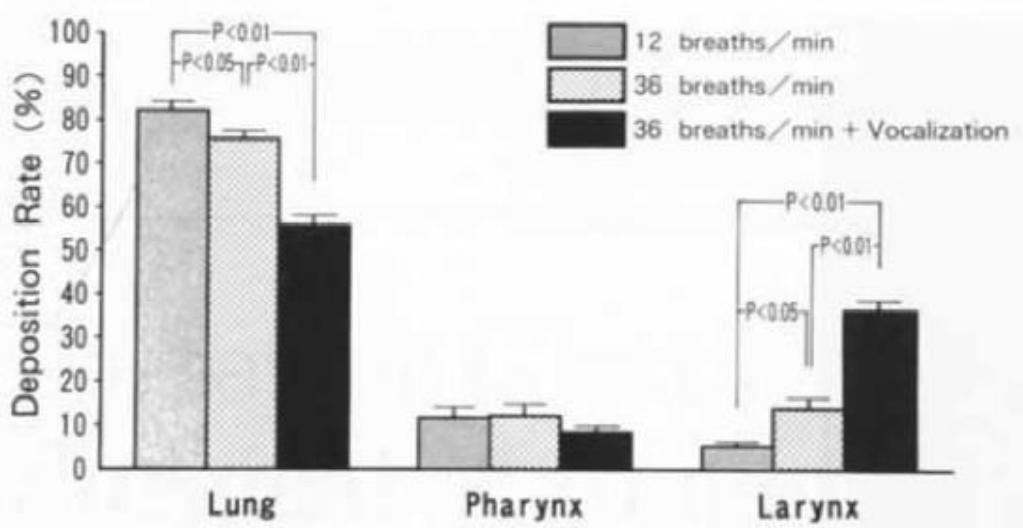


Figure 2.9: A comparison of the aerosol deposition in the lungs, the pharynx and the larynx of 6 healthy subjects following deep and slow inhalation (dark grey), fast inhalation (light grey) and fast inhalation with intermittent vocalisation (black) (Kumazawa et al., 1997).

The greatest change from the deep and slow depositions was found with a fast inhalation with vocalisation in which lung deposition decreased to ~56% and the larynx deposition increased to ~36% (Figure 2.9; Figure 2.10; Kumazawa et al., 1997).

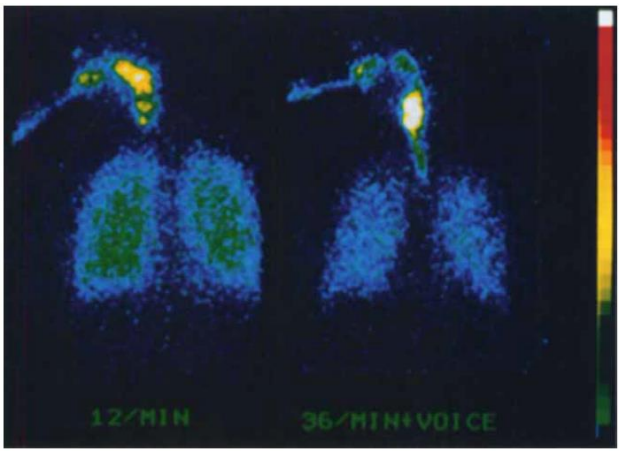


Figure 2.10: A comparison of 2 scintigraph images showing the deposition of ^{99m}Tc-DTPA labelled aerosol in 1 subject after deep and slow inhalation (left) and fast inhalation with intermittent vocalisation (right) (Kumazawa et al., 1997).

The comparison of the deposition in 1 subject (Figure 2.10) between a deep and slow inhalation *versus* a fast inhalation with vocalisation suggested according to the authors that the closing of the vocal cords by intermittent vocalisation led to deposition of the ^{99m}Tc-DTPA labeled aerosol on both sides of the vocal cords. The authors further suggested that part of the deposition of the aerosol on the vocal cords could emanate from the aerosol which was exhaled (Kumazawa et al., 1997).

2.5.2 Aerosol delivery through the upper airways to the lungs

As the upper airway is not a fixed, rigid tube but rather a structure in which the CSA and shape of the lumen can change (Brancatisano et al., 1983; Schwab et al., 1993a; Schwab et al., 1993b; Schwab et al., 1996; Schwab, 1998; Scheinherr et al., 2015), breathing pattern, inspiratory flow, airflow resistance and inhaler mouthpiece design could affect upper airway dimensions and hence lung deposition (Newman et al., 2009).

2.5.3 Impact of inhaler design and inspiratory manoeuvre on the upper airways

The impact of inhalation from different inhalation devices on the upper airway has been the focus of a number of clinical studies in which the upper airways have been measured by MRI during forced inspiration or tidal breathing with the subjects in a supine position (Ehtezazi et al., 2004; Ehtezazi et al., 2005; Pritchard et al., 2004; McRobbie et al., 2005). In the first study by Ehtezazi et al (2004) the authors investigated through MRI the impact of a pMDI, a pMDI with spacer, and a high-resistance DPI on the size of the oral cavity, oropharynx, larynx and trachea of 10 healthy adult subjects. The subjects inhaled through the pMDI, the pMDI with spacer, and the DPI as recommended by the manufacturer and were scanned in a supine position. The CSAs of the oral cavity, the oropharynx and the larynx were shown to have considerable variability during inhalation, which according to the authors was primarily due to the variability of the tongue position during the measurements, and secondarily due to differences in device airflow resistance and subject effort (Figure 2.11). Information regarding the diameter of the pMDI, DPI and spacer mouthpieces was not included but would have been of interest as at least the size of the oral cavity should have been affected by the opening of the mouth (Ehtezazi et al., 2004).

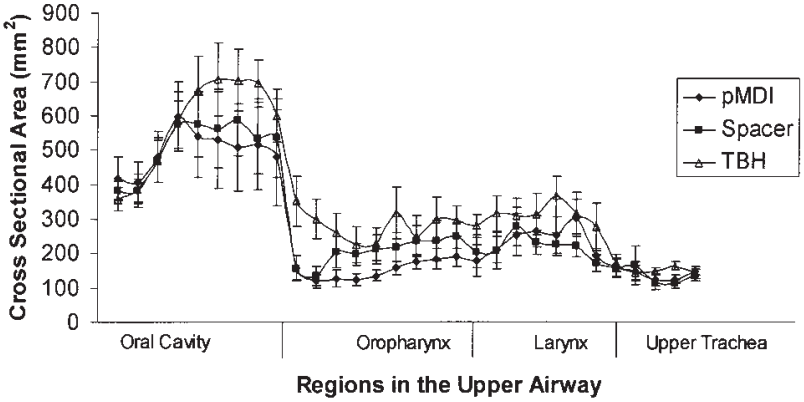


Figure 2.11: Mean CSAs of the upper airways of 10 healthy subjects inhaling through a pMDI, a spacer and a high-resistance DPI. The bars present SEM (Ehtezazi et al., 2004).

In the second study by Ehtezazi et al (2005) the authors investigated through MRI the impact of different inhaler airflow resistances on the upper airways of 7 healthy adult subjects in a supine position. The subjects inhaled deeply and forcefully through the test inhaler which had a 22 mm mouthpiece diameter and 6 different resistances. An increase in the CSAs of the oral cavity, oropharynx and larynx was observed following a decrease in inhaler resistance, whereas the CSAs of the upper trachea did not change. The mean volume of the upper airway increased with decreasing resistance from 72 cm^3 to 101 cm^3 (Ehtezazi et al., 2005).

In the study by Pritchard et al (2004) the authors investigated through an inhalation-gated MRI the impact of 4 dummy inhalers with varying mouthpiece diameters (14 mm and 25 mm; small and large) and resistances (bores 3.1 mm and 11.3 mm; low and high) on the upper airways of 20 healthy adult subjects (Figure 2.12).

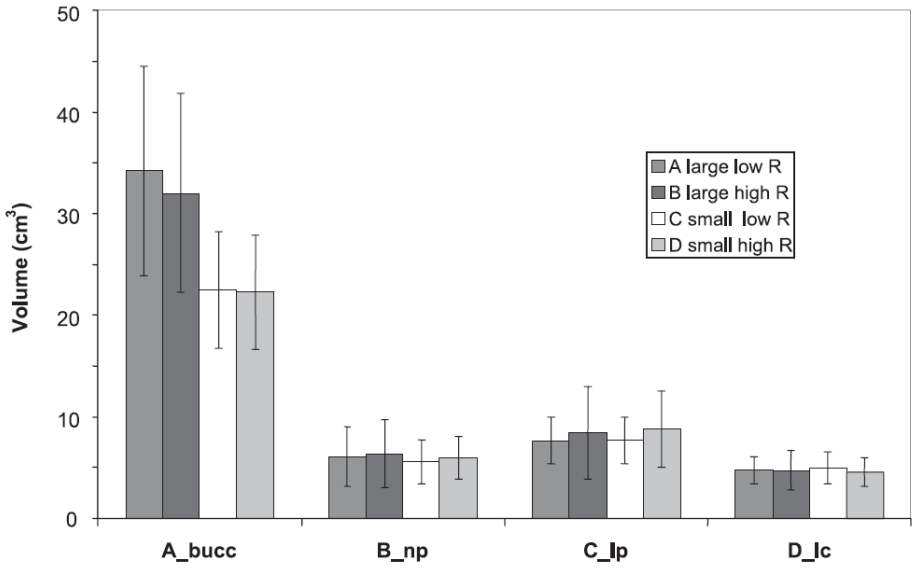


Figure 2.12: Mean regional volumes for 4 dummy inhalers with different resistances. A_bucc = buccal region from back of teeth to soft palate; B_np = naso-pharynx region including nasal airways (not part above roof of mouth) to tip of EG; C_lp = laryngeal-pharynx region, tip of EG to just above vocal cords; D_lc = laryngeal cavity just above vocal cords to trachea level with 5th intervertebral disc. Error bars represent ± 1 SD (Pritchard et al., 2004).

The subjects inhaled through the inhalers during tidal breathing without any instructions regarding tongue position, and were scanned in a supine position. Only the total airway and buccal volumes showed a consistent dependence on the dummy inhaler characteristics (Figure 2.12). Mean airway minimum and maximum CSAs and radii were not influenced by the dummy inhalers (Pritchard et al., 2004). In the study by McRobbie et al (2005) the authors investigated through an inhalation-gated MRI the impact of two dummy inhalers (mouthpiece diameter 14 mm) with different resistances on the upper airways of five healthy adult subjects. The subjects inhaled through the dummy inhalers with a forced inspiratory manoeuvre and were scanned in a supine position. The authors did not report the CSA values but compared upper airway volumes (Figure 2.13) to data from a previous study, in which the subjects inhaled through the 2 dummy devices with tidal breathing (Pritchard et al., 2004).

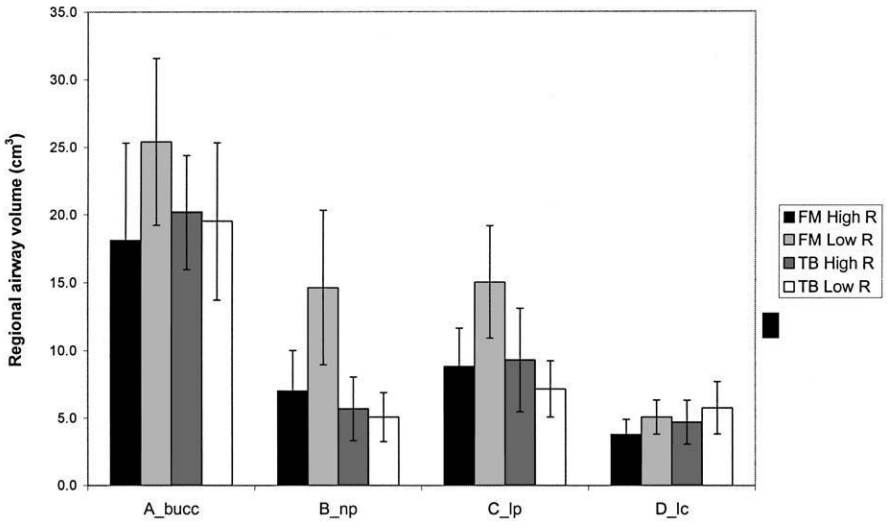


Figure 2.13: Mean regional volumes for combinations of devices and breathing strategies. A_bucc = buccal region from back of teeth to soft palate; B_np = naso-pharynx region including nasal airways to the tip of EG; C_lp = laryngeal-pharynx region, tip of EG to just above vocal cords; D_lc = laryngeal cavity just above vocal cords to trachea level with 5th intervertebral disc. FM = forced maneuver and TB = tidal breathing. Error bars represent ± 1 SD (McRobbie et al., 2005).

The mean upper airway volume was shown to be larger (60 cm^3) when the subjects inhaled with a forced manoeuvre through the low-resistance dummy inhaler in comparison with tidal inhalation (38 cm^3) through the same device (Figure 2.13). There were no significant changes in airway volume between the two breathing modes when using the high-resistance dummy inhaler (McRobbie et al., 2005).

2.5.4 Impact of mouthpiece design and inspiratory manoeuvre on lung deposition

In the study by Boyd et al (2004) the impact of mouthpiece cross-sectional shape, volume, and taper on oropharyngeal and lung deposition of inhaled insulin was tested using a prototype AER_x inhaler (Aradigm Corporation, Hayward, CA, USA). The 3 clinically tested mouthpieces were designed either as a cylindrical mouthpiece or as an elliptical mouthpiece, both with constant CSAs of 7.9 cm^2 and 7.5 cm^2, or as a tapered elliptical mouthpiece with an exit CSA equal (3.7 cm^2) to one half the entrance CSA (7.5 cm^2). Fifteen healthy subjects participated in the gamma scintigraphy study in which each inhalation of the radiolabelled aerosol was followed by a 5-s breath-hold. The MMAD ranged from 2.2 to 2.3 μm. There were no statistically significant differences in oropharyngeal or lung depositions between males and females, and the cross-sectional shapes of the mouthpieces had no significant effect on the oropharyngeal or lung depositions. The lack of effect of the cross-sectional shapes of the mouthpieces might have been related to the use of particles too small to be affected by the differences in mouthpiece designs.

Svartengren et al (1996) investigated whether the mouthpiece length, ~4 cm *versus* ~6.4 cm, would have an impact on oropharyngeal and lung depositions in 9 subjects diagnosed with obstructive airway diseases. The shorter mouthpiece was a standard mouthpiece, whereas the longer mouthpiece was designed to bypass part of the oral cavity and thereby reduce oropharyngeal deposition and was cut off at the level of the hard palate for each subject. The subjects inhaled at

0.5 L/s an aerosol consisting of monodisperse radiolabelled Teflon particles with a mean aerodynamic diameter of 3.5 µm. There were, however, no statistically significant differences in oropharyngeal or lung depositions between the mouthpieces.

2.5.5 Impact of mouthpiece design on the upper airway CSA

Van Holsbeke et al (2014b) have recently presented the results of a study in which the impact of mouthpiece design on the upper airway CSA was investigated. An ultrafast spoiled gradient echo sequence MRI was used in 12 healthy adult male subjects who were supine during the scans. The influence of mouthpiece height (12-27 mm), width (19-32.1 mm), protrusion (4-40 mm into the mouth), orifice size (3-7 mm) and resistance to airflow were investigated. The upper airways were divided into the oral cavity (zone 1), the oropharynx (zone 2), and the hypopharynx (zone 3). The results showed that mouthpiece protrusion and height had the most positive effect on CSA, whereas the impact of width and orifice size was minimal. The changes in CSA were mainly found in the oral cavity, whereas the changes in the oropharynx were small and inverse. The mouthpiece design parameters did not affect the hypopharynx (Figure 2.14 and Figure 2.15). The authors concluded that the influence of the mouthpiece protrusion on the CSAs of the oral cavity and the oropharynx was probably a consequence of the interaction between the mouthpiece and the tongue (Van Holsbeke et al., 2014b).

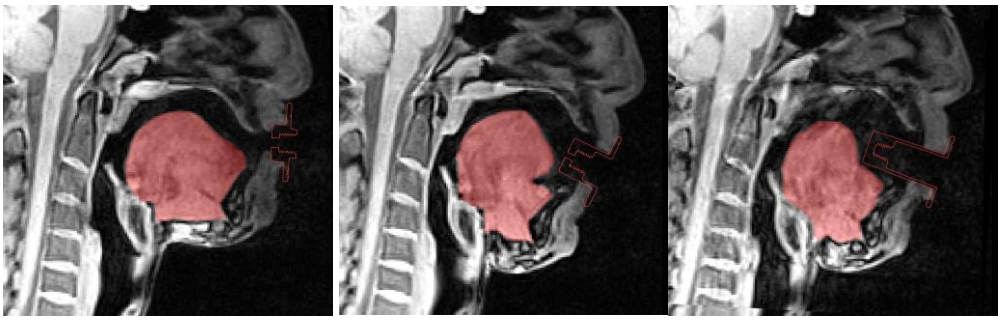


Figure 2.14: Interaction between mouthpiece protrusion and tongue position. In the left graph the mouthpiece protrusion is small, and large in the right graph (Van Holsbeke et al., 2014b; from poster at the European Respiratory Society congress 2014).

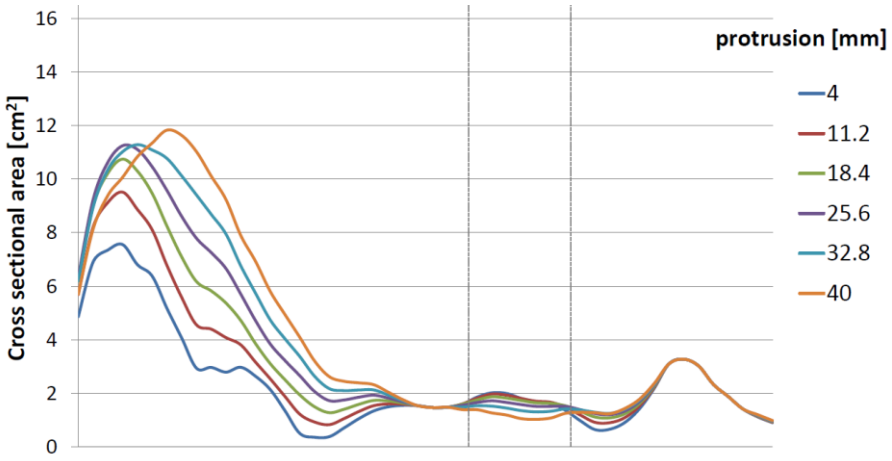


Figure 2.15: Influence of mouthpiece protrusion on the CSAs of the oral cavity, the oropharynx and the hypopharynx. The 3 sections represent the oral cavity (left, zone 1), the oropharynx (middle, zone 2), and the hypopharynx (right, zone 3) (Van Holsbeke et al., 2014b; from poster at the European Respiratory Society congress 2014).

2.6 Nebulisers

2.6.1 Atomisers and jet nebulisers

The evolution of the modern jet nebuliser, which was developed for aerosolisation of liquids, can be traced through available published sources to the mid-nineteenth century and the evolution of the atomisers. The early jet nebulisers were in essence atomisers and the terms “nebuliser” and “atomiser” seem to have been used synonymously during the nineteenth century. In the Oxford English Dictionary the term “nebulizer” was included in 1872, and both terms have the same definition and are attributed to late nineteenth century. The early atomisers – for example perfume atomisers - lacked a baffle system which would have created an aerosol with small droplets and therefore a respirable aerosol. May (1973) defined a nebuliser as a “baffled spray cloud-producing device”.

2.6.2 Early jet nebulisers

Several physicians published descriptions of early jet nebulisers designed with baffles and pumps to create compressed air (Waldenburg, 1864; Moeller, 1882; Tissier, 1903). In these descriptions the jet of liquid was directed against a baffle to create a respirable aerosol. Early information on droplet size was provided by Abramson (1946) who described the DeVilbiss No. 40 jet nebuliser (The DeVilbiss Company, PA, USA) as a nebuliser that baffled out the large droplets leaving a droplet spectrum of 0.3-2 µm. Abramson did not describe the technique for measuring the droplet size but defined "aerosol", "atomisation" and "nebulisation" and argued that "nebulization should be restricted to the special type of atomization in which the large particles are removed by the introduction of suitable baffle into the construction of the atomizer". Harsh (1948) compared 15 different jet nebulisers in terms of output per squeeze with the rubber bulb, the capacity of the bulb, droplet size and nebulisation time. The amount of solution delivered by one bulb compression was highly variable (range 0.4-13.0 mg) as was nebulisation time for the delivery of 1 mL (range 31-450 sec). An ocular micrometre in a microscope was used to determine the droplet size, and the median size ranged from 8 to 29 µm, whereas the largest droplets ranged from 40 to 308 µm.

2.6.3 Ultrasonic nebulisers

The introduction of ultrasonic nebulisers in the 1960ies created a new class of nebulisers with a higher output rate in comparison with jet nebulisers (Abramson, 1968). An early model of the DeVilbiss Ultrasonic Nebulizer nebulised 4-10 mL of solution per minute and created an aerosol with a median diameter of 7.7-9.6 µm (Goddard et al., 1968). Apart from a high output rate, the advantages of the ultrasonic nebulisers included independence of a compressed air flow through the nebuliser chamber, and the control of droplet size through adjustment of the ultrasonic

frequency (Goddard et al., 1968). A major drawback with modern ultrasonic nebulisers is the poor performance when nebulising for example suspensions with micronised particles (Nikander et al., 1999b) and viscous solutions (Newman et al., 2009).

2.6.4 Vibrating mesh nebulisers

The introduction of vibrating mesh nebulisers built on the experience with the ultrasonic nebulisers, and piezoelectric crystals are used to create the vibrations of the meshes. In vibrating mesh nebulisers the mesh contains hundreds or thousands of nozzles depending on technology and manufacturer (Newman et al., 2009). A number of vibrating mesh nebulisers have been introduced; the AeroNeb (Aerogen, CA, USA), the eFlow nebulisers (PARI, Germany), the I-neb Adaptive Aerosol Delivery (AAD) System (Philips Respironics, UK), and the MicroAir (Omron, Japan) nebuliser. In the eFlow nebuliser a stainless steel mesh is actuated by a battery powered annular piezoelectric element to vibrate at a frequency of ~100 kHz (Knoch et al., 2005). In the AeroNeb nebuliser a domed aperture plate is moved up and down by a battery powered ceramic piezoelectric element (Dhand, 2002). In the I-neb AAD System and the MicroAir nebulisers an ultrasonic horn transducer in the nebuliser cup is vibrated forcing liquid through a static mesh (Dhand, 2002). The MicroAir mesh is made of metal alloy with ~6000 holes with a diameter of 3 µm, and the aerosol droplets have a mean droplet diameter slightly larger than the diameter of the hole (Newman et al., 2009).

2.6.5 The Adaptive Aerosol Delivery (AAD) System

The Adaptive Aerosol Delivery (AAD) technology was developed to minimise wastage of drug during the patient's exhalation during jet nebulisation (Nikander, 1997; Denyer et al., 2004). The first nebuliser based on the AAD technology (HaloLite AAD System; Denyer, 1997) was based on jet nebuliser/compressor technology and was a breath activated, dosimetric jet nebuliser which

was co-developed by Medic-Aid Ltd. UK and Astra, Lund, Sweden, and made commercially available in 1997 (Denyer et al., 2010b). In the system a flow sensor monitored the subject's breathing pattern, and after an analysis by the AAD software, aerosol was pulsed from the beginning of the inspiration during 50% of the inspiration minimising waste of aerosol during expiration. The analysis of the breathing pattern continued during the whole nebulisation and adapted the pulse of aerosol continuously to the subject's breathing pattern. The background to the development of the HaloLite AAD System was partly *in vitro* and clinical studies performed by Astra during the development of nebulised budesonide.

The advantage of a breath-synchronised, dosimetric jet nebuliser with lack of wastage of drug during exhalation had in the early 1990ies been highlighted in an *in vitro* study of the differences in nebulisation of budesonide between breath-activated, breath-enhanced and conventional jet nebulisers (Nikander, 1994; O'Callaghan, 1997). Filter studies with nebulised budesonide in which children with asthma (age range 0.5-15.7 years) were inhaling through filters attached to the nebuliser inhalation port, showed that the inhaled mass (amount of drug on filter) could be increased with a breath-activated jet nebuliser and the waste of aerosol during exhalation reduced (Nikander, 1994; Nikander et al., 1999a; Nikander et al., 2000a). In a follow-up filter study in asthmatic children (2.5-5.8 years), adolescents and adults (13-52 years), continuous jet nebulisation, breath-activated jet nebulisation during the whole inspiration and breath activated pulsed jet nebulisation for up to 1 sec from start of inspiration were compared (Nikander et al., 2000c). The authors concluded that the results supported breath-activated jet nebulisation during the whole inspiration but not pulsed jet nebulisation with conventional jet nebulisers, and that pulsed nebulisation in children required further studies (Nikander et al., 2000c).

The use of the HaloLite AAD System was investigated in subjects with CF (Kastelik et al., 2002; Byrne et al., 2003), with hereditary α_1–proteinase inhibitor deficiency (Brand et al., 2003), and

with pulmonary hypertension (Olschewski et al., 2003). The AAD technology was further developed with the introduction of the 2nd generation AAD system, the Prodose AAD System (Denyer et al., 2004) – also based on jet nebuliser/compressor technology - in which the maximal length of the aerosol pulse time was set to 8 sec. The aerosol pulse was also made dependent on the tidal volume; aerosol was pulsed into the first 50% of the subjects tidal volume if the volume was <1 L, if it was larger the pulse time was longer. Due to the introduction of the AAD Disc technology, a plastic disc with a microchip and an antenna with information regarding aerosol dosage, dosing frequency, number of doses to be delivered, drug lot number and expiry date could be introduced (Denyer et al., 2004). The new technology made the use of the ProDose AAD System in a telehealth setting possible (Nikander et al., 2010a).

2.6.6 The I-neb Adaptive Aerosol Delivery (AAD) System

The I-neb AAD System (I-neb nebuliser; Figure 2.16) was the 3rd generation AAD system which was developed with Omron Healthcare (Kyoto, Japan) based on a vibrating mesh technology (Denyer et al., 2004).

Figure 2.16: The I-neb nebuliser shown with the AAD disc. Available at and accessed October, 2015: http://www.healthcare.philips.com/main/homehealth/respiratory_drug_delivery/index.wpd

The main parts of the I-neb nebuliser are the body, the medication chamber assembly including the metering chamber, the mesh and the mouthpiece (Figure 2.17).

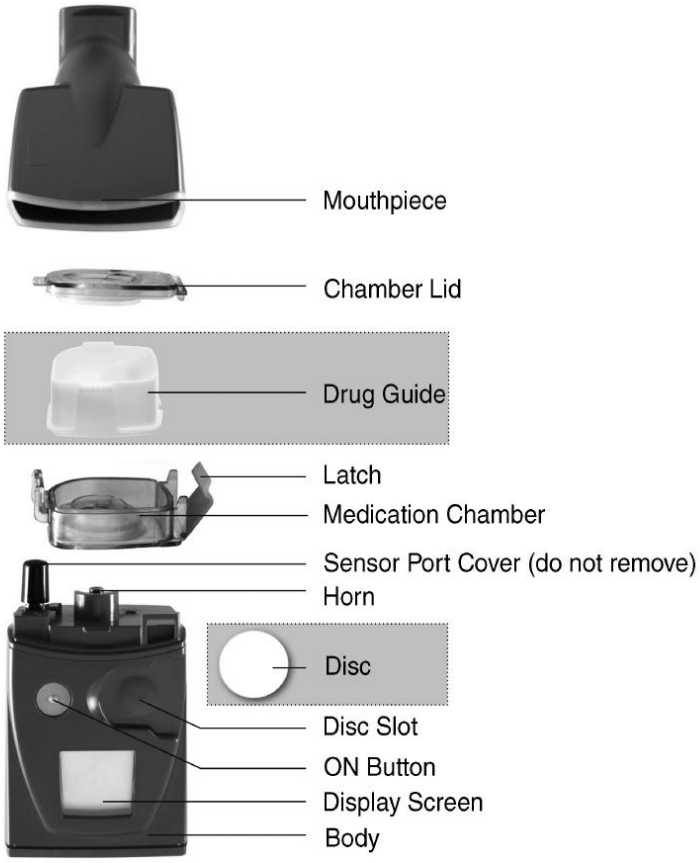


Figure 2.17: The figure shows the different parts of the I-neb nebuliser (Nikander et al., 2008).

2.6.6.1 The breathing modes, TBM and TIM

Two different breathing modes, the Tidal Breathing Mode (TBM) and the Target Inhalation Mode (TIM) are used with the I-neb nebuliser. In TBM the subject breathes tidally through the TBM mouthpiece, and through the function of the AAD algorithm a pulse of aerosol is delivered during 50% of the first part of each inspiration (Figure 2.18).

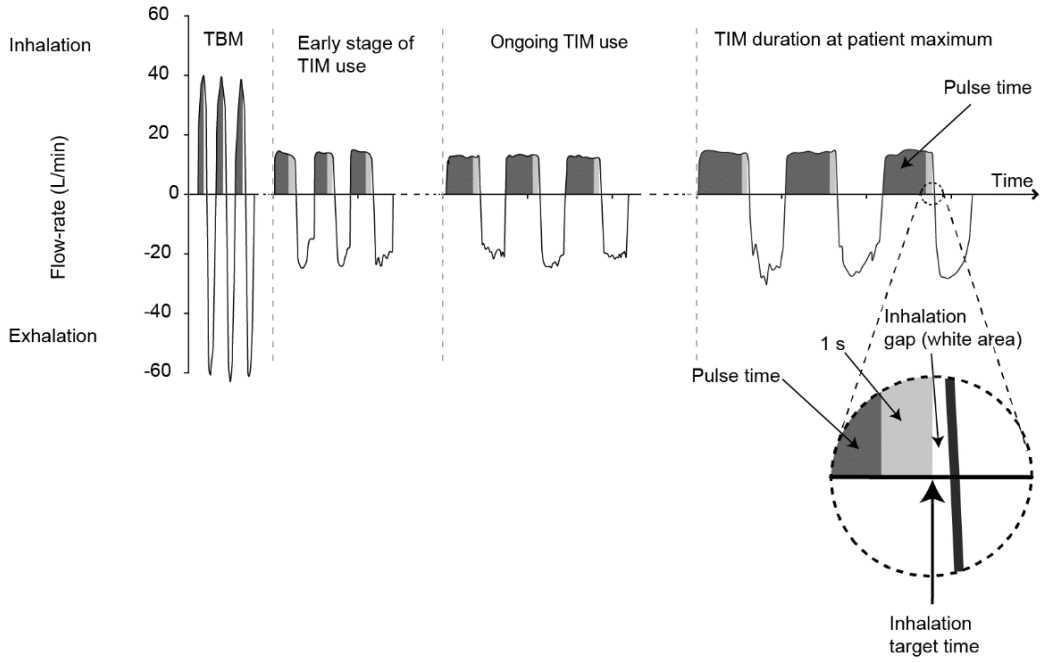


Figure 2.18: A schematic presentation of the two breathing modes used with the I-neb nebuliser, the TBM and the TIM. In the TBM graph the aerosol is pulsed in 50-80% of the inspiration (grey area). In the TIM graphs (2nd, 3rd, last graphs) the gradual extension of the inspiration from a 3-sec inhalation (2-sec aerosol pulse) to an 8-sec inhalation (7-sec aerosol pulse) is shown. Reproduced from (Denyer et al, 2010a).

The pulsed aerosol delivery is based on a continuous calculation of the average of the past three tidal inspirations, and from these the length of the following inspiration is predicted. The pulse time is continuously monitored and adjusted depending on variability in the subject's inspirations. If the inspiration is extended past 2 sec, the pulse time is extended beyond the 50% of the predicted inspiration time and up to ~1sec before the start of the predicted expiration (Denyer et al., 2010a; Denyer et al., 2010b; Denyer et al., 2010c).

In TIM a slow and deep inspiration is performed through the TIM mouthpiece guided by feedback from the device, and achieved via a magnet in the TIM mouthpiece which activates the TIM algorithm. The peak inspiratory flow through the TIM mouthpiece is restricted to ~20 L/min by an elastomeric valve in the mouthpiece with no resistance on expiration (Figure 2.19).

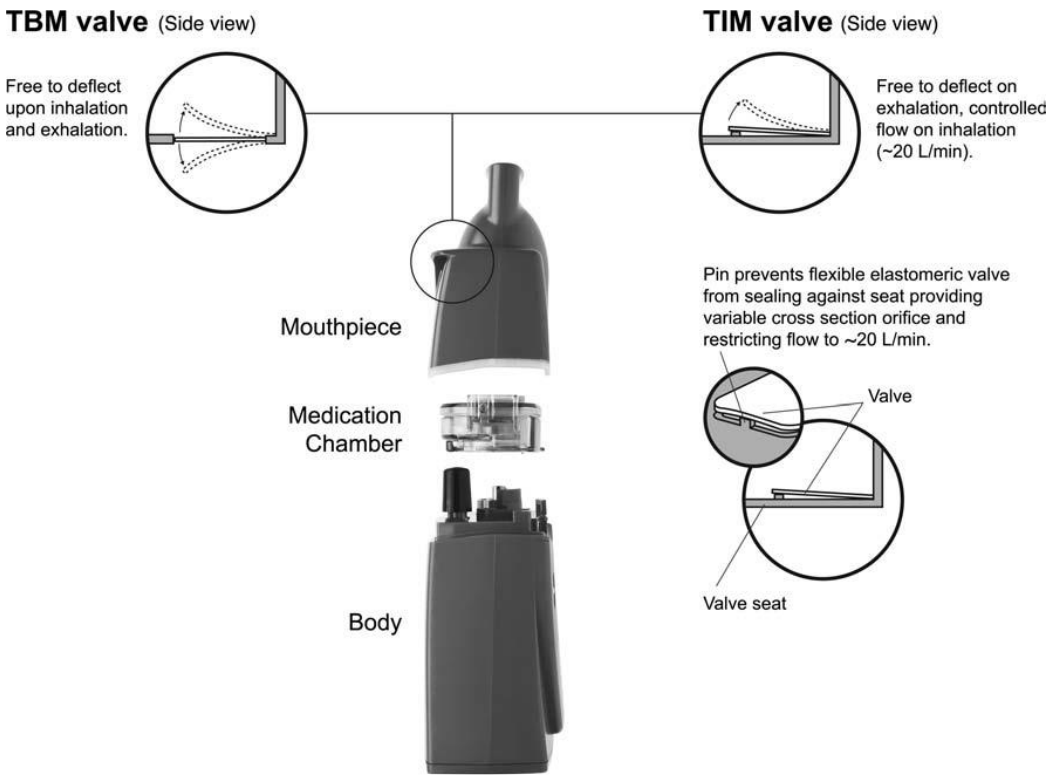


Figure 2.19: A schematic presentation of the main components of the I-neb nebuliser; the mouthpiece, the medication chamber assembly, and the body. The valve system in the mouthpiece is shown for the two different breathing modes, the TBM (left), and the TIM (right) (Denyer et al., 2010a; Nikander et al., 2010c).

2.6.6.2 The mouthpiece

The aerosol generated by the horn and the mesh passes the mesh at a low velocity, and the subject's inspiration carries the aerosol through the mouthpiece (Figure 2.20). The inspiratory airflow through the inhalation and exhalation valve in the mouthpiece mixes with the aerosol during inhalation (Denyer et al., 2010b).

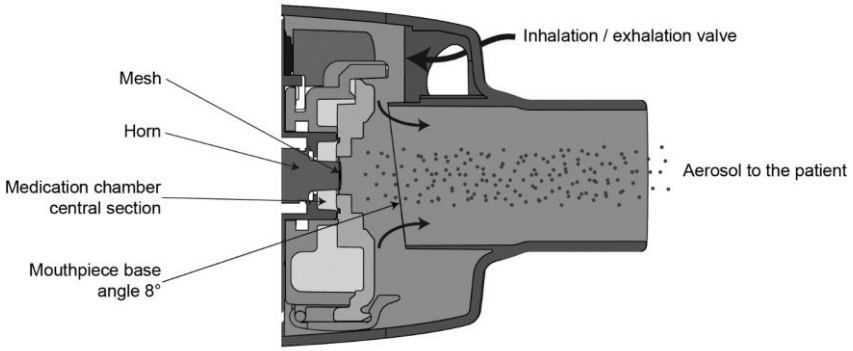


Figure 2.20: The mouthpiece of the I-neb nebuliser shown with the medication chamber (light grey), the mesh in the middle of the medication chamber, and aerosol flowing through the mouthpiece (Denyer et al., 2010b).

2.6.6.3 The vibrating mesh technology

The mesh in the I-neb nebuliser is made of platinum with ~7000 holes with an average diameter of 2 µm. The solution or suspension poured into the medication chamber fills the gap between the ultrasonic horn and the mesh by gravity, and is pumped through the mesh at a frequency of 178 kHz. At the end of nebulisation when the liquid in the medication chamber has been aerosolised, an electronic control circuit detects the change in power required by the horn and signals the end of nebulisation. The aerosol output rate can be adjusted depending on the requirements of different drug formulations as the piezoelectric element connected to the horn has a variable power range (Denyer et al., 2010b).

2.6.6.4 The medication chambers

The I-neb nebuliser medication chambers were designed for metering or non-metering purposes (Figure 2.21). The metering chamber was designed with a central section for a metered dose of liquid drug formulations in volumes of 0.25-0.75 mL. If a commercially available drug vial with a volume of 2-5 mL was used to fill the metering chamber, the remaining liquid flowed into an outer chamber and this part of the liquid was not aerosolised.

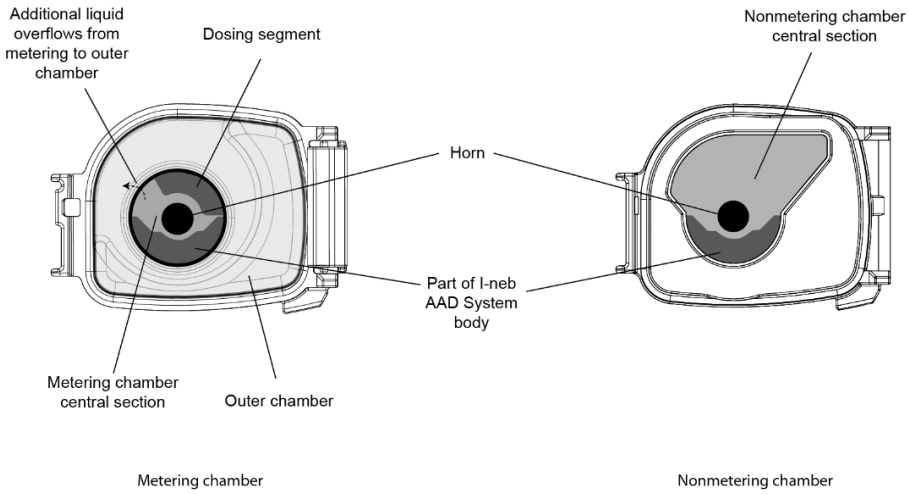


Figure 2.21: The medication chambers designed for the I-neb nebuliser; the metering chamber (left) and the non-metering chamber (right) (Denyer et al., 2010b).

The metered dose was defined ex-mouthpiece which meant that the metered dose was calculated to compensate for drug losses in the mouthpiece. The non-metering chamber was designed for liquid volumes ranging from 0.25 mL to 1.7 mL and was filled by using a pipette (Denyer et al., 2010b).

2.6.6.5 Upper airway and lung deposition with the I-neb nebuliser

The upper airway and lung deposition with the I-neb nebuliser has been investigated in several studies both in healthy subjects (Nikander et al., 2010c), and in subjects diagnosed with IPF (Diaz et al., 2012), and in subjects diagnosed with CF (van Velzen et al., 2015). In the study by van Velzen et al (2015) the bioavailability of nebulised tobramycin was used as a surrogate marker for lung deposition in adult subjects diagnosed with CF. Eighteen subjects (10 male) aged 19-57 years were included in a randomised, open-label, crossover study in which the I-neb nebuliser was used with a 1 mL medication chamber. The subjects nebulised 1 mL of a ~10% tobramycin solution in both TBM and TIM breathing modes. The results showed that lung deposition when inhaling

tobramycin in the TIM breathing mode was 53% higher compared to inhalation in the TBM breathing mode. Due to the study design, data on upper airway deposition was not reported.

In the study by Diaz et al (2012) the safety, and lung and upper airway deposition of nebulised INF-γ was investigated in subjects diagnosed with IPF. Ten subjects (majority male) with a mean age of 68 years were included in a non-randomised, interventional pilot study in which the I-neb nebuliser was used in the TIM breathing mode. INF-γ was nebulised in a dose of 100 µg 3 times a week for 80 weeks. Lung and stomach deposition was investigated using radiolabelled INF-γ solution. The subjects drank a glass of water immediately after nebulisation of the radiolabelled INF-γ solution in order to wash the aerosol deposited in the oropharynx into the stomach. A scan of both the lungs and the stomach defined lung and upper airway (stomach activity) deposition. The mean lung deposition was 65.4 ± 4.8% (± SEM) of the nebuliser charge and ranged from 21.6 to 95.1%, whereas the mean upper airway deposition was 12.6 ± 3.0% and ranged from -2.8 to 35.3%. The mean ratio between central and peripheral lung zones (sC/P) was 1.20 ± 0.06 and ranged from 1.00 to 2.21; a ratio of 1.0 indicated deposition in the small airways and alveoli and a ratio >1.0 more central airway deposition.

In the study by Nikander et al (2010c) the lung and upper airway deposition of nebulised ^{99m}Tc-DTPA in saline was investigated in healthy adult subjects. Twelve subjects (3 male) with a mean age of 33.8 years (range 20-65 years) were included in a randomised, open-label, crossover study in which the I-neb nebuliser with a power level 10 AAD Disc was used both in TBM and TIM. An exhalation filter was fitted to the inhalation/exhalation port of the mouthpiece to capture exhaled ^{99m}Tc-DTPA. The TIM breathing mode had a maximum length of 9 sec with an aerosol pulse of to 7 sec, and with no aerosol delivered during the last 2 sec. This is in contrast with the present TIM breathing mode with a maximum length of 8 sec with no aerosol delivered during the last 1 sec. All subjects were in a seated position during nebulisation and nose clips were used

during the nebulisation process. Inspiratory and expiratory flows through the mouthpiece, aerosol pulse times during nebulisation, time spent in inspiration, number of breaths and minute volume were monitored electronically during the study (Table 2.3).

Table 2.3: Summary of a number of parameters recorded during the nebulisation with the I-neb nebuliser: inspiratory and expiratory flows through mouthpiece, aerosol pulse times during nebulisation, length of inspiration, number of breaths and inspiratory minute volume (Nikander et al., 2010c). The data are shown as means ± SD.

Mean	**TBM**	**SD**	**TIM**	**± SD**
Inspiratory flow (L/min)	23.92	8.23	12.95	4.29
Expiratory flow (L/min)	27.51	10.89	18.39	7.57
Aerosol pulse time (sec)	2.13	0.89	5.83	1.06
Length of inspiration (sec)	3.53	1.32	8.75	0.92
Number of breaths	44.80	23.19	11.60	3.20
Inspiratory minute volume (L)	7.19	2.44	7.77	2.36

The lung deposition of ^{99m}Tc-DTPA (with central and peripheral lung deposition shown separately), the upper airway deposition, and the exhaled fraction caught on the filter attached to the mouthpiece are shown in Figure 2.22. The data is presented in percent of emitted dose ex-mouthpiece. A lung deposition image is shown in Figure 2.23. The lung deposition in TIM (mean 73.29%, SD 16.3) was statistically significantly (p=0.0020) higher than the lung deposition in TBM (62.82%, 19.6). The upper airway deposition in TBM (36.18%; 19.7) was statistically significantly (p=0.0039) higher than the upper airway lung deposition in TIM (26.49%; 16.3). The central lung deposition was 17.95% (3.89) in TIM and 16.50% (5.2) in TBM, whereas the peripheral lung deposition was 34.83% (8.38) in TIM and 28.73% (9.62) in TBM. The mean sC/P was in TBM 1.66 (0.33) and in TIM 1.57 (0.32) with no statistically significant difference. The

amount of aerosol on the exhalation filter was in TBM (0.99%; 0.43) statistically significantly ($p<0.0001$) higher than in TIM (0.20%; 0.13). The mean deposition in the mouthpiece in percentage of loaded dose was 5.1% (1.8) in TBM and 5.0% (1.7) in TIM.

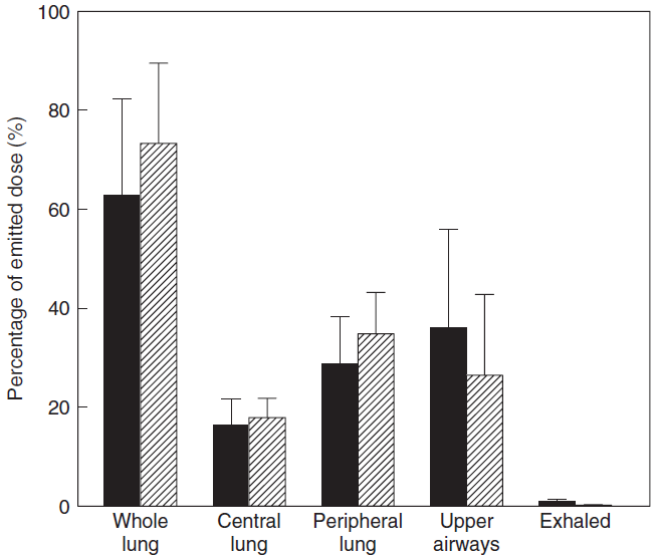


Figure 2.22: The lung, central lung, peripheral lung, and upper airways deposition of ^{99m}Tc-DTPA plus the exhaled amount of ^{99m}Tc-DTPA in healthy subjects with TBM data in black and TIM in gray (Nikander et al., 2010c).

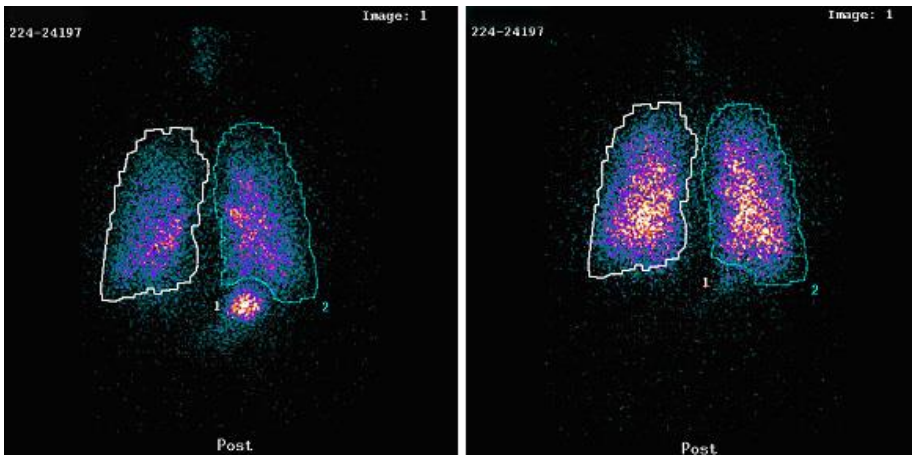


Figure 2.23: Posterior lung deposition images of ^{99m}Tc-DTPA delivered with the I-neb nebuliser in TBM (left) and TIM (right) for subject 4 (Nikander et al., 2010c).

The MMD of the saline aerosol emitted from the I-neb nebuliser was for the 14 devices allocated for the study 4.6 µm with a FPF of 56.8% which was substantially lower than the lung deposition in either breathing mode.

2.7 Acoustic pharyngometry

2.7.1 Early acoustic reflection (AR) method, development and studies

The Sondhi et al (1971) paper has been described as the original description of acoustic pulse-response analysis (Buenting et al., 1994; Kamal, 2004c). The first clinical study of the AR method has been attributed to Fredberg et al (1980) who tested the hypothesis that upper airway and tracheal geometry could be determined through AR at the mouth (Kamal, 2004c). The acoustic equipment consisted of a mouthpiece, a sliding 2-position valve, a 5 m long wavetube of stainless steel, a microphone, and a loudspeaker (Figure 2.24). The mouthpiece was designed to limit variability due to tongue movement, and jaw position, and filled the oral interstices between the posterior margin of the hard palate and the lips.

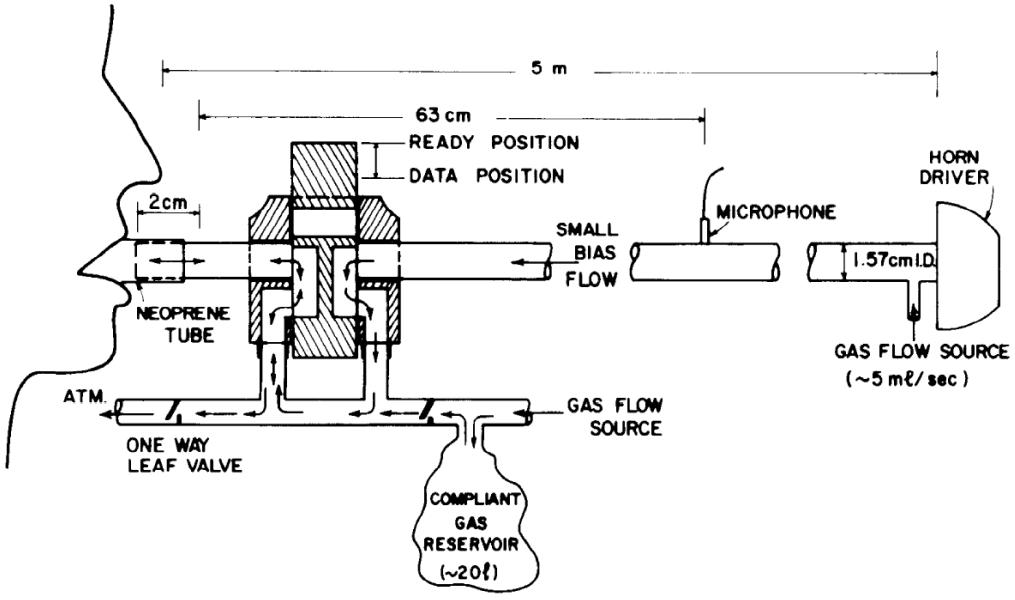


Figure 2.24: Diagram of the test equipment consisting of a mouthpiece, a 2-position valve, a 5 m long wave tube of stainless steel, a microphone, and a loudspeaker (Fredberg et al., 1980).

The 6 healthy adult subjects breathed either room air or a humidified mixture of helium (80%) and oxygen (20%). The authors concluded that the study suggested that the geometry of the upper airways between mouth and carina could be determined accurately with the equipment (Fredberg et al., 1980). The equipment was later used in several clinical studies due to lack of commercially available equipment (Marshall et al., 1991).

The early AR studies were focused on measuring the upper airways, the trachea and part of the lungs (Hoffstein et al., 1991). An example of an airway echogram of the upper airway, the trachea and the lungs is shown in Figure 2.25 (Hoffstein et al., 1991).

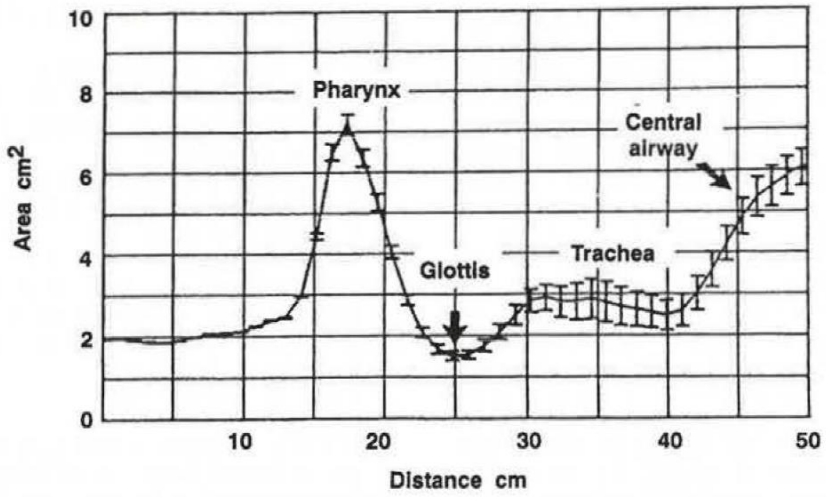


Figure 2.25: An echogram acquired during tidal breathing. Some major anatomical landmarks can be identified. The first ~6 cm correspond to the end of the wave tube and the mouthpiece, the large peak is the pharynx, the 1st minimum following the pharynx is the GL and the plateau region distal to the GL is the trachea followed by the central airway (Hoffstein et al., 1991).

The authors highlighted the reproducibility of the echogram from the wavetube to the trachea as evidenced by the small standard deviations, but also noted that the reproducibility decreased for the distal structures as the assumptions of the method were satisfied only for the central airways (Hoffstein et al., 1991).

In 1991 Marshall et al (1991) presented a paper in which they described the theory and limitations of AR, and suggested some modifications to the equipment used by Fredberg et al (1980; Figure 2.26). The equipment was "closed" as the subject could not breathe during the measurement in contrast to the older Fredberg et al (1980) equipment (Marshall et al, 1991)

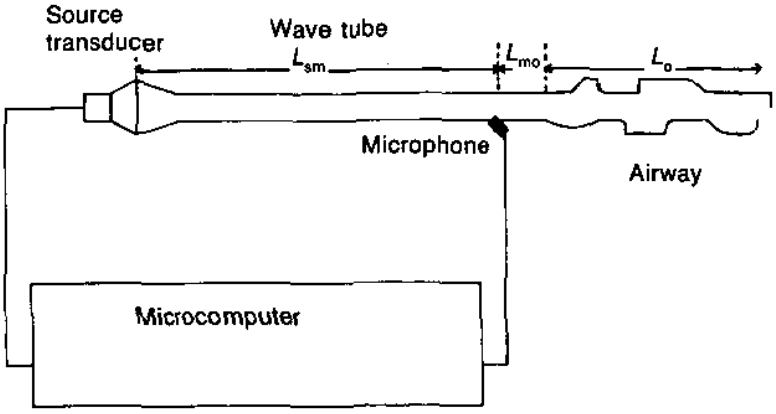


Figure 2.26: A diagram of the closed acoustic reflectometer equipment (Marshall et al., 1991).

In 1993 Marshall et al (1993) presented a new AR equipment which allowed the subject to breathe during the measurement (Figure 2.27). This was achieved by a hole in the wavetube wall immediately proximal to the mouthpiece and the hole was closed just before a measurement was made. A flexible wavetube made of PVC with a 16 mm internal diameter with a loudspeaker-microphone distance of 1130 mm and a microphone-mouthpiece distance of 130 mm was included in the equipment (Figure 2.27). Marshall et al (1993) focused on the upper airways from the mouth to the hypopharynx and started using room air for the AR measurements instead of the helium/oxygen mixture used in previous studies with the Fredberg et al (1980) equipment (Marshall et al., 1993).

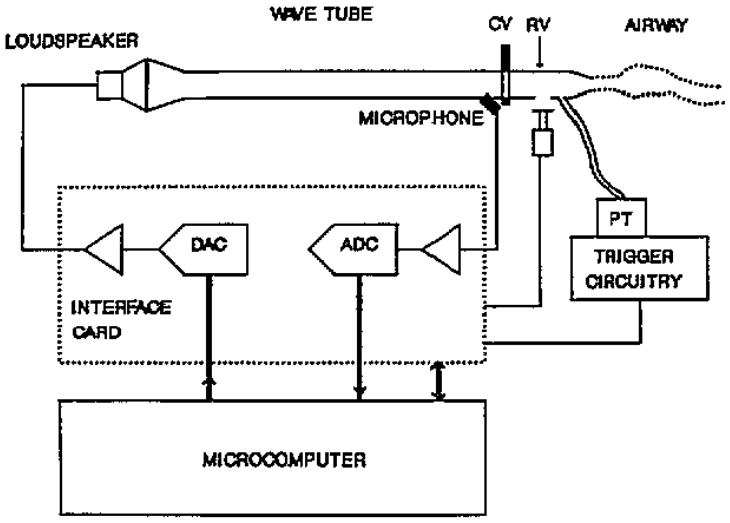


Figure 2.27: A diagram of the new AR equipment. PT = pressure transducer, CV = calibration (slide) valve, RV = respiratory (shutter) valve (Marshall et al., 1993).

In 1994 Louis et al suggested a two-microphone method in order to be able to use a shorter wavetube. They published a year later the results of *in vitro* and clinical tests in 3 healthy subjects in which they compared a one-microphone method *versus* the two-microphone method, and a helium/oxygen mixture *versus* room air (Figure 2.28; Louis et al., 1994).

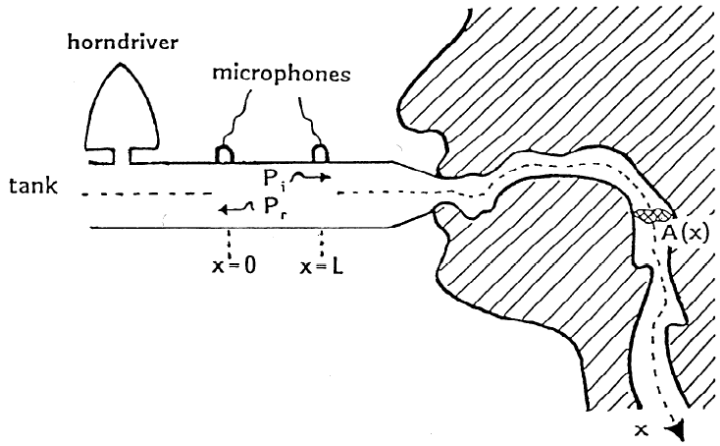


Figure 2.28: A diagram of the Louis et al (1994) wave tube developed for measurement of upper airway area by a two-microphone AR method. P_i stands for an incident pressure wave which impinges on airway opening and gives rise to a reflected wave, P_r. The pressure sum of the incident and the reflected waves was recorded in 2 loci of the wavetube to infer area (A) *versus* axial position (x) along the airway. L = length of wave tube (Louis et al., 1994).

The new equipment that Louis et al (1994) tested was considerably smaller than the equipment used by Marshall et al (1993), with a 30 cm long wavetube with a 1.89 cm internal diameter (Figure 2.28). The results of the tests indicated that the two-microphone method with a helium/oxygen mixture was equivalent with the one-microphone method over a distance up to 60 cm. The AR method was further developed into the commercially available Eccovision acoustic pharyngometer used in this thesis. The focus of the pharyngogram was limited to the upper airways between the wavetube and the GL.

2.7.2 Principles of acoustic reflectometry

The principles of the acoustic method has been explained by a number of authors (Fredberg et al., 1980; Brooks et al., 1984; Hoffstein et al., 1991; Marshall et al., 1991; Marshall et al., 1993, Louis et al., 1994, Kamal, 2004c). The basic principle of the AR method has been described as follows by Hoffstein (Hoffstein et al., 1991):

- As a sound pulse travels along a tube and comes across a change in area from A_1 to A_2, part of the pulse is reflected and travels back along the tube, and part is transmitted.
- With known wavespeed (c) and travel time (t), the length of the tube (d) can be calculated to be $d = ct$.
- With one-dimensional wave propagation, the measurement of wave travel time is equivalent to the measurement of distance.
- The amplitude of the reflected pulse (Pr) is determined by the amplitude of the incident pulse (Po), and the physical property of the tube. Considering a tube with a single discrete area change from A_1 to A_2, and assuming constant and uniform gas composition, the amplitude of the reflected pulse is given by: $Pr = Po[(A_1 - A_2) / (A_1 + A_2)]$.

The CSA of A_2 can be calculated by measuring the amplitude of the incident and reflected pulses, since A_1 is presumed known. Therefore, the determination of the length and area of the straight tube is reduced to measuring the travel time of the pressure pulses from the area change of the tube, and the amplitudes of the incident and reflected waves. In case of a duct consisting of many

segments - each with different area – the incident sound wave (pressure wave) will be reflected in part every time for each new segment. Determination of the lengths and areas of the individual segments is based on measurement of arrival times and amplitudes of the reflections. This gives the area of the duct *versus* distance from inlet that is the area-distance function or the airway echogram (Hoffstein et al., 1991).

Kamal expanded on the principles of the AR method as used in the Eccovision equipment (Kamal, 2004c) as follows:

- An acoustic impulse traveling through a wavetube into an upper airway will undergo partial reflection and partial transmission at each change in the CSA creating a reflection sequence which will return through the wavetube without further reflection. The passage of the impulse is recorded through a microphone in the wavetube close to the connection between wavetube and the upper airway, the input impulse response. An area-distance relationship of the upper airway geometry can be created by comparing the incident and the reflected acoustic impulse.
- The input impulse response is a series of reflections created by changes in the impedance within the upper airway. The reflection can be either single due to a single change of a tube CSA or multiple as in a human upper airway. The input impulse response and the input impedance are closely related.
- A straight tube with a single change in CSA can be used as an example to highlight how acoustic reflection is used to obtain an area distance function (Figure 2.29).
- The pulse is recorded as it passes the microphone and when the pulse reaches the area of discontinuity some of it is reflected back from right to left (r0) and some continues through the discontinuity (1-r0). The amplitude of the reflected part is calculated as follows: $r0 = (A0 - A1) / (A0 + A1)$ which can be rearranged as: $A1 = A0 \times (1 - r0) / (1 + r0)$. Assuming the pulse travels at a constant speed (C, meters per second) in the wavetube, the distance from the microphone to area change can be computed (Kamal, 2004c).

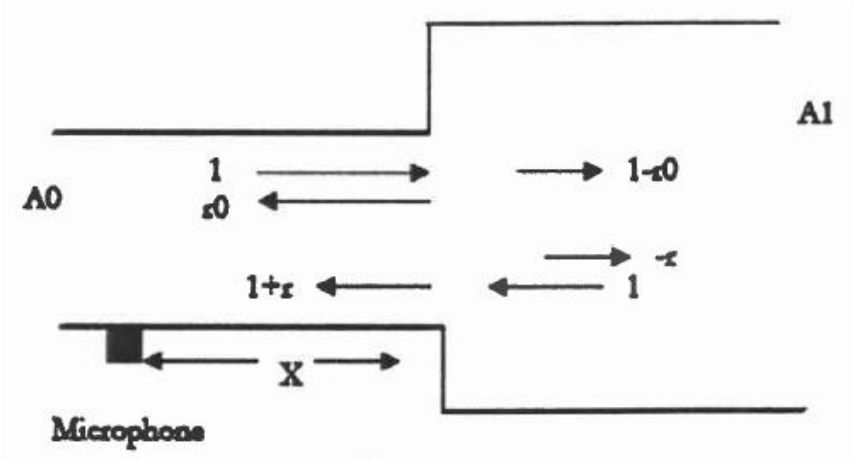


Figure 2.29: The amplitudes of reflected and transmitted impulses (waves) for unit pulse arriving from left (top) and right (bottom) of a single area change (Kamal, 2004c).

Kamal expanded on the principles for a tube with variable CSAs as follows:

- A tube with variable CSAs can represent the upper airway as highlighted in a schematic space-time diagram (Figure 2.30). In this diagram the first reflection has amplitude *r0*, and with *r0* and *A0* we can get *A1*. Thus the amplitude of the pulse transmitted through the first area change is *1 –r0*. In the second area change the reflected portion becomes *(1 – r0)* × *r1*. The pulse travels back and reaches the first area change and the amplitude which reaches the microphone is *(1 – r0)* × *r1* × *(1 + r0).* As *r0* is known *r1* can be computed given the amplitude of the pulse reaching the microphone at time *2* × *2L / C* where L is the length, and with *r1* and *A1* known, *A2* can be computed. This is more complex with increasing number of segments as there are two components of the pulse arriving at the microphone at time *2* × *3L / C.* The first is the part of the original impulse which is transmitted through the first two area changes, is reflected from the third area change and is then transmitted again through the first two area changes and reaches the microphone. This component has amplitude r2 × $(1 – r1^2)$ × $(1 – r0^2)$. The second component is due to the part of the impulse which was transmitted through area change 1 (*A1*), then reflected back and forth from *A2* to *A1* again and then through *A1* to the microphone. As this component is determined by the known *r0* and *r1* , this can be subtracted from the impulse and solve for *r2*.
- In summary, the impulse response of an upper airway with multiple area changes consists of a series of impulses arriving at times 2 × *n* × *L / C.* The impulse arriving at *tn = n* × *2L*

/ *C* consists of two components of which one is due to the original impulse transmitted through area changes *A1* through *n-1*, reflected back at area change *n* and then transmitted back through area change *n-1* to *A1* to the microphone. The amplitude of this component is $rn\text{-}1 \times (1 - rn\text{-}2^2) \times (1 - rn - 2^2) \times \ldots \times (1 - r0^2)$. The other component is caused by reverberations between area changes *A1* through *n-1* and this component is determined by *r0* through *r n-2*. The major assumption is that once a reflected impulse passes the microphone it does not return which can be assured by having a wavetube which is at least as long as the farthest area change measured (Kamal, 2004c). Thus the wavetube should be at least as long as the upper airway measured.

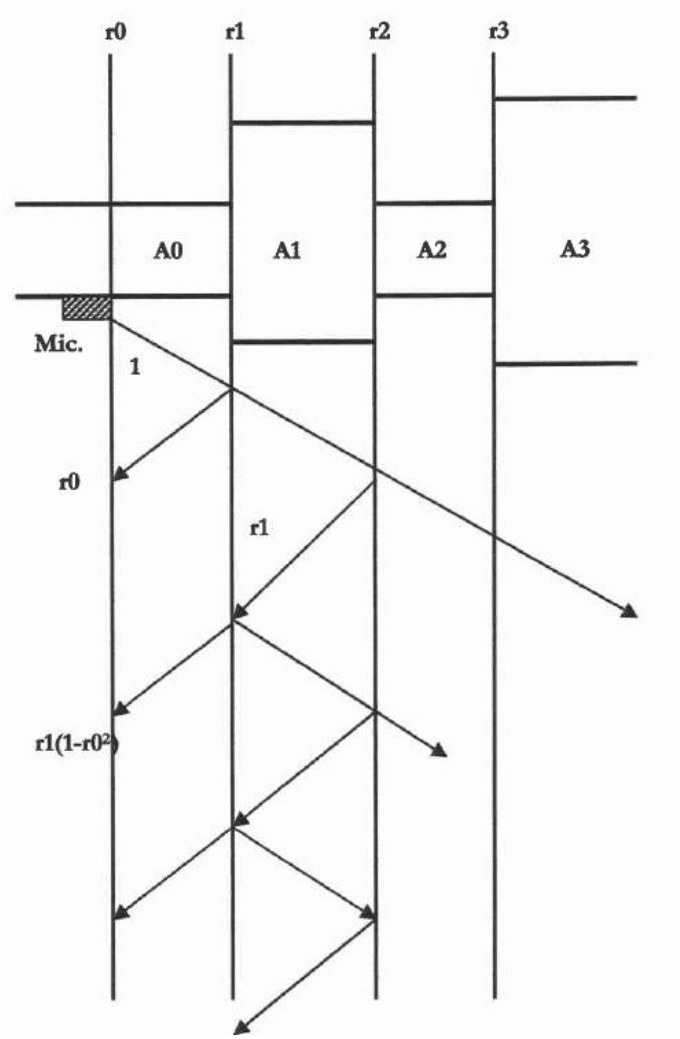


Figure 2.30: A schematic drawing of components of reflected waves as a function of multiple discontinuities with the microphone highlighted as "Mic" (Kamal, 2004c).

2.7.3 Early AR method - accuracy

The early clinical studies that investigated the accuracy of the AR method used the equipment tested by Fredberg et al (1980) and are listed in Table 2.4 (Hoffstein et al., 1991). All but 2 of the studies - including both clinical studies, studies in dogs and *in vitro* studies - were focused on the trachea and showed close correspondence between the areas measured with the AR method, and the radiographic techniques with ratios ranging from 0.88 to 1.15.

Table 2.4: Early comparative clinical studies between the AR technique, and the X-ray and the CT techniques. The comparison is presented as a ratio between area determined by AR and the other methods (Adapted from Hoffstein et al., 1991).

Study	**Region examined**	**Comparison technique**	**Ratio**
Brooks et al., 1984	Trachea	PA, lateral X-rays	1.06
D'Urzo et al., 1987	Trachea	CT scans	0.96
D'Urzo et al., 1988	GL	CT scans	1.06

In the study by D'Urzo et al (1988; Table 2.4), the authors compared the results of the AR measurements of the upper airways of 11 subjects with "glottal pathology" with CT scans of the GL. Both measurements were performed during different days with the subjects in a supine position, during tidal breathing and at FRC. The mean (± SD) CSA values for the GL were 1.8 ± 0.8 cm^2 (AR) and 1.7 ± 0.9 cm^2 (CT), and there was a statistically significant correlation between the two measurements. The authors discussed the suggestion by Brooks et al (1984) – based on *in vitro* glass tube model analysis - that in normal subjects the lower limit of the GL area that might be resolved by the AR would be in the range 0.9-1.0 cm^2. As a number of subjects in the D'Urzo et al study had smaller GL areas confirmed by the CT scans, the results indicated that at least in subjects with glottal pathology the AR method could be used to measure quite small CSAs down to 0.4 cm^2. It should, however, be noted that the smallest GL areas measured by CT scan (0.3, 0.6

and 0.8 cm^2; 3 subjects) were somewhat larger when measured with the AR method (0.4, 0.7 and 1.2 cm^2; D'Urzo et al., 1988).

In their 1993 paper Marshall et al (1993) presented a comparison between AR and MRI CSAs of the upper airways of 10 subjects (Table 2.5). The comparison for 1 subject is presented in Figure 2.31 and the authors pointed out the apparent "smoothing" effect of the AR method. The hypopharynx maximum was the only comparison which was statistically significantly different ($p = 0.04$) between the 2 methods. The AR measurement underestimated the hypopharynx maximum by 35% in comparison with the MRI measurement. However, the minimum values of the OPJ and the GL were similar.

Table 2.5: CSAs (cm^2) of the OPJ, the hypopharynx (HP) maximum and the GL, and the pharynx volume (cm^3) measured by MRI and by AR. Data from 10 subjects presented as mean ± SD (Marshall et al., 1993).

	OPJ, cm^2	**HP, cm^2**	**GL, cm^2**	**Pharynx, cm^3**
MRI	0.9 ± 0.5	2.6 ± 0.9	1.1 ± 6.0	14.9 ± 6.0
AR	0.3	1.6 ± 0.6	1.3 ± 0.3	13.2 ± 2.7
MRI - AR	-0.04 ± 0.42	0.92 ± 1.18	-0.22 ± 0.36	1.6 ± 5.8
p-values	0.77	0.04	0.09	0.40

One reason for the differences could be found in the measurement techniques (Table 2.5). The MRI measurement took several minutes to perform with data averaged over several breaths and might have included swallowing, whereas the AR measurement was essentially instantaneous (Marshall et al., 1993).

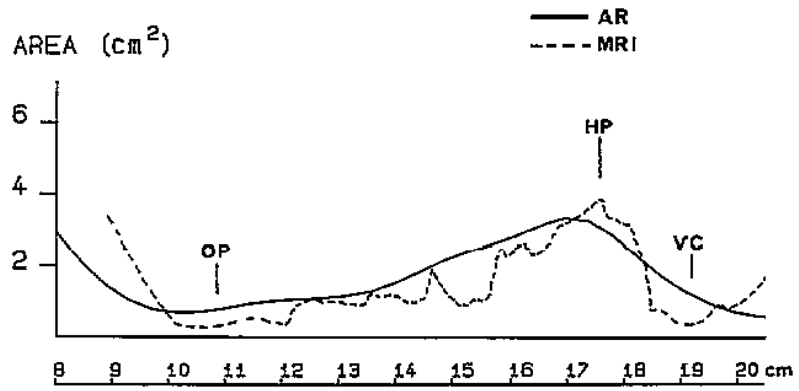


Figure 2.31: A comparison of AR and MRI estimates of an airway area in a supine, gently breathing subject. The estimates were aligned at the vocal cords and the positions of the landmarks: the oropharynx (OP), the hypopharynx (HP) and the vocal cords (VC). These were determined from a midline sagittal image (Marshall et al., 1993).

2.7.4 Early AR method - reproducibility

The reproducibility of the AR method in humans was evaluated by Brooks et al (1984) in 10 healthy adult male subjects using the Fredberg et al (1980) equipment with a shorter (2 m) wave tube. The subjects were seated and the measurements performed during tidal breathing near FRC. The AR measurements were repeated during 3 days to assess reproducibility which was calculated as a CV expressed as a percentage (100 × SD / mean) for the average area of the tracheal segment 6-10 cm beyond the GL. The within-run tracheal variability (90 measurements; ± SD) for all subjects was 10 ± 4%, and the day-to-day variability (270 measurements) was 9 ± 4%.

In 1989 Brooks et al (1989) published a second study on the reproducibility of the AR in measurements of the upper airway area, using the same kind of technique as in their study published in 1984 (Figure 2.32). The main difference in the technique was related to the wave tube inner diameter of 1.95 cm^2, instead of 1.57 cm^2 as in the first study (1984).

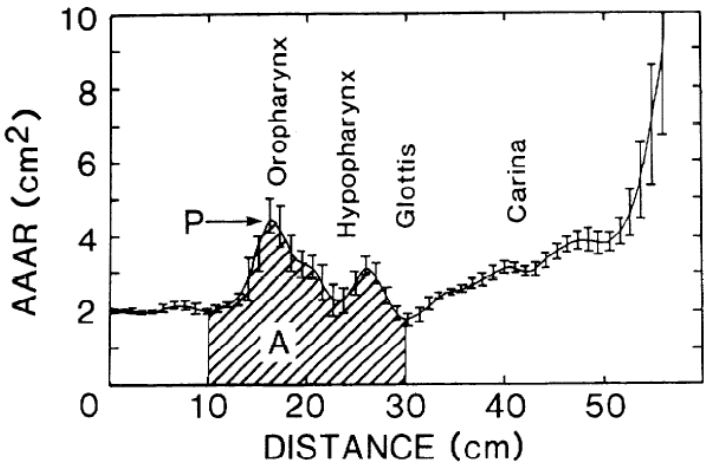


Figure 2.32: An echogram acquired during tidal breathing from a single subject with the airway CSA measured by AR plotted *versus* distance from the mouth. The landmarks (oropharynx, hypopharynx, GL and carina) were identified by X-ray. Peak pharyngeal area (P), and mean pharyngeal area (A; end of mouthpiece to GL) are highlighted (Brooks et al., 1989).

Ninety AR measurements were performed in 10 healthy adult subjects, and the mean echograms were analysed in terms of peak pharyngeal area (P), mean pharyngeal area (A) (end of mouthpiece to GL), and pharyngeal volume (V) (A × distance mouthpiece to GL; Figure 2.30). The within-run variability (CV = SD/mean) for all subjects was for P, A and V: 0.11, 0.08 and 0.08, and the day-to-day variability for P, A and V: 0.08, 0.08 and 0.12.

Marshall et al (1993) tested a new AR equipment in 10 subjects to measure reproducibility and focused on the upper airway area from mouth to hypopharynx. The subjects were breathing room air during the measurements. An example of the typical within-run reproducibility for 1 subject is show in Figure 2.33. The CV of the measurements was ~10% and close to the CV reported by Brooks et al (1984).

The within-run CV was 10% (range 2-25%) for the 10 subjects. The day-to-day CV in 5 subjects over 21 days (± SD) was 13 ± 3% at the oropharynx minimum and 11 ± 3% at the hypopharynx maximum and GL mimimum, close to the 9 ± 4% reported by Brooks et al (1984).

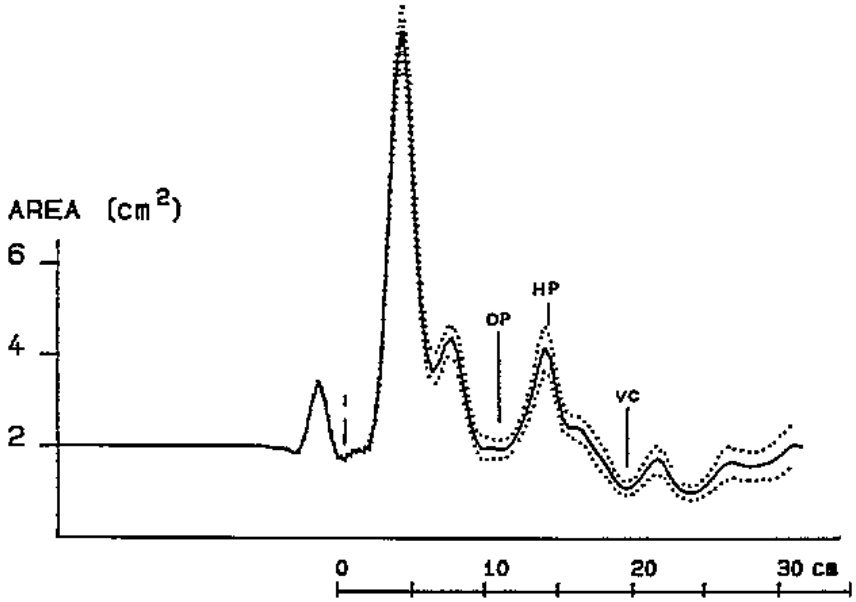


Figure 2.33: Example of the within-run reproducibility of the AR technique developed by Marshall et al (1993). The mean (solid line) and ±1 SD limits (dotted line) of 10 consecutive measurements are shown. The approximate position of the incisors (I), the oropharynx (OP), the hypopharynx (HP) and the vocal cords (VC) are highlighted (Marshall et al., 1993).

2.7.5 Sources of artefacts of the AR method

A number of authors have highlighted the risk of artefacts when performing AR measurements of the upper airways (Molfino et al., 1990; Marshall et al., 1993; Kamal, 2004c).

2.7.5.1 Position of subject and wavetube during measurement

Posture control of the subject during measurements with an acoustic pharyngometer is important as the pharyngeal volume may be affected by the position of the subject's head and neck (Rubinstein et al., 1987; Eckmann et al., 1996; Walsh et al., 2008; Kamal, 2004c). In order to avoid movement of the head during the measurement, the subject should be asked to fix the gaze at a spot at the opposite wall on the same level. Monitoring of the subject's posture is important during the measurements, and the position and height of the wavetube has to be adjusted in relation to the

subject so that the wavetube can be kept in a fixed position during the measurement (Kamal, 2004c).

2.7.5.2 Physiological variations of the pharynx CSA during breathing

As breathing related motion of soft tissue in the pharynx and related bony structures has been shown to create dimensional changes in the pharynx (check 2.2.8), the subject's breathing during the acoustic pharyngometer measurement should be observed (Kamal, 2004c). During tidal breathing the FRC is the volume of air in the lungs at the end of the expiration. In order to ensure reproducible acoustic pharyngometer measurements of the upper airway area at its most narrow point, acoustic pharyngometer measurements should be made at FRC (Kamal, 2004c; Viviano, 2004).

2.7.5.3 The impact of an open velum

In one of the early publications regarding the AR measurements by Fredberg et al (1980), the authors commented upon the necessity to ensure that the "nasopharyngeal aperture (velum)" was closed during data acquisition so that the airway could be "modeled as a one-dimensional duct of varying area". Brooks et al (1984) did also include a comment regarding the open velum (soft palate not closed) in their paper. Molfino et al (1990) presented perhaps the first published example of an echogram measured with an open (A) and a closed (B) velum in their letter to the Editor of the American Review of Respiratory Diseases (Figure 2.34).

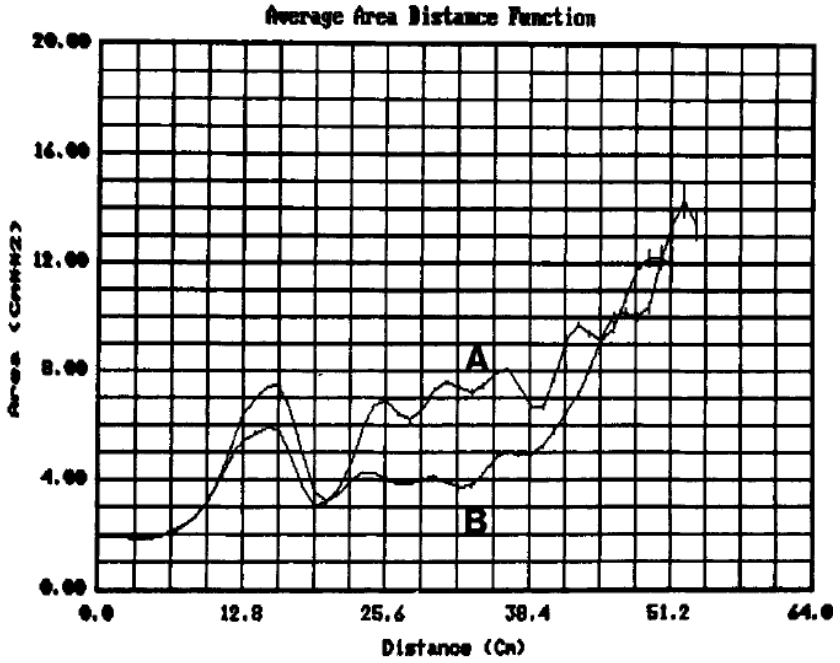


Figure 2.34: The figure shows an echogram ("Average Area Distance Function"; area in cm^2 plotted *versus* distance in cm) measured at FRC in a subject wearing a nose clip (A), and 10 s after removal of the nose clip (B) (Molfino et al., 1990).

Hoffstein et al (1991) discussed the open velum in terms of "branching". If the velum was open there were 2 parallel pathways for the sound pulse, mouth to subglottic airways and mouth to nasal airways. The AR algorithm would interpret the mouth to nasal airways area as an increase in the subglottic airway area, and the echogram would be meaningless. Hoffstein et al (1991) stated that the artefact was "easily recognized in practice and measures may be taken to prevent the opening of the velum (e.g. removing the noseclips or instructing the subject to breathe through the mouth)". Marshall et al (1993) included 2 figures of interest relating to the open velum artefact, Figures 2.35 and 2.36, in their 1993 paper. In Figure 2.35 the upper airway anatomy is shown with the soft palate (velum) in 2 positions, when breathing through the mouth (the velum closes off the nasopharynx from the pharyngeal area) and when breathing through the nose (the velum is open between the nasopharynx and the pharyngeal area).

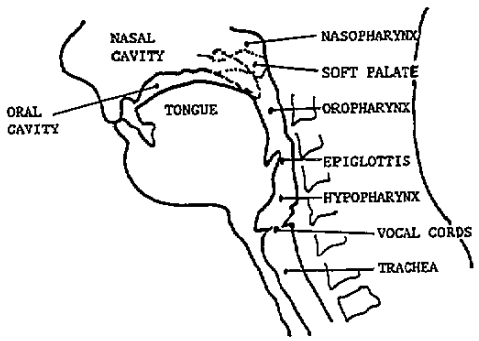


Figure 2.35: The figure highlights the soft palate (velum) position when breathing through the mouth (dotted curve, the passage between nasopharynx and the pharyngeal area closed) and when breathing through the nose (broken curve, the passage between nasopharynx and the pharyngeal area open (Marshall et al., 1993).

Marshall et al (1993) included an echogram showing the effect of the open velum in comparison with a closed velum, Figure 2.36. The measurement with an open velum (nasal) created a curve with a larger CSA in comparison with the curve acquired with a closed velum (oral).

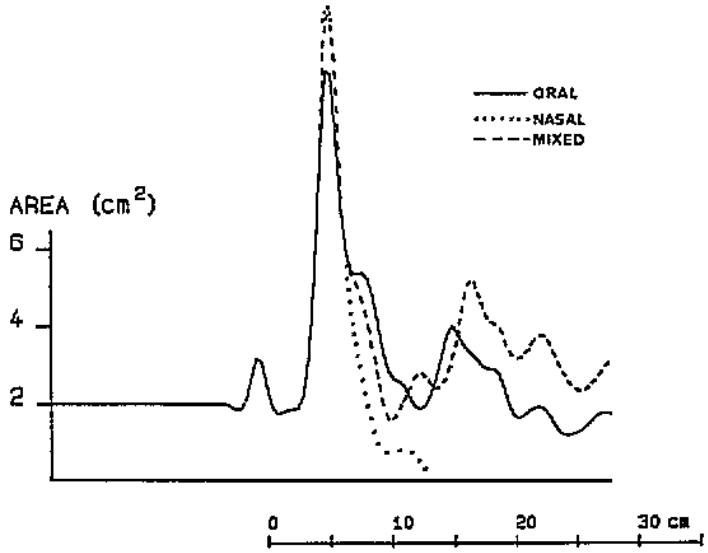


Figure 2.36: The figure shows the effect of the soft palate (velum) position (open or closed) on the echogram. The open velum (nasal) created an echogram with a larger CSA in comparison with the curve acquired with a closed velum (oral). The main differences in the 2 echograms occur from the OPJ at ~10 cm (x-axis) onwards to the right. The broken curve shows the effect of a mixed oral/nasal (closed velum/open velum) breathing (Marshall et al., 1993).

In order to avoid an open velum during the acoustic pharyngometer measurement, the subject could be asked to silently think of making an "oooh" sound. This should be helpful in keeping the tongue relaxed on the floor of the mouth (Kamal, 2004c).

2.7.6 The Eccovision Acoustic Reflection Pharyngometer (ARP)

The first FDA approved ARP – used in the studies presented in this thesis – was the Eccovision ARP (Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com). The Eccovision ARP is composed of a mouthpiece, wavetube, speaker, microphone, filter strips, acoustic device, C.P.U., printer, monitor, PC, board, software and source code (www.sleepgroupsolutions.com). A recording with the Eccovision ARP creates a pharyngogram and the different upper airway anatomic structures on the pharyngogram is shown in Figure 2.37.

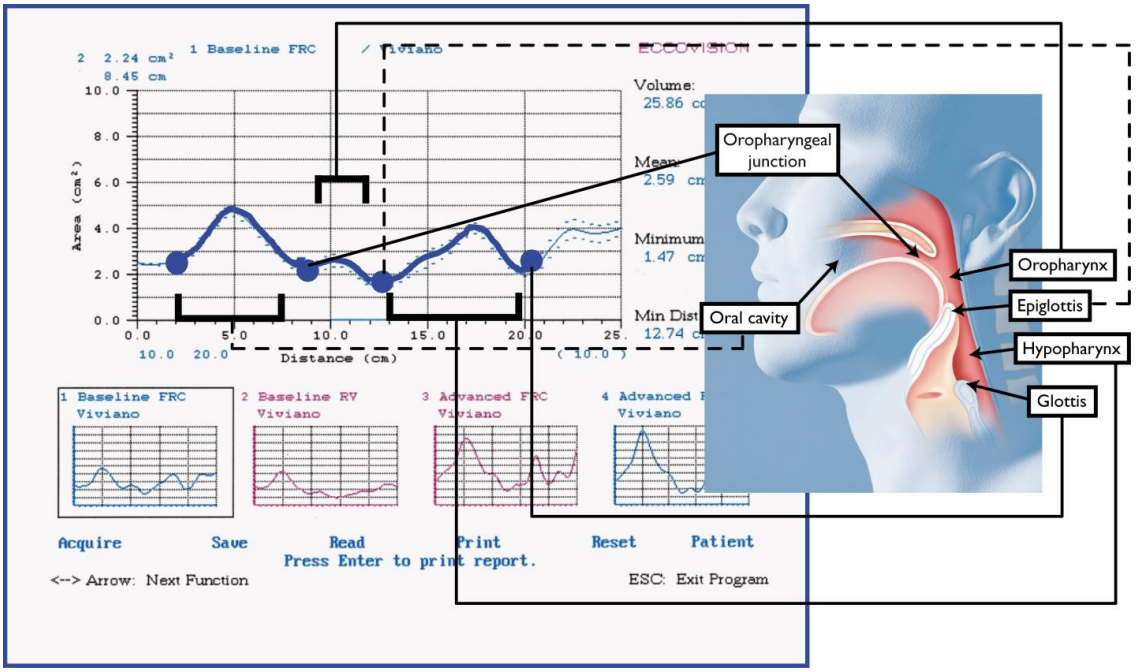


Figure 2.37: The figure shows a pharyngogram obtained from an Eccovision ARP measurement (y-axis shows CSA in cm^2, x-axis shows distance from mouth in cm). Along the pharyngogram different anatomic structures can be identified, and the CSA of the upper airways can be measured at several anatomical levels. The oral cavity is recorded as a peak from 0 to ~7.5 cm, the OPJ is located at the dip of the curve at ~9 cm followed by the oropharynx from ~9 to ~12 cm, the EG is recorded as the second dip at ~13 cm followed by the hypopharynx from ~13 to ~20 cm and the GL between ~20 to ~21 cm (Viviano, 2004).

The Eccovision ARP has been used in a number of clinical studies in healthy subjects (Kamal, 2001; Kamal, 2002; Kamal, 2004b; Monahan et al., 2005; Shiota et al., 2007), and in adults (Jung et al., 2004; Kamal, 2004a; Gelardi et al., 2007; DeYoung et al., 2013) and children diagnosed with OSA for measurements of upper airway CSAs and volumes (Monahan et al., 2005; Kumar et al., 2015).

A number of clinical studies with the Eccovision ARP have reported mean CSA values for the upper airways for healthy subjects of both genders. In one of the largest studies with the Eccovision ARP Kamal (2001) measured the upper airways of 350 healthy subjects, and showed a mean pharyngeal CSA of ~3.2 cm^2 (minimum 2.7 cm^2, maximum 3.8 cm^2) in males and of ~2.8 cm^2 (minimum 2.1 cm^2, maximum 3.4 cm^2) in females (Kamal, 2001). In the same study the mean GL CSA were in males 1.06 cm^2 (minimum 0.9 cm^2, maximum 1.2 cm^2), and in females 0.94 cm^2 (minimum 0.75 cm^2, maximum 1.09 cm^2) (Kamal, 2001).

The repeatability of pharyngeal CSA measurements was investigated by Kamal (2004b) in 20 healthy adult subjects (16 men) using the Eccovision ARP and following a developed SOP. The SOP highlighted 5 general pharyngometry test related points, as follows:

- Position of subject. The subject should be seated in a firm chair in order to keep the head in a neutral position and the wavetube in proper position.
- Subject considerations. The test is performed during normal quiet breathing. The subject is told to think silently of "oooh" in order to place the tongue in a relaxed position on the floor of the mouth and keepthe velum closed.
- Mouthpiece. The mouthpiece is made of rubber and is designed to be placed with the teeth against the flange. The subject is told to bite down on the protruding tabs and to place the lips over the flange to form an acoustic seal.
- Position of the wavetube. The wavetube should be kept horizontally parallel to the floor.
- Operator. Training and familiarity with the equipment is important. Accuracy of the test is improved by performing 4 tests on the same session in order to be able to calculate the CV.

The results of 3 separate measurements - consisting of several recorded pharyngograms - of the mean pharyngeal CSAs in the 20 subjects showed a good repeatability (Kamal, 2004b). The first

2 measurements were performed the same day and showed mean pharyngeal CSAs of 3.187 cm^2 (SD 0.249) and 3.239 cm^2 (SD 0.0790). The 3rd test was performed 7-10 days later and showed a pharyngeal mean CSA of 3.245 cm^2 (SD 0.0811). There was no statistically significant difference ($p = 0.440$) among the measurements of the pharyngeal mean CSAs made during the 3 sessions. Kamal (2004b) concluded that provided an SOP is adopted and maintained, repeatability of pharyngometry measurements could be achieved.

Kamal (2004c) discussed the need to standardize the use of the acoustic pharyngometry equipment and reproducibility. The argument was that this could be achieved by performing 3-4 pharyngometry recordings and a CV of 5-10% of these seems to have been accepted by most authors in the field (Kamal, 2004c). An understanding of the "true" reproducibility" of the pharyngometry recordings can be found in the *in vitro* study presented in Chapter 6. The four pharyngometer recordings per measurement were in the *in vitro* study design with a cast of a human airway not affected by any subject related movement.

Searches during October 2015 at www.pubmed.gov for "pharyngometry", "acoustic pharyngometry", for "acoustic pharyngometry and sleep apnoea" and for "acoustic pharyngometry and sleep apnea" provided numerous hits whereas searches for "acoustic pharyngometry and upper airway aerosol deposition", "acoustic pharyngometry and lung deposition of aerosol", "acoustic pharyngometry and upper airway deposition of aerosol" and "acoustic pharyngometry and aerosol inhalation" provided no hits. Thus, the use of the ARP methodology seems to have been mainly published in relation to studies of sleep apnoea, but not in studies of upper airway and lung deposition of inhaled aerosols. As the existing studies do not cover acoustic pharyngometry and lung deposition **and**/or breathing modes, a power calculation could not be made regarding the use of acoustic pharyngometry and lung deposition and/or breathing modes.

2.7.7 Analysis of the pharyngogram

As highlighted in Figure 2.37, different anatomic structures can be identified in the pharyngogram, and the CSA of the upper airway can be measured at several anatomical levels (and volumes between these) including the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx, and the GL. A number of authors have developed the analysis further for diagnostic purposes, and examples from five published articles have been included (Jung et al., 2004, Monahan et al., 2005, Gelardi et al., 2007; Patel et al., 2008; Friedman et al., 2014). In the study by Jung et al (2004), the authors defined 5 upper airway landmarks in the pharyngograms; the OPJ, the maximum pharyngeal area (Apmax), the GL, the mean pharyngeal area from OPJ to GL (Apmean), and the pharyngeal volume between the OPJ and the GL (Vp) (Figure 2.38).

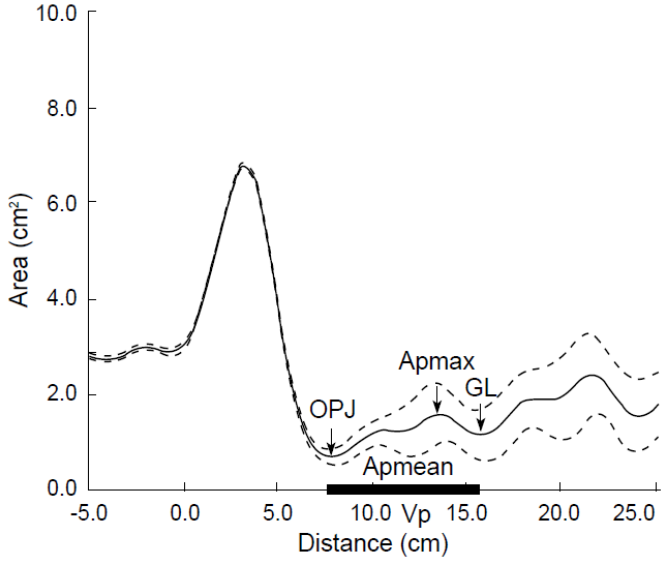


Figure 2.38: A representative pharyngogram by Jung et al. Five parameters are shown through arrows and a thick line: the OPJ, the Apmax (maximum pharyngeal area), the GL, the Apmean (mean pharyngeal area from OPJ to GL), and the Vp (pharyngeal volume between OPJ and GL) (Jung et al., 2004).

Monahan et al (2005) defined several features of the pharyngogram as additional descriptors of the oropharyngeal anatomy with the maximum CSA as the reference point (Figure 2.39).

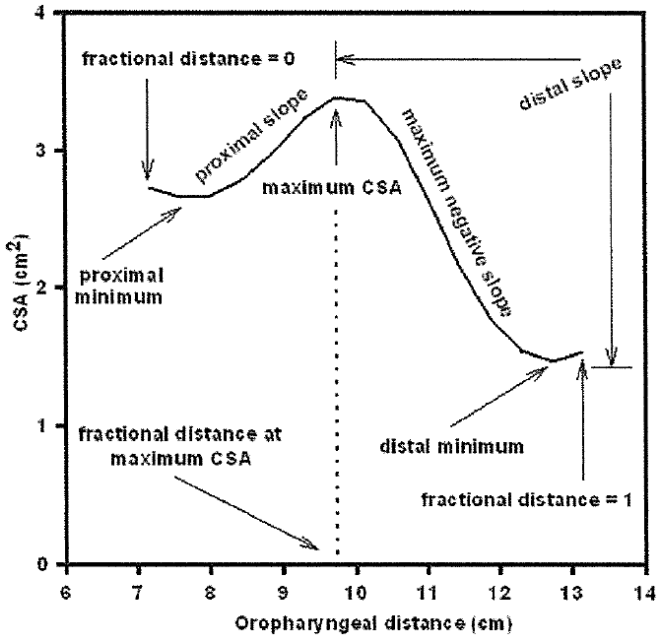


Figure 2.39: Sample pharyngogram by Monahan et al (2005). The proximal and distal minima referred to the values before and after the maximum CSA. The proximal and distal slopes defined the rates of change in CSAs around the maximum CSA. The fractional increase and decrease represented the relative amounts that the CSA changed between a respective minimum and the maximum CSA. The maximum negative slope and the fractional distance at which it occurred facilitated the detection of changes in calibre (Monahan et al., 2005).

In the study by Gelardi et al (2007) the authors defined several dimensions from each pharyngogram (Figure 2.40); wave I amplitude (changes in the volume of the tongue), extension and amplitude of the oropharyngeal segment (OP) and the area of the hypopharynx.

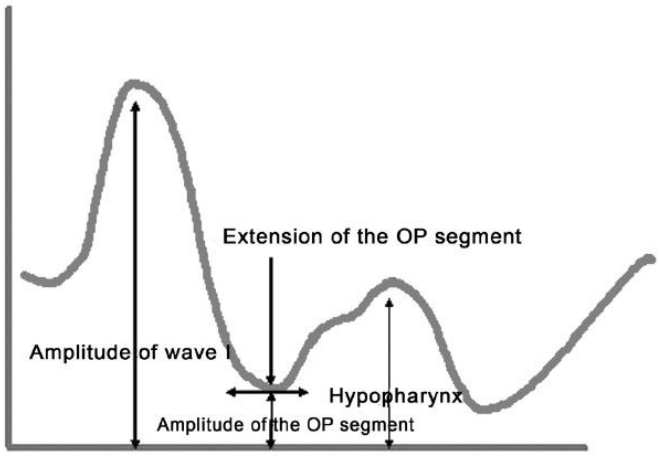


Figure 2.40: Main pharyngometric parameters (Gelardi et al., 2007).

Patel et al (2008) defined 8 dimensions from each pharyngogram (Figure 2.41); 5 CSAs (OPJ, EG, overall minimum CSA, maximum CSA, mean CSA), and 3 axial dimensions (oropharyngeal segment length, relative position of maximum CSA over segment length and segment volume).

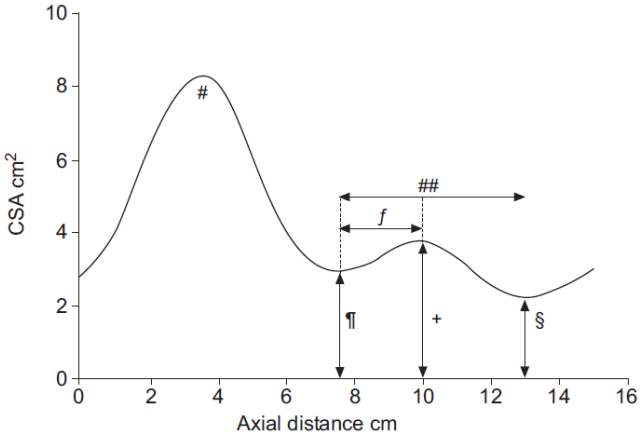


Figure 2.41: Schematic pharyngogram by Patel et al. The oral cavity is shown as (#). The mean CSA was obtained by averaging the OPJ (¶) and the EG (§), the volume was calculated from the mean CSA and the oropharyngeal length (##). The relative maximum location was defined as the ratio of maximum location (f) to oropharyngeal length. Maximum CSA (+) (Patel et al., 2008).

Friedman et al (2014) used the pharyngogram to identify the anatomical locations (OPJ, EG, GL) of the maximal collapse (Figure 2.42), which were classified into a retropalatal, a retroglossal or a retroepiglottic obstruction.

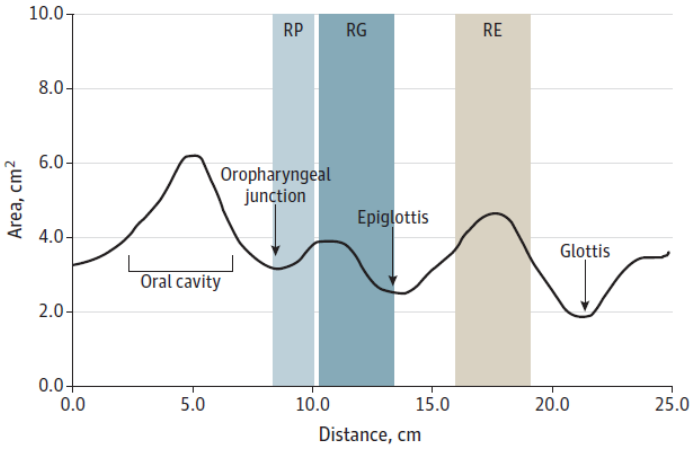


Figure 2.42: A normal pharyngogram by Friedman et al highlighting the OPJ, the EG and the GL. The authors used these landmarks to measure the CSAs of the retropalatal (RP), the retroglossal (RG), and the retroepiglottic (RE) anatomical regions (Friedman et al., 2014).

Examples of the 3 anatomical regions in the pharyngograms based on the Friedman et al paper (2014) have been included, as follows:

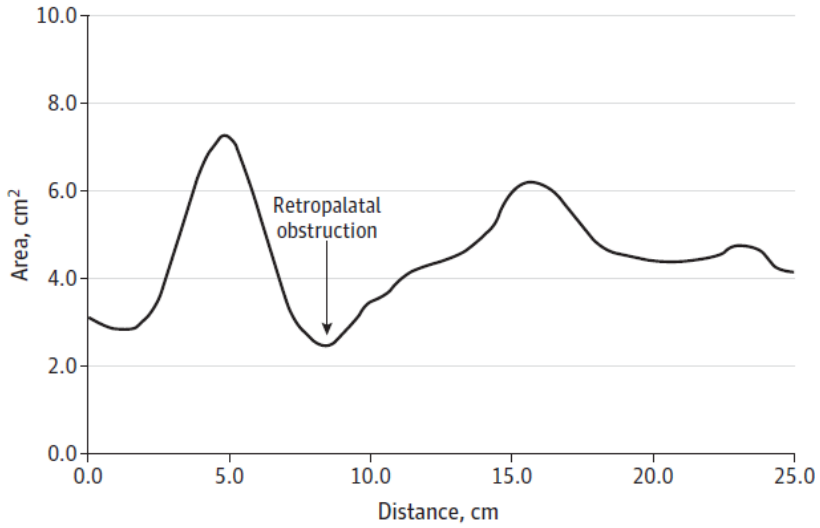


Figure 2.43: The pharyngogram shows the minimal CSA ~8-10 cm from the mouth indicating a retropalatal obstruction (Friedman et al., 2014).

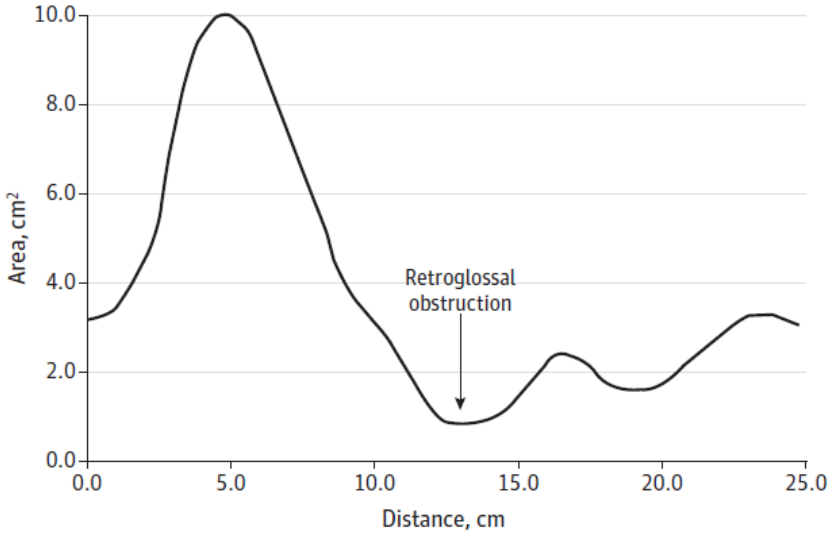


Figure 2.44: The pharyngogram shows the minimal CSA ~12-14 cm from the mouth indicating a retroglossal obstruction (Friedman et al., 2014).

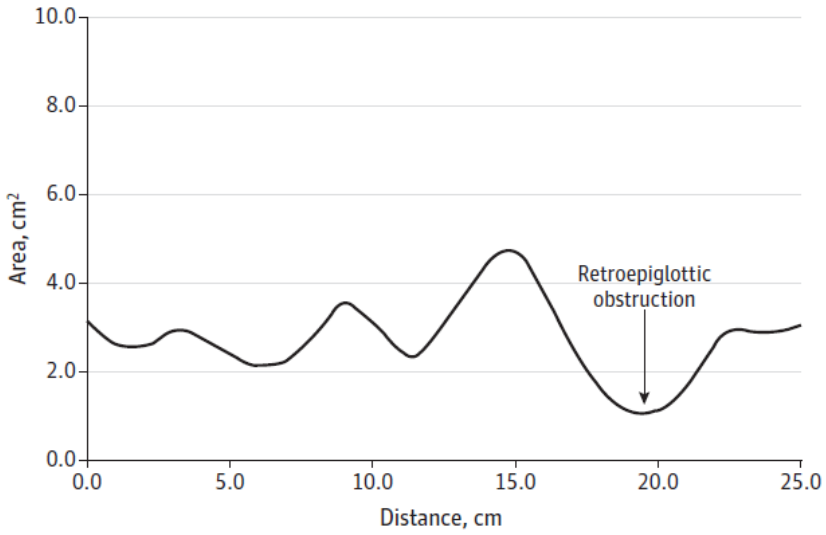


Figure 2.45: The pharyngogram shows the minimal CSA ~19-20 cm from the mouth indicating a retroepiglottic obstruction (Friedman et al., 2014).

2.8 Measurements when seated or supine

The impact of the seated position in comparison with the supine position on the size of the upper airways has been measured in healthy subjects using AR (Fouke et al., 1987; Jan et al., 1994), ARP (Eccovision; Jung et al., 2004), and CT/CBCT scans (Van Holsbeke et al., 2014a). All 4 studies showed that the CSAs of the upper airway were smaller in the supine than in the seated position.

Fouke et al (1987) showed that the CSA was smaller in the supine than in the upright position in 9 out of 10 subjects, and that the overall pharyngeal CSA was 23% smaller in the supine position. They also showed that the mechanism of change was independent of the change in FRC. Jan et al (1994) showed that the CSAs of the OPJ were larger in the seated *versus* the supine position (1.65 cm^2 *versus* 1.31 cm^2), which was in agreement with the results achieved by Jung et al (2004) for the CSAs of the OPJ (1.61 cm^2 *versus* 1.25 cm^2). Jung et al (2004) also reported mean CSA results for the GL which were 1.78 cm^2 (seated) *versus* 1.35 cm^2 (supine). Van Holsbeke et al (2014a) showed that for the upper airway the average CSA was ~10% and the minimal CSA 26.90% larger

in the seated position in comparison with the supine position. The largest difference of ~50% in favour of the seated position was shown for the region between the hard palate and the bottom of the uvula.

In the study by Walsh et al (2008) the authors compared the pharyngeal shape and size in the supine *versus* the lateral recumbent posture in subjects with and without OSA using anatomical optical coherent tomography. The airway CSAs, and anteroposterior and lateral diameters of the velopharynx and the oropharynx were measured. The ratio of anteroposterior to lateral diameter in the velopharynx was significantly less for the supine than for the lateral recumbent posture in both groups. CSA was smaller in subjects with OSA than in healthy subjects and was unaffected by posture.

2.9 Mandibular advancement

The right angle bend of the lumen at the back of the mouth, the variable position of the tongue during inhalation, the variable size and shape of the lumen in the pharynx and larynx, and the breathing pattern (tidal *versus* slow and deep) could – in addition to aerosol characteristics - promote upper airway deposition and restrict lung deposition (Kumazawa et al., 1997; Borgström et al., 2006; Newman et al., 2009; Nikander et al., 2010c; Scheuch et al., 2010; Diaz et al, 2012; van Velzen et al., 2015). The part of the pharynx located behind the tongue, and mainly between the OPJ and the EG, seems to present the narrowest part of the upper airways (Fajdiga, 2005). Mandibular advancement has in the past been practiced as a means to open up the upper airway behind the tongue during inhalation (Tissier, 1903). Tissier discussed the opposition of the EG to the penetration of atomised liquids into the larynx. He also described an interesting "general method" of practicing inhalation of atomised liquid, gas, or vapour, as follows:

"In the more general method, patients are first instructed to project the tongue as far as possible. It is then grasped with a cloth held in the fingers, preferably between the thumb and forefinger of

the patient's right hand, and pulled downward as far as possible. Lazarus recommends that the organ be rolled, as it were, around the lower lip. In this way is prevented the arching of the base of the tongue that often causes a narrowing of the ostium of the pharynx, while the lingual traction causes the epiglottis to be lifted up and well forward. The patient throws his head slightly forward, at the same time tilting it a trifle backward and upward, bringing his lower jaw as far as possible. These manoeuvres have for their object the greatest possible widening out of the angle between the axes of the buccal and laryngeal cavities. In this position the medication may be made to reach the vestibule of the larynx, even in the most difficult cases."

Mandibular advancement was already used during the late 1800s in cases of mandibular retrusion, and is still used as a means to prevent collapse up the upper airway during sleep in subjects with OSA through the use of oral appliances (Bailey, 2005; Fleetham et al., 2010).

2.9.1 Oral appliances for the treatment of OSA

In subjects with enough teeth in order to retain an oral appliance, the appliance is a simple and non-invasive device for treatment of snoring and mild to moderate OSA (Wee, 2012). There are presently a number of different oral appliances available for the treatment of OSA, which are used to increase the size - and prevent a collapse - of the upper airway by either advancing the mandible or the tongue (Ferguson et al., 2006; Fleetham et al., 2010; Wee, 2012).

There is a wide variety of oral appliances in terms of design, material, location of coupling mechanism, and amount of mandibular advancement and incisal opening (Ferguson et al., 1997b; Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Chan et al., 2007; Hoffstein, 2007; Fleetham et al., 2010; Dort et al., 2012; Wee, 2012). The terminology regarding the oral appliances is somewhat variable and some of the labels in English include: oral appliances, functional appliances, mandibular advancement devices, mandibular advancement splints, mandibular repositioning devices, anterior mandibular positioners, oral airway dilators and airway orthotic devices (Viviano, 2002a; Bailey, 2005; Ferguson et al., 2006; Horchover, 2007; Fleetham et al., 2010; Wee, 2012, Friedman et al., 2014). Two of the main proposed mechanisms of action during

sleep for these devices are increasing the size of the upper airway (Ryan et al., 1999), and decreasing the collapsibility of the upper airway (Ng et al., 2003; Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Hoffstein, 2007; Fleetham et al., 2010; Wee, 2012). In order to get an understanding of the degree of mandibular advancement and vertical incisal opening achieved with oral appliances, a number of studies in subjects with OSA have been listed in Table 2.6.

Table 2.6: The table presents a number of studies in which data on mandibular advancement and incisal opening achieved with oral appliances in subjects with OSA or snorers are reported (mm; means ± SD).

First author, year published	**Subjects (male)**	**Maximal mandibular protrusion***	**Vertical opening**
Barnes et al., 2004	114 (NA)	10.3 (0.3)	NA**
Barthlen et al., 2000	8 (7)	3-5	NA**
Bloch et al., 2000	24 (NA)	10.0 (0.4) = 75%*	5-10, 4-6
Chan et al., 2010a	35 (28)	5.8 (2.2) = 75%*	NA**
Chan et al., 2010b	69 (47)	6.5 (2.3) = 76.1% (12.8)*	NA**
Chan et al., 2011	35 (29)	4.8 (1.6) = 70.9% (20.5)*	NA**
Dort et al., 2012	41 (29)	6 and 8	NA**
Ferguson et al., 1996	27 (24)	7	NA**
Fleury et al., 2004	44 (36), 3 groups	12.0 (3.0), 11.0 (4.0), 12.0 (4.0)	NA**
Fransson et al., 2003	77 (63)	10.7 (2.3)	6.9 (2.1)
Gale et al., 2000	32 (27)	5.7 (2.5)	NA**
Johnston et al., 2002	20 (16)	5.7 = 75%*	4
Kyung et al., 2005	14 (12)	7.1 (1.9)	7.7 (2.5)
Lazard et al., 2009	84 (64)	7.0 (1.5)	NA**
Lettieri et al., 2011	805 (698), 2 groups	NA	3-5
Marklund et al., 2004	630 (508)	4-6	At least 5
Mehta et al., 2001	24 (19)	7.5 (1.8) = 75%*	NA**
Sari et al., 2011	24 (NA)	9.4 (1.3)	5
Tsuiki et al., 2004	20 (20), 2 groups	10.5 (1.5), 10.5 (1.4)	NA**
Vroegop et al., 2012	40 (32)	7.2 (1.8)	6.8-20
Walker-Engström et al., 2002	95 (95)	9.7	2.0
Walker-Engström et al., 2003	86 (86), 2 groups	9.8 (0.6) 9.6 (0.6)	2.0

*If maximum advancement not reported the mean protrusion at a specific percentage has been included;
** NA, if no data on vertical bite opening reported.

The mandibular protrusion ranged from 3 to 12 mm, and the incisal opening ranged from 2 to 20 mm in the studies included in Table 2.6. In the Walker-Engström studies (2002; 2003) the mouth opening capacity was reported as 51.2 mm (2002), and 49.9 mm and 52.0 mm, respectively (2003). In order to achieve a degree of mandibular advancement which is tolerable or free of discomfort, a titration process is used as shown in Figure 2.46 (Wee, 2012). The subject is initially given an oral appliance set at 50-75% of the subject's maximal mandibular advancement, which is later changed with incremental steps of 0.5 to 1.0 mm every week based on the comfort or the discomfort with the appliance and the advancement. Assuming the therapeutically effective position for the treatment of OSA is at A, the use of the oral appliance should be tolerable as this is between the initial advancement and the maximum discomfort free advancement (Figure 2.44).

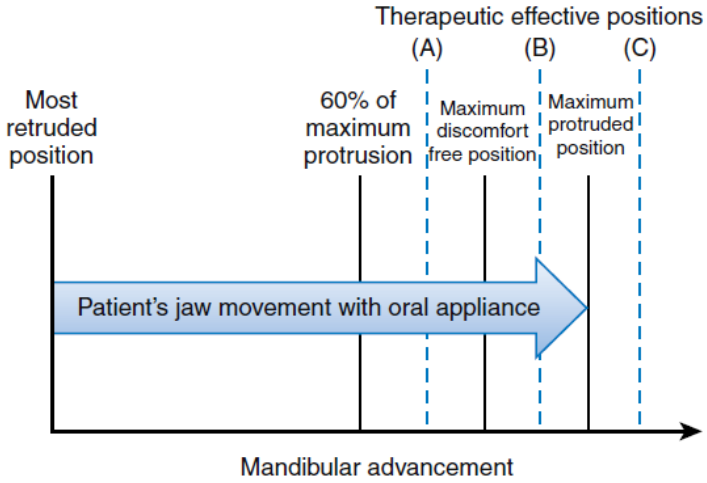


Figure 2.46: Diagram of mandibular advancement (protrusion) (Wee, 2012).

If the therapeutically effective position for the treatment of OSA is at B or C, the subject could not tolerate the maximal mandibular advancement, and the treatment would be either suboptimal (B) or ineffective (C) (Ferguson et al., 2006; Wee, 2012).

2.9.2 Examples of oral appliances

A number of different oral appliances available for the treatment of OSA are used to increase the size - and prevent a collapse - of the upper airway by either advancing the mandible or the tongue as shown in Figure 2.47 (Hoekema et al., 2004; Hoffstein, 2007; Fleetham et al., 2010; Vanderveken et al., 2010; Ahrens et al., 2011; Randerath et al., 2011; Sutherland et al., 2014).

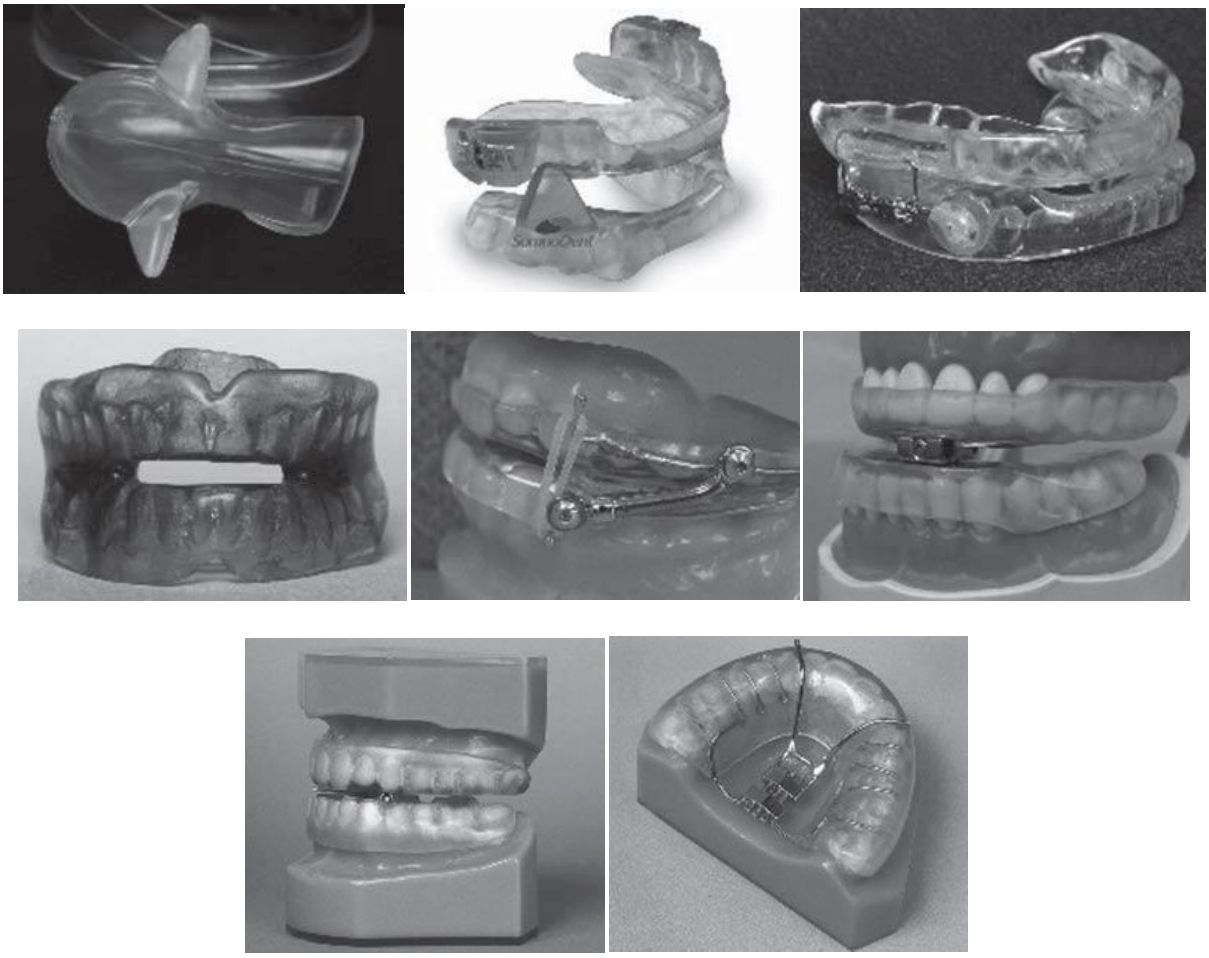


Figure 2.47: Eight different brands of oral appliances used for the treatment of OSA are shown (Fleetham et al., 2010). From top left to bottom right: Aveo-TSD1 (Innovative Health Technologies, Christchurch, New Zealand); SomnoDent1 MAS (SomnoMed, Denton, TX, USA); PM positioner1 (Great Lakes Orthodontics Ltd, Tonawanda, NY, USA); Monoblock appliance (courtesy of M. Marklund, Umeå University, Sweden); HerbstTM (Great Lakes Orthodontics Ltd); MDSA1 (Medical Dental Sleep Appliance; R.J. and V.K. Bird Pty Ltd, Melbourne, VIC, Australia); KlearwayTM (Great Lakes Orthodontics Ltd), lateral view; KlearwayTM, hinge view.

2.9.3 Impact of mandibular advancement on the pharyngeal dimensions

The efficacy of oral appliances has been tested in a number of clinical studies in subjects with OSA using the AHI as the main outcome variable (Barthlen et al., 2000, Johnston et al., 2002; Walker-Engström et al., 2002; Walker-Engström et al., 2003; Petri et al., 2008, Aarab et al., 2010; Lazard et al., 2009; Lettieri et al., 2011; Sari et al., 2011; Marklund et al., 2012; Friedman et al., 2014).

Airway-imaging studies have been performed in both healthy subjects and in subjects with OSA using oral appliances. The imaging techniques used included cephalometry, CT, MRI, and videoendoscopy (Fleetham et al., 2010). Mandibular and tongue advancement have been shown to increase the size of the upper airway, and to alter the shape of the upper airways particularly in the velopharynx in both healthy subjects and in subjects with OSA (Ferguson et al., 1997a; Johal et al., 1999).

The use of oral appliances have in other studies been shown to increase the anteroposterior diameter of the oropharynx and hypopharynx (Ng et al., 2003), to increase the total volume of the upper airway, and the CSAs of the retropalatal and retroglossal regions (Sam et al., 2006; Kyung et al., 2005), to increase the lateral dimensions of the velopharynx (Tsuiki et al., 2004; Zhao et al., 2008; Chan et al., 2010a; Chan et al., 2010b; Marklund et al., 2012), and to move the entire tongue forward (Brown et al., 2013). Mandibular advancement has also been shown to decrease respiratory resistance in subjects with OSA (Lorino et al., 2000).

There are, however, studies showing that in some subjects the use of an oral appliance might not create an enlargement of the upper airway (Gale et al., 2000; Mostafiz et al., 2011; Sutherland et al., 2011).

2.9.4 Impact of incisal opening on the pharyngeal dimensions

Whereas the impact of mandibular advancement on the upper airways has been the focus of a large number of studies, only a few have focused on the potential impact of the vertical incisal opening on the size of the upper airways mainly in subjects with OSA (Meurice et al., 1996; Pitsis et al., 2002; Ahrens et al., 2011; Nikolopoulou et al., 2011; Vroegop et al., 2012). In the study by Meurice et al (1996) the authors investigated the influence of a 15 mm bite opening on the collapsibility of the upper airways in 6 sleeping healthy subjects. Pressure-flow measurements of the subjects' upper airways showed that the bite opening increased the upper airway collapsibility. The study by Nikolopoulou et al (2011) in 18 subjects with OSA investigated the effect of an incisal opening of 6 mm using an oral appliance without mandibular advancement on the AHI. The results indicated that an increased incisal opening without mandibular advancement might be associated with aggravation of OSA in some subjects.

Pitsis et al (2002) investigated the impact of 2 incisal openings (4 mm and 14 mm) in 2 oral appliances in a randomised, cross-over study in 23 subjects with OSA using a reduction in AHI after 2 weeks of usage as a measure of response. Both incisal openings reduced AHI in a similar fashion, although 78% of the subjects preferred the smaller incisal opening. The authors concluded that the results suggested that the amount of incisal opening did not have a significant impact on treatment efficacy of the oral appliances.

Vroegop et al (2012) investigated the impact of the incisal opening on the pharyngeal collapsibility in 40 subjects with OSA using video-endoscopy during sleep endoscopy. During the sleep endoscopy the incisal opening was increased from a baseline mean of 6.8 (SD 1.0) mm, and a mean maximal comfortable advancement of 7.2 (1.8) mm, to an incisal opening of 20 mm by manual downwards movement of the subjects' mandibles (Figure 2.48).

The effects of the incisal opening on the CSA of the upper airway at the level of the tongue base was scored as either adverse (narrowing), positive (widening) or indifferent (no change in pharyngeal dimensions). Thirty-two subjects had an adverse effect of the incisal opening (top row of pictures in Figure 2.48), 1 had a positive effect (middle row of pictures in Figure 2.48), and 7 subjects had an indifferent effect (bottom row of pictures in Figure 2.48).

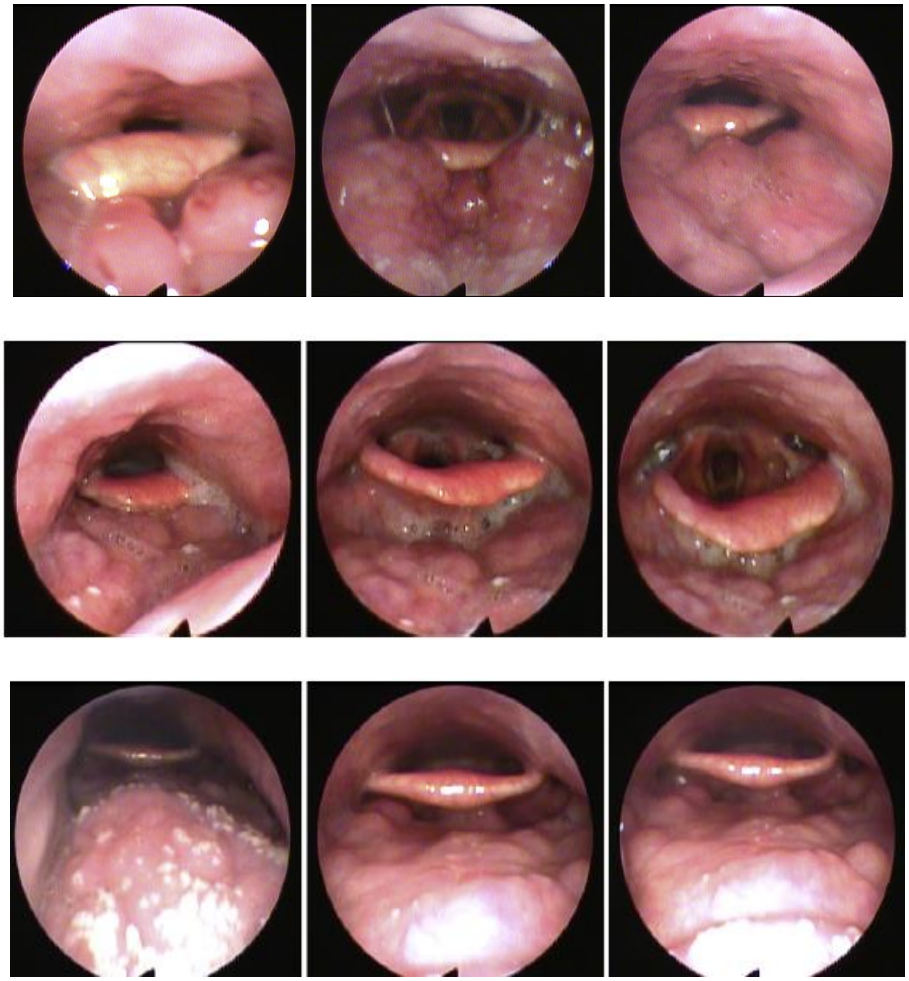


Figure 2.48: Effects of vertical incisal opening of the mouth on pharyngeal dimensions in subjects assessed video-endoscopically during sleep endoscopy. The left panel shows the baseline, the middle panel the impact of the maximal comfortable protrusion and the right panel the impact of the vertical opening. The top row of photos shows adverse effects of vertical incisal opening on pharyngeal dimensions, the middle row the positive effects and the bottom row the indifferent effects of vertical opening on pharyngeal dimensions (Vroegop et al., 2012).

2.9.5 Combination of incisal opening and mandibular advancement

In the studies reviewed in 2.9.2 the subjects were either diagnosed with OSA (Meurice et al., 1996; Pitsis et al., 2002; Vroegop et al., 2012) or were sleeping when the impact of the increase in incisal opening was registered (Nikolopoulou et al., 2011; Vroegop et al., 2012). In contrast the study by Gao et al (2004) examined in 14 non-apnoeic men adaptive changes in the CSAs of the upper airways following mandibular advancement and incisal opening. A custom made oral appliance was used to keep the mandible at 0%, 50%, 75% and 100% of maximal mandibular advancement and at 50%, 75% and 100% of maximum incisal opening at 75% mandibular advancement. The incisal openings were 4 mm (V_0), 9.8 ± 4.1 mm (V_{50}), 14.7 ± 4.1 mm (V_{75}) and 19.6 ± 4.1 mm (V_{100}). An MRI was used to examine differences in the CSAs of the upper airways in the 7 possible mandibular advancement and incisal opening positions with the study subjects in a supine position. The percent changes in the minimum and mean CSA of the whole upper airway, the velopharynx, the oropharynx and the hypopharynx were reported. The changes of the minimum CSAs of the whole upper airway ($p = 0.0004$), the velopharynx ($p = 0.0006$) and the oropharynx ($p = 0.0258$) were statistically significant during mandibular advancement. In contrast, the changes of the mean CSAs of the upper airway ($p = 0.0434$) and the velopharynx ($p = 0.0027$) were the only statistically significant changes during mandibular advancement. The relative changes in the minimum CSA for the whole upper airway, the velopharynx, the oropharynx and the hypopharynx are shown in Figure 2.49.

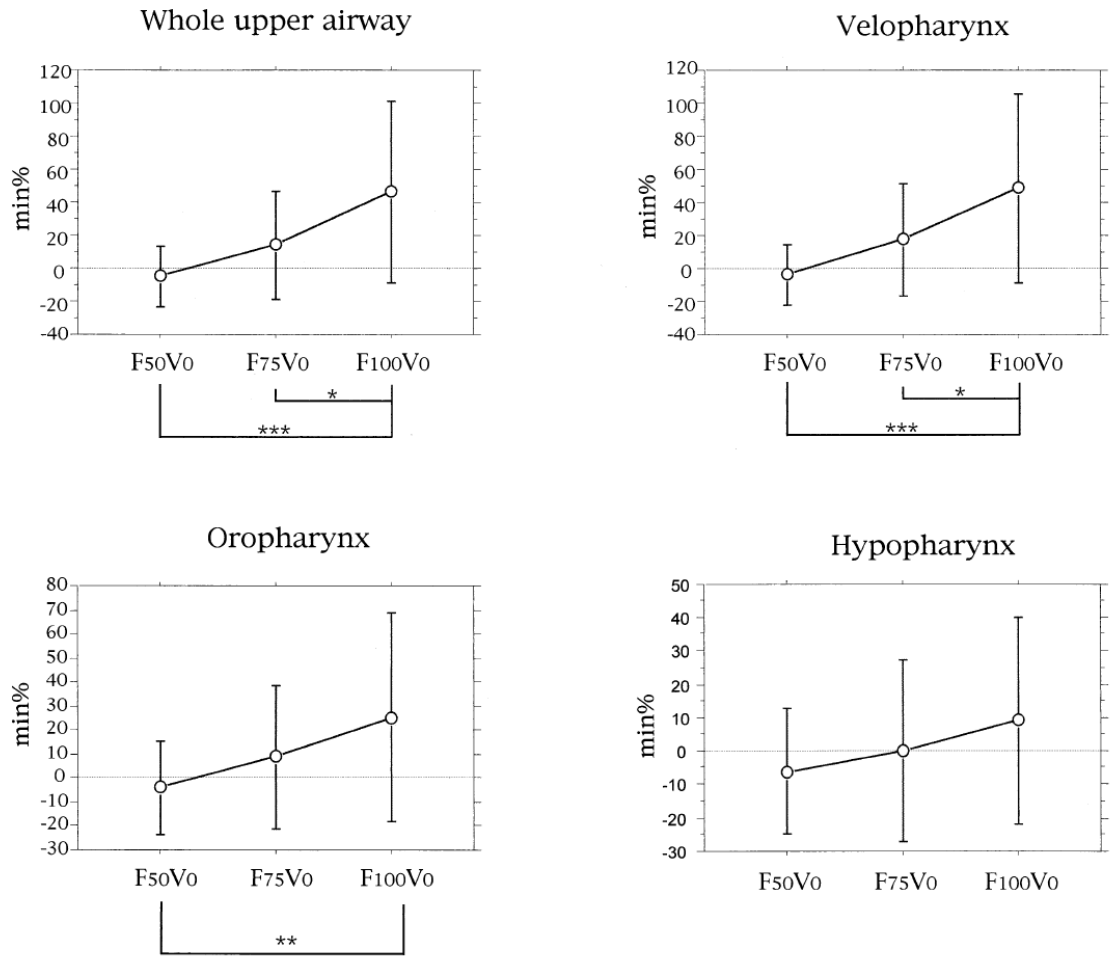


Figure 2.49: The relative changes in the minimum CSA (min%) for the whole upper airway, the velopharynx, the oropharynx, and hypopharynx following mandibular advancement at 50%, 75% and 100% (F_{50}, F_{75} and F_{100}) without increase in incisal opening (V_0) (Gao et al., 2004).

However, when the incisal openings were included in the measurements there were no statistically significant differences in either the minimum or the mean CSAs for any of the mandibular advancements due to the large inter-individual variability (Figure 2.50).

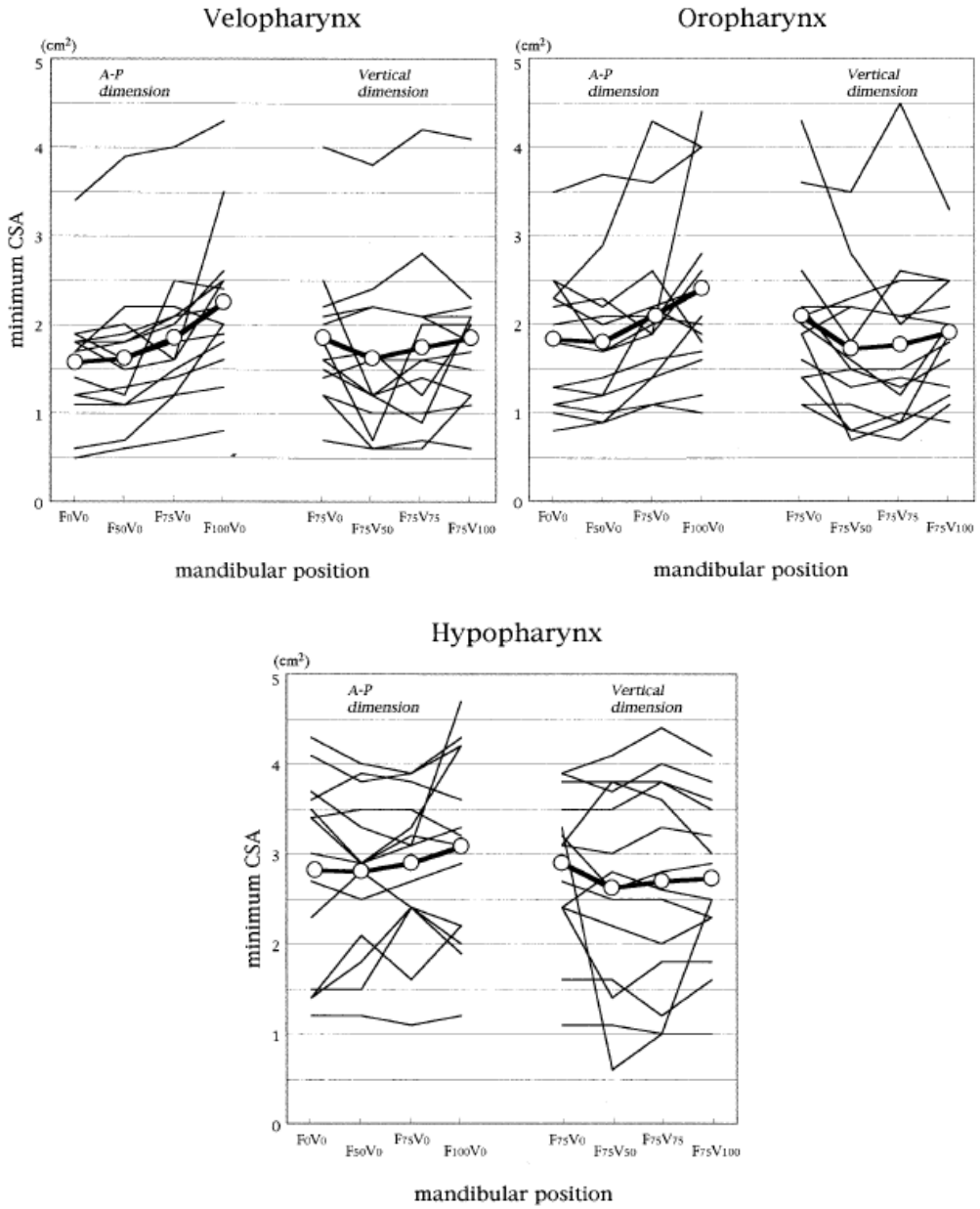


Figure 2.50: The actual minimum CSA of the velopharynx, the oropharynx and the hypopharynx per subject for the different mandibular positions. The "A-P dimensions" refer to the mandibular advancements ($F_{0\text{-}100}$), and the "Vertical dimensions" to the incisal openings ($V_{0\text{-}100}$). The mean values are shown by open circles and thick lines (Gao et al., 2004).

The minimum CSAs for the velopharynx, the oropharynx and the hypopharynx are shown in Figure 2.50. Some of the individual changes were actually negative when compared with baseline (F_0; V_0) further highlighting the large variability of the individual results especially in the

hypopharynx. As the impact of the incisal opening was limited in comparison with the mandibular advancement it would have been of interest to investigate the vertical incisal opening also at F_0 and F_{50} mandibular protrusions instead of only at F_{75}.

2.9.6 The distensibility of the upper airway

The human upper airway lacks a fixed rigid structural support and the position of structures like the soft palate, the tongue and the wall of the oropharynx determine the shape and size of the upper airway. The anterior wall of the oropharynx is primarily composed of the soft palate, the tongue and the lingual tonsils, and the posterior wall is bounded by the superior, middle and inferior constrictor muscles. The lateral pharyngeal walls are made up of muscles, lymphoid tissue and pharyngeal mucosa. Due to the structure of the upper airway, the collapsibility of it and the impact of dilator muscles, it does not have a fixed CSA (Ayappa et al., 2003).

The distensibility of the upper airways has been investigated in response to nasal CPAP, and the collapsibility of it in response to nasal CNAP (Shepard et al., 1990, Schwab et al., 1996). Shepard et al included 13 healthy subject and 17 subjects with moderately severe OSA in a study of the distensibility and the collapsibility of the upper airways. The airway pressures ranged from -5 cm H_2O to 0 cm H_2O and +5 cm H_2O and the upper airway size was measured through CT with the subjects awake and in a supine position. In the healthy subjects the CNAP had little impact and only decreased the CSAs in the most caudal segments of the hypopharynx (Figure 2.51). The impact of CNAP was similar in the subjects with OSA (Figure 2.52). The CPAP increased the CSAs of the upper airways at most levels (Figure 2.51; Figure 2.52) (Shepard et al., 1990).

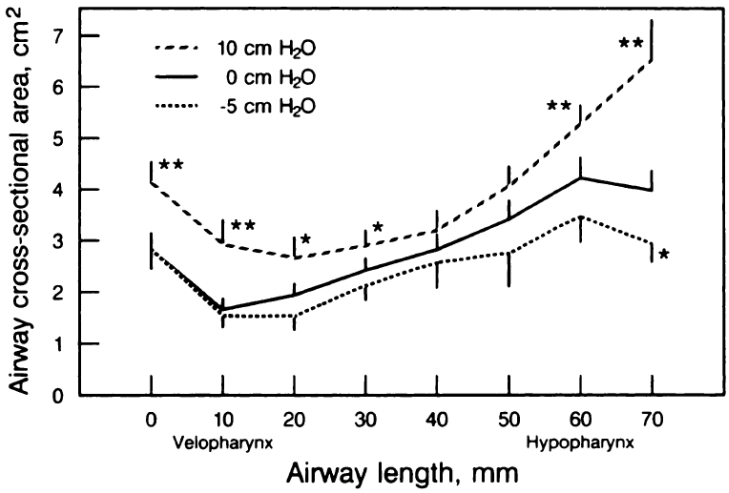


Figure 2.51: CSAs of upper airway regions from velopharynx to hypopharynx measured through CT under conditions of -5, 0 and +10 cm H_2O of CAP ventilation in healthy subjects. Data are presented as means ± SE, * = p<0.05, and ** = p>0.01) (Shepard et al., 1990).

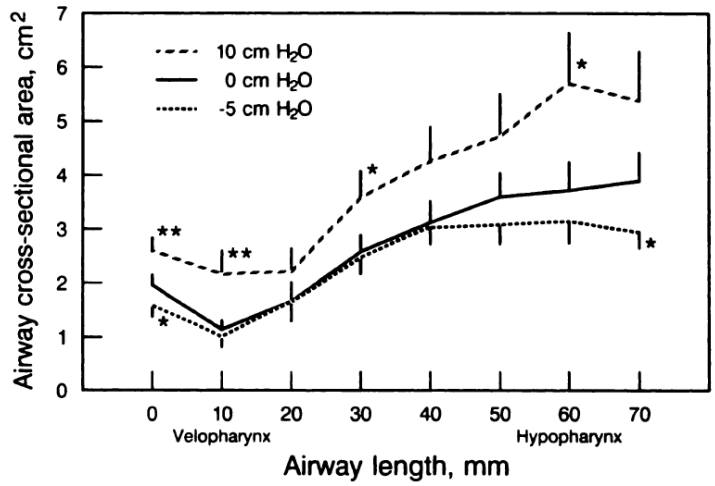


Figure 2.52: CSAs of upper airway regions from velopharynx to hypopharynx measured through CT under conditions of -5, 0 and +10 cm H_2O of CAP ventilation in subjects with OSA. Data are presented as means ± SE, * = p<0.05, and ** = p>0.01) (Shepard et al., 1990).

Schwab et al (1996) used MRI to investigate the effects of increasing levels of nasal CPAP (0, 5, 10, and 15 cm H_2O) on the upper airway size in 10 healthy adult subjects awake and in supine position (Figure 2.53).

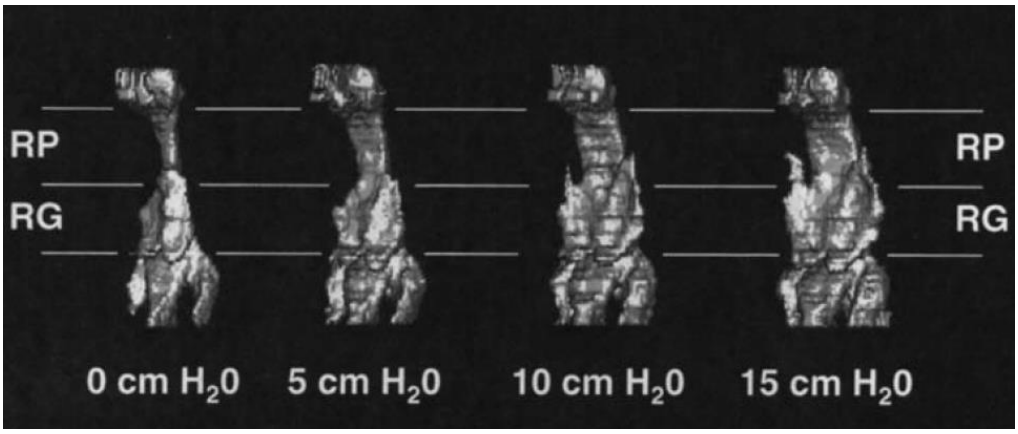


Figure 2.53: Three-dimensional surface renderings of the upper airway in one subject. The effects of different levels of CPAP pressure (0-15 cm H_2O) on the volume of the upper airways is shown with focus on the retropalatal and the retroglossal regions. The lateral widening is obvious (Schwab et al., 1996).

The results of the progressive increases in CPAP showed an increase in both volume and area within the retropalatal and retroglossal regions of the pharynx. The volume was almost doubled in both regions, whereas the changes in the CSAs were even more pronounced, especially in the retropalatal region (Table 2.7).

Table 2.7: Mean airway CSAs (cm^2 ± SD) in the retropalatal (RP) and retroglossal (RG) regions at three anatomic locations (midregion, minimal, and maximal) with increasing levels of nasal CPAP (0, 5, 10, and 15 cm H2O). (Adapted from Schwab et al., 1996).

Anatomic site	**0 cm H_2O**	**5 cm H_2O**	**10 cm H_2O**	**15 cm H_2O**
RP midregion	1.0 ± 0.5	1.3 ± 0.7	2.0 ± 0.8	2.8 ± 1.1
RP minimal	0.8 ± 0.5	1.0 ± 0.6	1.5 ± 0.8	2.1 ± 1.1
RP maximal	2.5 ± 0.6	2.6 ± 0.6	3.1 ± 0.8	3.6 ± 0.9
RG midregion	1.9 ± 0.7	1.8 ± 0.6	2.3 ± 0.7	2.8 ± 0.6
RG minimal	1.4 ± 0.7	1.4 ± 0.5	1.9 ± 0.5	2.2 ± 0.6
RG maximal	2.1 ± 0.7	2.1 ± 0.7	2.7 ± 0.9	3.1 ± 0.8

The lateral dimensional changes were greater than the anteroposterior changes, and the structural changes in the lateral upper airway soft tissue were significantly greater than the anteroposterior changes. Overall, the changes in the CSAs were similar to those found in the study by Shepard et

al (1990). The distance between the lateral parapharyngeal fat pads increased and the lateral pharyngeal wall thickness decreased. The authors concluded that the study provided further evidence that the lateral pharyngeal walls play an important role in the mediation of the size of the upper airway (Schwab et al., 1996). The distensibility of the upper airways in the studies by Shepard et al (1990), and Schwab et al (1996) proved to be considerable. The CSAs ranged from ~1 cm^2 to ~7 cm^2 and were close to some of the values on CSAs in studies in which oral appliances (Gao et al., 2004), and the ARP technique had been used (Busetto et al., 2009; Table 2.1).

2.10 The stepped mouthpieces

During the analysis of the study presented in Chapter 3, the question regarding the variability in the size of the subjects' upper airways and the impact of the subjects' anatomy of the upper airways on lung deposition was discussed. The possibility to enlarge the upper airway through mandibular advancement led to the development of a new stepped mouthpiece as a tool to achieve mandibular advancement when using an inhaler. The inhaler could be a pMDI (with spacer or VHC), a DPI or a nebuliser. The stepped mouthpiece without tongue depressor is shown in Figure 1.1 (patent US 2011/0240015 A1; published 6 October, 2011; PCT filed 23 November, 2009).

The abstract of the new stepped mouthpiece in the patent US 2011/0240015 A1 stated:

"The invention of the present application relates to an apparatus to aid in administering inhaled pharmaceutical aerosol to a patient. The apparatus is used in conjunction with an aerosol delivery device. The apparatus comprises steps on the top and bottom of the apparatus, which when used aid the patient causes mandibular advancement, and opening of the mouth, causing opening of patient's airway, resulting in improved aerosol lung deposition. The invention also relates to a method of using such apparatus in a combination with an aerosol delivery device or a system, and to the mouthpiece of said apparatus."

The stepped mouthpiece without a tongue depressor was tested in the proof-of-concept study presented in Chapter 4. It was designed in several sizes with front ends with 10 mm, 15 mm and 20 mm orifices (vertical diameters). These front orifices were also designed with a single

protrusion on the upper side for the upper incisors and 1 of 4 protrusions on the lower side at different distances (-3 mm, ±0 mm, +3 mm and +6 mm) in relation to the protrusion on the upper side for horizontal movement of the mandible. The horizontal offsets were -3 mm (lower jaw moved back from an incisal edge-to-edge position), ±0 (incisal edge-to-edge position), + 3 mm and +6 mm (mandible moved forward from an incisal edge-to-edge position). The stepped mouthpiece was 40 mm long.

The stepped mouthpiece with a tongue depressor was developed based on the experience from the proof-of-concept study in Chapter 4 and is shown in Figure 1.2 (patent US 2012/0240922 A1; published 27 September, 2012; PCT filed 9 November, 2010).

The abstract of the new stepped mouthpiece in the patent US 2012/0240922 A1 stated:

"An apparatus and method to aid in administering inhaled pharmaceutical aerosol to a patient is configured to maintain a tongue in proper position and offset the patient's upper and lower jaws during aerosol delivery. An adjustable member is provided adjacent a mouthpiece and at least partially surrounds and moves with respect to the body of the apparatus. The adjustable member has a step structure to impart a selected amount of mandibular advancement to a patient during aerosol delivery. A tongue depressor which may be integrally formed with the adjustable member configured to prevent a tongue from occluding a flow of aerosol is also provided."

The stepped mouthpiece with a tongue depressor was tested in the study presented in Chapter 5. The tongue depressor and the related part of the mouthpiece to be held in the mouth were 33 mm long, and the external horizontal and vertical diameters 34 mm and 18 mm, respectively. The 18 mm vertical external diameter was chosen partly based on the results of the previous proof-of-concept study in which the largest mouthpiece had an external vertical mouthpiece diameter of 20 mm, and partly as this is a common vertical size of jet nebuliser mouthpieces.

Chapter 3 Assessment of the upper airways in healthy subjects when using acoustic pharyngometry

3.1 Introduction

3.1.1 Deposition of aerosol in the upper airway

Deposition of aerosol in the upper airways is the major anatomical determinant for deposition of aerosol in the lungs (Svartengren et al., 1996), and the relative variability in lung deposition seems to be high for low lung deposition and low for high lung deposition (Borgström et al., 2006).

Deposition of aerosol in the upper airway can be the purpose of inhaled drug therapy as highlighted in the study by Kumazawa et al (1997), in which the authors aimed for a high upper airway deposition (pharynx and the larynx) in a scintigraphic study in 6 healthy subjects. An ultrasonic nebuliser was used for the aerosolisation of a saline solution labelled with ^{99m}Tc-DTPA. The subjects inhaled the aerosol during either deep and slow breathing with 12 breaths/minute, fast breathing with 36 breaths/minute, or fast breathing with 36 breaths/minute with intermittent vocalisation. The lung deposition decreased, and deposition in larynx increased, when the subjects changed from deep and slow breathing to fast breathing with vocalisation.

Slow and deep inhalation of aerosol has in a number of studies been shown to increase deposition of aerosol in the lungs (Svartengren et al., 1996; Brand et al., 2000; Nikander et al., 2010c; van Velzen et al., 2015). In the study by Nikander et al (2010c) performed in the summer of 2006, the lung and upper airway deposition of ^{99m}Tc-DTPA in a saline solution was investigated in 12 healthy adult subjects in a randomised, open-label, crossover study with the I-neb nebuliser in TBM and TIM breathing modes. All subjects were in a seated position during nebulisation, used nose clips during nebulisation, and inspiratory and expiratory flows through the mouthpiece were

monitored electronically during the study. The mean lung deposition of ^{99m}Tc-DTPA, expressed in percent of emitted dose ex-mouthpiece, was in TIM 73.29% (SD 16.3) and in TBM 62.82% (19.6), and the difference was statistically significant (Figure 3.1). The mean upper airway deposition in TIM was 26.49% (16.3) and in TBM 36.18% (19.7), and the difference was statistically significant (Figure 3.1).

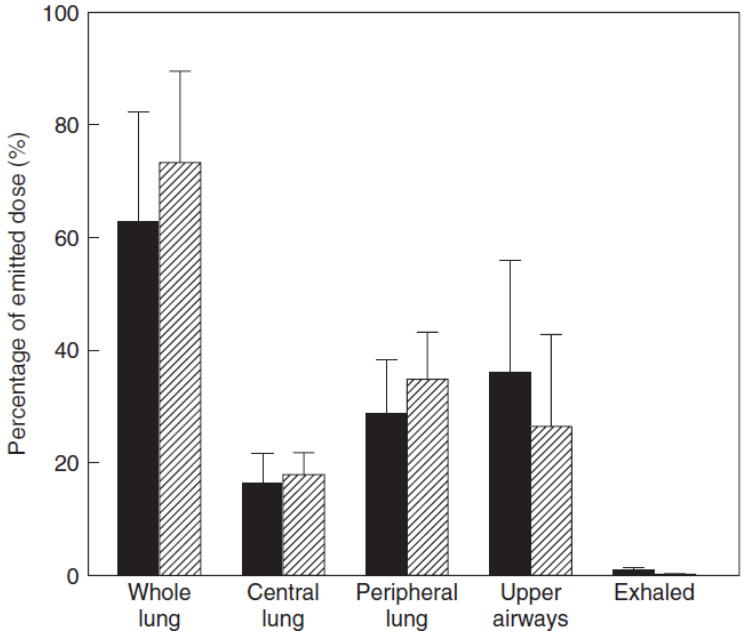


Figure 3.1: The lung, the central lung, the peripheral lung, and the upper airway deposition of ^{99m}Tc-DTPA plus the exhaled amount of ^{99m}Tc-DTPA is presented in percentage of the emitted dose ex-mouthpiece of the I-neb nebuliser. TBM in black and TIM in grey bars, respectively (Nikander et al., 2010c).

The variability in the upper airway deposition is difficult to explain considering the slow and deep breathing pattern and the relatively small droplet size of 4.6 µm. It would therefore be of interest to investigate the subjects' upper airway CSAs and volumes using acoustic pharyngometry and to measure the oral cavity and the size of the tongue using other techniques.

3.1.2 Acoustic pharyngometry

As described in Chapter 2, section 2.7 of this thesis.

The Eccovision Acoustic Pharyngometer (Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com) has been used in a large number of clinical studies of the upper airways (section 2.7.6-2.7.7). The pharyngogram obtained from the measurements with the acoustic pharyngometer were analysed in terms of the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx and the GL (Chapter 2, section 2.7.6 and Figure 2.35).

3.1.3 The oral cavity

As acoustic pharyngometry only measures the CSAs and volumes of the upper airways, other tools are required for more detailed measurements of for example displaced mandibles, highly arched palates, and disproportionately large amounts of oral soft tissue (i.e., an oversized tongue and/or soft palate). These tools cover the assessment of the pharyngeal space (Tsai et al., 2003), the Mallampati scoring technique for assessment of the tongue size (Berkow, 2004), the measurement of the cricomental space (Tsai et al., 2003), and tongue scalloping (Weiss et al., 2005).

A number of other variables such as palatal height, maxillary inter-molar distance, mandibular inter-molar distance, incisor overjet, and tongue length might affect deposition of aerosol in the oral cavity and in the upper airway. These variables could be measured with a prototype Oral Mez device (Philips Respironics, PA, USA).

3.1.3.1 Assessment of the pharyngeal space

Tsai et al (2003) described a grading system of the pharyngeal space which was based on a four-point ordinal scale (Tsai et al., 2003; Figure 3.2). The grading system created 4 pharyngeal grades from left to right in Figure 3.3 as follows:

- Class I - the palatopharyngeal arch intersects at the edge of the tongue.
- Class II - the palatopharyngeal arch intersects at 25% or more of the tongue diameter.
- Class III - the palatopharyngeal arch intersects at 50% or more of the tongue diameter.
- Class IV - the palatopharyngeal arch intersects at 75% or more of the tongue diameter.

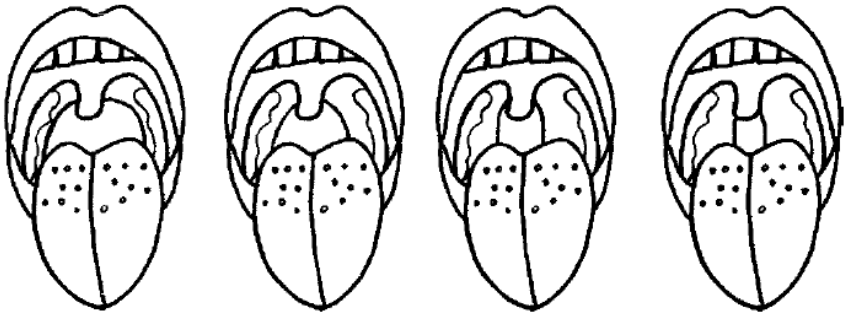


Figure 3.2: The pharyngeal grading system by Tsai et al (2003). The grading system created 4 pharyngeal grades, Class I-IV, from left to right.

3.1.3.2 Mallampati scoring technique for assessment of tongue size

Mallampati initially described his classification of airway assessment in 1985 (Mallampati, 1985) and hypothesized that a large tongue would cause difficulty in exposing the larynx leading to difficult laryngoscopy. Since a large tongue also obscures the view of the uvula and tonsillar pillars, 3 classes were created which were shown to be correlated with the degree of difficulties experienced at laryngoscopy. With the subject sitting up and with maximal protrusion of the tongue, visibility of the faucial pillars, soft palate and uvula were noted. The classification was as follows:

- Class I - described full visualization of all three structures.
- Class II - allowed visualization of only the faucial pillars and soft palate.
- Class III - only the soft palate was visible.

Samsoon et al (1987) modified the original classification to add a 4th class in which not even the soft palate was visible (Figure 3.3).

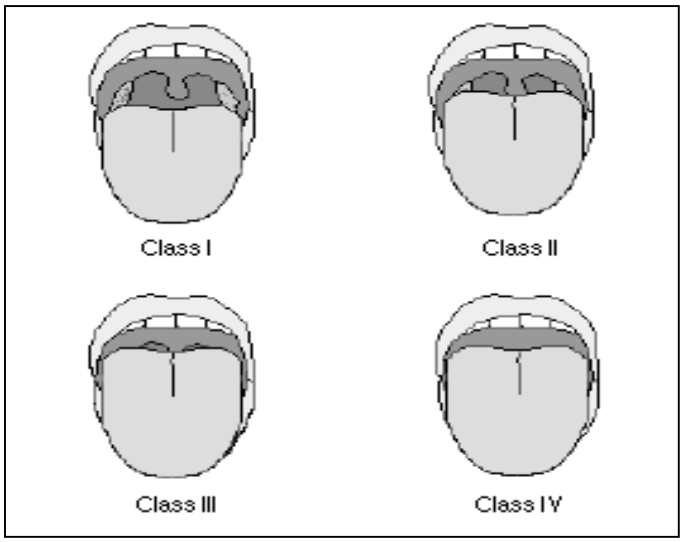


Figure 3.3: The modified Mallampati scoring system (Lam et al., 2005).

3.1.3.3 Measurement of the cricomental space

The cricomental space can be determined using a thin ruler to connect the cricoid cartilage to the inner mentum with the head in a neutral position. The cricomental line should be bisected, and the perpendicular distance to the skin of the neck should be measured (Figure 3.4). The use of a thin ruler (1 mm or less) has been considered essential because thicker devices might influence measurement (Tsai et al., 2003; Persaud, 2010).

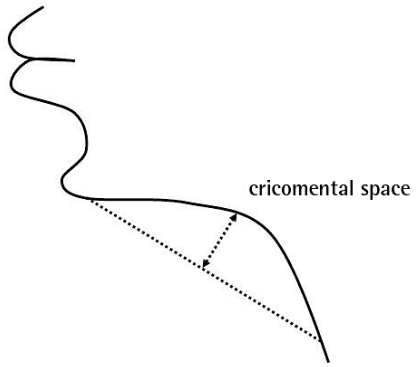


Figure 3.4: Assessment of the cricomental space, which is defined as the distance between the neck and the bisection of a line from the chin to the cricoid membrane, when the head is in a neutral position (Persaud, 2010).

3.1.3.4 Tongue scalloping

Tongue scalloping can be measured using the grades (0-3) published by Weiss et al (2005) as follows (Figure 3.5):

- 0 - complete absence of scalloping.
- 1 - scalloping evident but not pronounced.
- 2 - scalloping pronounced but resolved with tongue protrusion.
- 3 - scalloping pronounced and unresolved with tongue protrusion.

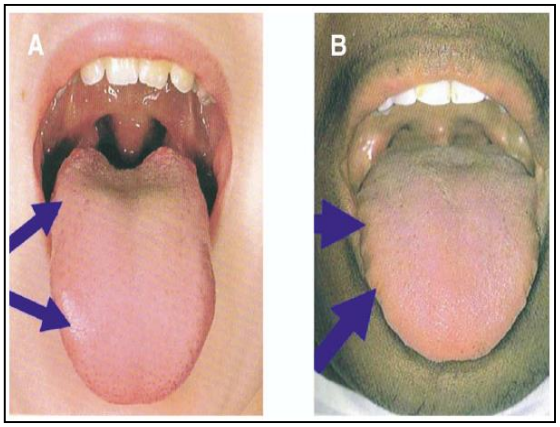


Figure 3.5: The lateral glossal margin in a normal subject is shown in (A), and the same area in a subject with grade III tongue scalloping evident during tongue protrusion in (B) (Weiss et al., 2005).

3.1.3.5 The Oral Mez

The Oral Mez device (Figures 3.6 and 3.7) was a prototype device developed by Philips Respironics (PA, USA) for measurements of the palatal height, maxillary inter-molar distance, mandibular inter-molar distance, incisor overjet, and tongue length in the oral cavity. There are no published validation data on the Oral Mez device and it has not been commercialised. It has been described in the abstract of the US patent US 7632238 B1 (published 2009), as follows:

"A device for taking measurements associated with an oral cavity of an individual. The device comprises a body and measuring indicia formed on the body which can be used to measure at least one parameter of the individual's mouth. An upper surface and a lower surface of the body are formed from an markable material capable of being marked by application of force from the individual's teeth. The body may also comprise a pallet measuring member constructed and

arranged to extend operably from the body. The pallet measuring member has indicia formed thereon to enable measurement of the height of the individual's hard pallet. The body may also be provided with indicia to measure the length of the individual's tongue."

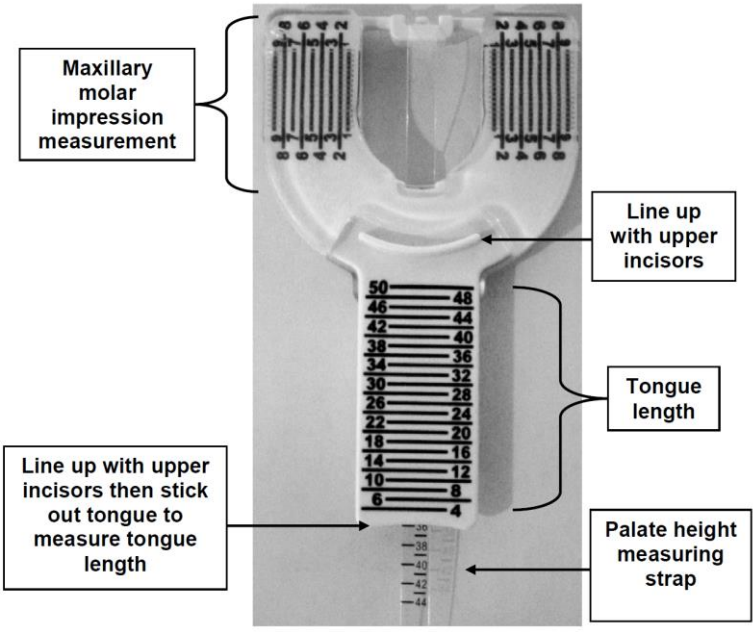


Figure 3.6: Top view of the Oral Mez device (Philips Respironics, PA, USA).

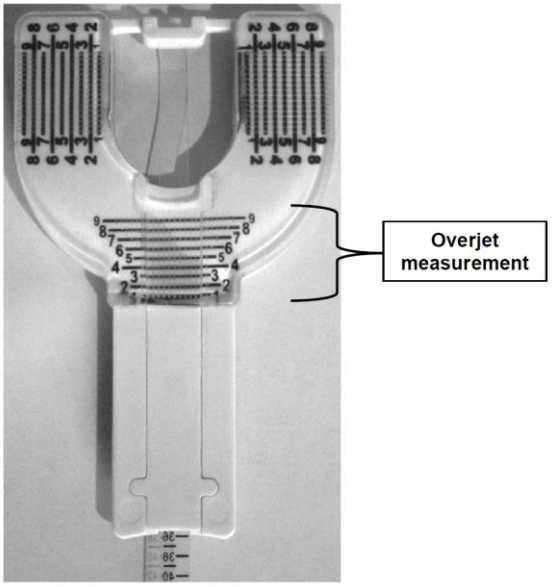


Figure 3.7: Bottom view of the Oral Mez device (Philips Respironics, PA, USA).

Before the measurement the Oral Mez was placed in hot water for some minutes, then cooled in running cold tap water and then placed in the subject´s mouth. The procedure with hot water was necessary in order to make the plastic soft enough for the teeth to leave marks on it.

3.1.4 Study hypothesis

The anatomy of the upper airway (CSAs, and volumes) determines subsequent deposition of aerosol in the upper airway and therefore the deposition of aerosol in the lung.

3.2 Study objectives

3.2.1 Primary Objective

The primary objective of the study was to measure the upper airways of 12 healthy subjects - enrolled in the previous lung deposition study (Nikander et al., 2010c) - by means of AR using an acoustic pharyngometer (Eccovision ARP). The measurements were performed with the subjects seated in the same upright position as when they were inhaling through the I-neb nebuliser in the previous lung deposition study. The subjects were also instructed to inhale with the same inspiratory flows as in the previous study.

3.2.2 Secondary Objectives

The secondary objectives included several assessments and measurements of the oral cavity which were performed with the healthy subjects seated in the same upright position as when inhaling through the I-neb nebuliser in the previous lung deposition study. The assessments and measurements were based on the techniques described in the previous section (3.1.3.1. – 3.1.3.5.).

3.3 Methods

The study was performed as an open investigation including one study group. The healthy subjects included had all participated in a lung deposition study using the I-neb nebuliser (Nikander et al., 2010c). The subjects attended the clinic (Cardiff Scintigraphics Ltd., Cardiff, UK) once for

eligibility confirmation, consent, measurements of the upper airways by acoustic pharyngometry, and assessments and measurements of the oral cavity.

3.3.1 Study design and study variables

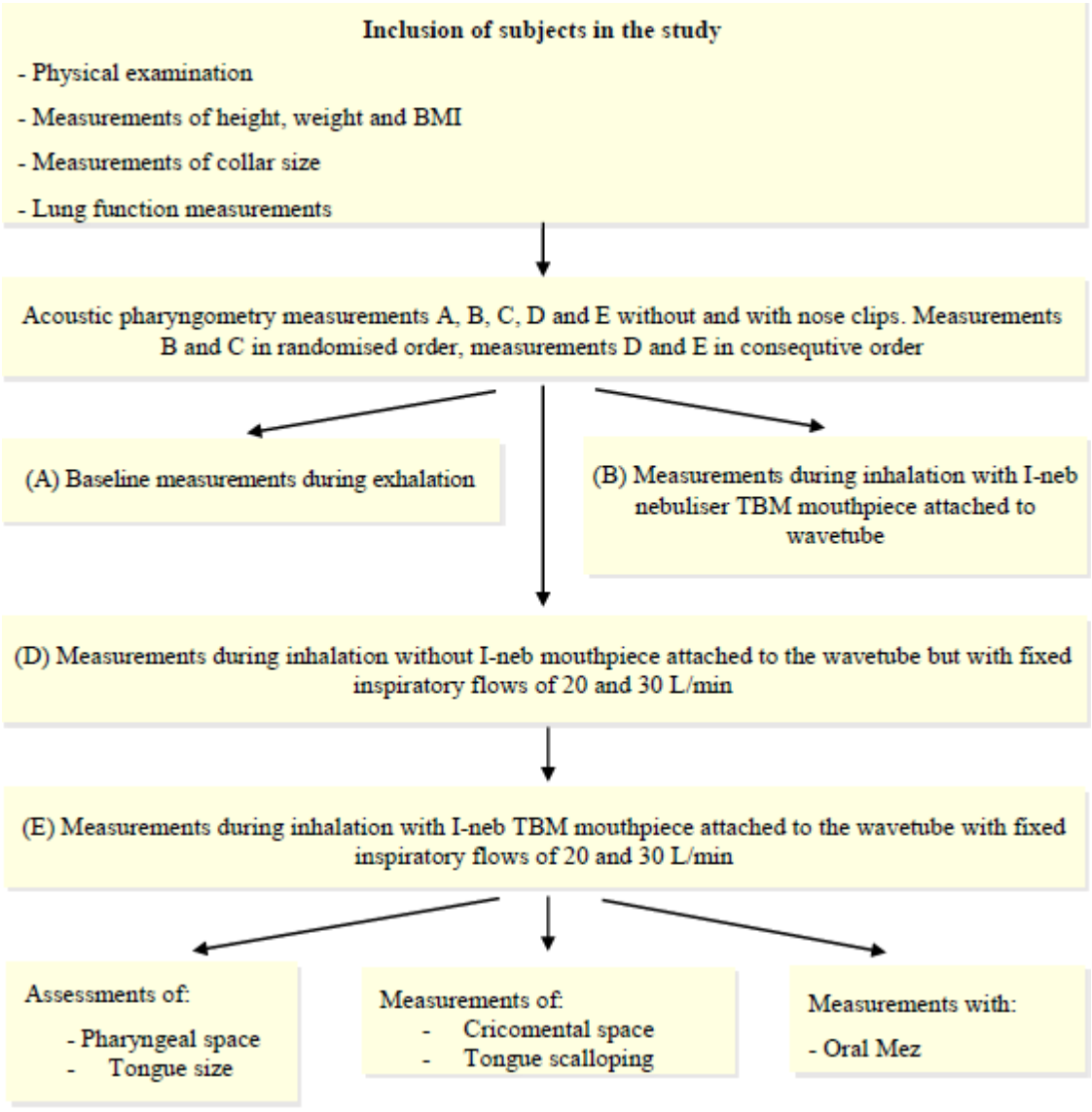
- Physical examination including measurement of vital signs (supine blood pressure and pulse rate).
- Height (cm) and weight (kg) for calculation of BMI [weight in kg/(height in m^2)].
- Collar size (cm).
- Lung function. In order to be able to compare each subject's lung function to that of the previous lung deposition study, lung function was measured following the recommendations of the ATS Standardization of Spirometry 1994 Update to establish reproducibility of FEV_1. (ATS, 1994).
- Measurement of the upper airways by acoustic pharyngometry without and with nose clip as nose clips were used in the previous lung deposition study to prevent inhalation and exhalation via the nose. The primary acoustic pharyngometer measurements each consisting of 4 pharyngogram recordings are outlined in A, B and C (B and C were applied in the randomised order used in the previous lung deposition study; Nikander et al., 2010c), and the secondary acoustic pharyngometer measurements are outlined in D and E (applied in that order).
    - **(A)** Measurement with 4 baseline recordings with the subject exhaling air through the pharyngometer (without and with nose clip).
    - **B** and **C** were then applied in the randomised order used in the previous lung deposition study:
    - **(B)** Measurement with 4 recordings with the subject inhaling air through the pharyngometer wavetube with the I-neb nebuliser **TBM** mouthpiece attached to the back

end of the wavetube. The subject was trained to inhale with an inspiratory flow similar to the one recorded in the original lung deposition study in TBM (without and with nose clip).

- **(C)** Measurement with 4 recordings with the subject inhaling air through the pharyngometer wavetube with the I-neb nebuliser **TIM** mouthpiece attached to the back end of the wavetube. The subject was trained to inhale with an inspiratory flow similar to the one recorded in the original lung deposition study in TIM (without and with nose clip).
- **D** and **E** were then applied in the order outlined below:
- **(D)** Measurements (4 recordings at each flow) with the subject inhaling air through the pharyngometer wavetube with inspiratory flows of 10, 20, 30, 40 and 60 L/min (without and with nose clip).
- **(E)** Measurements (4 recordings at each flow) with the subject inhaling air through the pharyngometer wavetube with the I-neb nebuliser TBM mouthpiece attached to the wavetube with inspiratory flows of 10, 20, 30, 40 and 60 L/min (without and with nose clip).
- Assessment of the tongue size using a modified Mallampati score.
- Measurement of the cricomental space.
- Measurement of tongue scalloping.
- Measurement of palatal height, maxillary intermolar distance, mandibular intermolar distance, incisor overjet, and tongue length using the Oral Mez.

The study procedures have been outlined in a diagram (Table 3.1).

Table 3.1: Diagram over study measurements and assessments.



3.3.2 Study equipment and timing of pharyngometer measurements

3.3.2.1 Acoustic pharyngometer

The acoustic pharyngometer measurements followed the instructions regarding measurements with the device as outlined in the Eccovision Acoustic Pharyngometry Operator Manual. The acoustic pharyngometer measurements were performed with the subjects seated on a straight-

backed chair. The aim was to keep the wavetube horizontally parallel to the floor and prevent head, neck and shoulder movement by instructing the subjects to keep their gaze fixed at a point on the wall. A comfortable position was important in order to avoid any increase in muscle tonus through heavy occlusion on the mouthpieces (Viviano, 2002a). The recordings during exhalation followed the instructions to a subject in the Eccovision Acoustic Pharyngometry Operator Manual (Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com):

- You will sit in a chair and hold a wand with a mouthpiece on it.
- You will place the mouthpiece in your mouth and do various breathing on the mouthpiece as instructed by the technologist.
- Breathing through the mouth normally for 10 to 12 seconds.
- Breathing through the nose for 10 to 12 seconds.
- Closing your glottis and exhaling.
- Closing your glottis and inhaling.
- A technologist will instruct you on how to perform the test and coach and encourage you to do your best.

3.3.2.2 Acoustic pharyngometer wavetube modifications

Due to the design of the study it was important that the acoustic pharyngometer measurements of the upper airways of the subjects were recorded with the subjects inhaling through the I-neb nebuliser mouthpieces in both TBM and TIM breathing modes. In order to mimic the TBM and TIM breathing modes during the acoustic pharyngometer measurements, the I-neb nebuliser mouthpieces were attached to the back end of the pharyngometer wavetube (Figure 3.8). The subjects' inhalation and exhalation flow rates during both TBM and TIM breathing had in the previous lung deposition study been recorded through an I-neb Function Monitor (Philips Respironics, Chichester, UK) (Nikander et al., 2010c). As the I-neb Function Monitor was not available for the present study, a pneumotachograph was connected between the I-neb nebuliser mouthpiece and the wavetube in series with a Mimic Breathing Monitor (Philips Respironics,

Chichester, UK) (Nikander et al., 2000a; Nikander et al., 2000b) which was connected to a laptop. During breathing through the wavetube the subjects could follow their breathing patterns on a PC laptop screen and were guided to use their mean peak inspiratory flow rates from the previous study. In order to guide the subjects to the right peak inspiratory flow rates a transparent plastic sheet was placed on the PC laptop screen with horizontal lines highlighting flows of either 20 L/min or 30 L/min.

The back end of the I-neb nebuliser mouthpiece, which in normal use is connected to the body of the nebuliser, was covered by a plastic wrapping so that the subject's inspiratory flow was directed through the inhalation and exhalation valve of the mouthpiece (Figure 3.8).

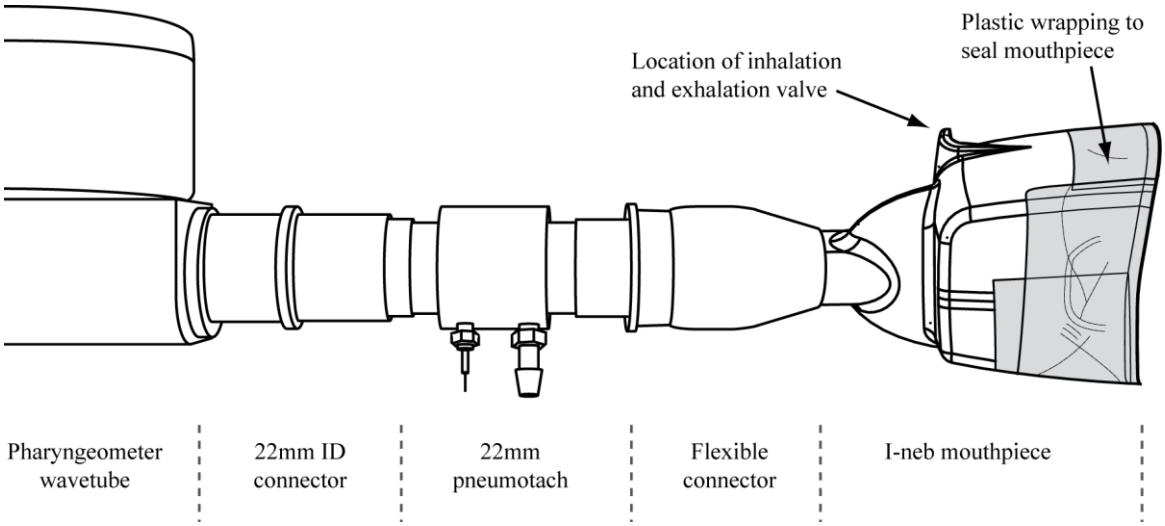


Figure 3.8: The back end of the pharyngometer wavetube is shown with a pneumotachograph and an I-neb nebuliser mouthpiece attached. The back end of the mouthpiece was covered by a plastic wrapping so that the subject's inspiratory flow was directed through the inhalation and exhalation valve of the mouthpiece.

3.3.2.3 Timing of acoustic pharyngometer measurements

Timing of the pharyngometry measurement – when to start and when to stop during the subject's breathing – could be challenging when timing is restricted to an observation of the subject. The

use of the Mimic Breathing Monitor pneumotachograph connected to the wavetube offered both a method to follow the subject's breathing breath-by-breath, and a tool for when to start and when to stop the acoustic pharyngometer measurement. As the acoustic pharyngometer measurements were performed both during exhalation and inhalation, the timing of the measurements was of importance as motion of soft tissue in the upper airway and related bony structures has been shown to create dimensional changes in the upper airways (Figure 2.5; Schwab, 1998).

Interestingly the dimensional changes were predominantly found in the lateral dimension and Schwab concluded that this suggested "that the lateral walls may have an important role in modulating airway caliber", and that "those studies indicate that significant changes in upper airway caliber occur during the respiratory cycle" (Schwab, 1998). The upper airway has been shown to be significantly smaller in apnoeic subjects than in healthy subjects (Schwab et al., 1993a), but similar dimensional changes in the upper airways were shown to occur in healthy subjects (Schwab et al., 1993b).

Although the dimensional changes in the upper airway area reported by Schwab (1998) occurring during breathing were recorded with the subjects in a supine position the results might be relevant for the current study. The possible impact of the supine position in comparison with the seated position on the size of the upper airways has been measured in healthy subjects using AR (Fouke et al., 1987; Jan et al., 1994), ARP (Eccovision; Jung et al, 2004) and CT/CBCT techniques (Van Holsbeke et al., 2014a) as discussed in section 2.8. The CSAs were shown to be 23% (Fouke et al., 1987), ~21% (OPJ; Jan et al., 1994), ~22% (OPJ) and ~24% (GL; Jung et al, 2004) smaller in the supine position. Van Holsbeke et al (2014a) showed that the region between the hard palate and the bottom of the uvula was ~50% larger in the seated position.

As the subject's breathing pattern through the wavetube could be followed on a PC laptop screen, the technician could plan the recording of the pharyngogram based on each individual breathing

pattern. As shown in Figure 3.9, the measurement during exhalation (measurement A; 3.3.1) was started at mid-inhalation and stopped at end of exhalation with the pharyngograms recorded from mid to end of exhalation.

The pharyngometer measurements during inhalation without the addition of an I-neb nebuliser mouthpiece (measurement D; 3.3.1.) were started at mid-exhalation and stopped at end of inhalation with the pharyngograms recorded during mid to end of inhalation. The same start and stop points were followed when performing the measurements with the I-neb TBM mouthpiece attached to the wavetube (measurements B and E; 3.3.1.). When the I-neb TIM mouthpiece (measurement C; 3.3.1.) was attached to the wavetube the pharyngometer measurement followed different start and stop points with start early during the slow and deep inhalation, and with stop at the end of the inhalation and with the pharyngograms recorded during mid to end of inhalation.

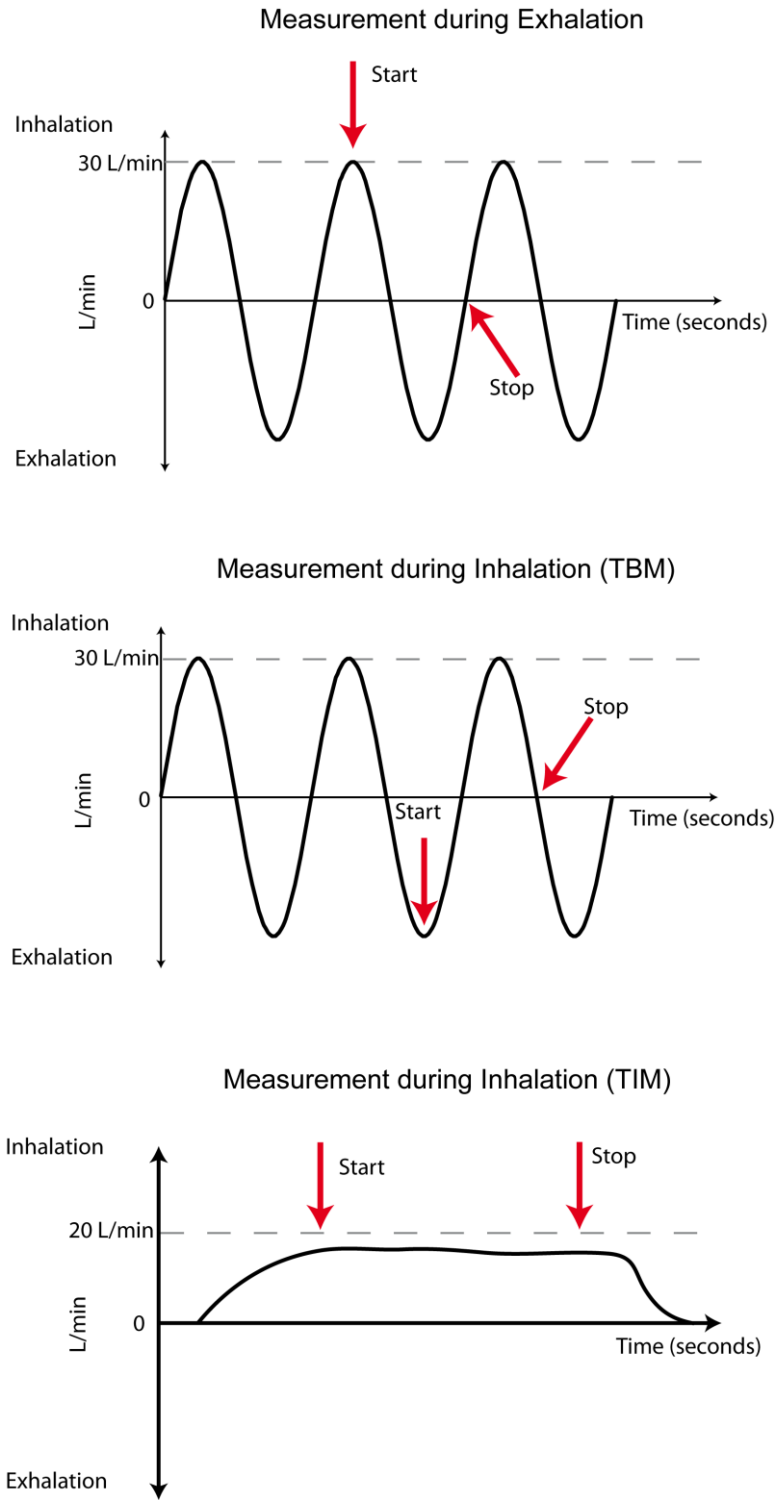


Figure 3.9: An example of TBM and TIM breathing patterns with red arrows showing when the acoustic pharyngometer measurements should be started and stopped in the different measurements during exhalation (measurement A; 3.3.1.), and inhalation (measurements B, C, D, and E; 3.3.1.).

3.3.3 Study Subjects

3.3.3.1 Inclusion Criteria

- Subjects had to provide written informed consent to participate in the study.
- Healthy male or female subjects who had participated in the previous lung deposition study (Nikander et al., 2010c).
- Subjects with no clinically significant findings in vital signs.
- Subjects must be available to complete the study.
- Subjects must satisfy a medical examiner about their fitness to participate in the study.

3.3.3.2 Exclusion Criteria

- Subjects not compliant with the instructions for use of the acoustic pharyngometer wavetube.
- Subjects who had participated in a clinical study in the previous month.

3.3.3.3 Withdrawal Criteria

- If the Investigator considered that the subject's health was compromised by remaining in the study or the subject was not sufficiently cooperative.
- On request from the subject for any reason.

3.4 Adverse events

AEs were recorded on the CRFs and defined as any untoward medical occurrence in a subject or clinical investigation subject undergoing an investigational procedure, and which did not necessarily have a causal relationship with the device under investigation. An AE could therefore be any unfavourable and unintended sign (for example including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a device whether or not considered related to the device under investigation.

3.5 Ethical considerations

The study was performed in the summer of 2008 according to the principles of the Declaration of Helsinki (South Africa, 1996) and the ABPI Guidelines for Medical Experiments in Non-Patient Human Volunteers - 1988, amended May 1990 and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP).

Ethics Committee approval was obtained from the South East Wales Research Ethics Committee prior to the start of the study and prior to any communication with potential study subjects. No study related procedures were carried out before ethics committee approval had been granted (APPENDIX A.1).

Both verbal and written information was given to the subjects. Sufficient time was allowed for the subject to consider participation in the study and providing consent for inclusion, if they decided to enter the study. Written consent was obtained prior to commencement of any study procedures.

3.6 Statistical analysis

3.6.1 Data analysis

A formal plan for the statistical analysis was not pre-specified. Due to the exploratory nature of the study the main statistical analysis (ANOVA; Pearson correlation) was focused on the analysis of the pharyngograms and possible correlations between the outcomes of the acoustic pharyngometer measurements and the lung and the upper airway deposition of ^{99m}Tc-DTPA in the previous lung deposition study (Nikander et al., 2010c). The study data has been analysed by SAS 9.2 for Windows (W32_VSPRO platform), running on a Lenovo L412 under Windows 7 Professional. The significance level was established at 0.05.

3.6.2 Deviations from study protocol

The study protocol stated that acoustic pharyngometer measurements D and E were to be made at inspiratory flows of 10, 20, 30, 40 and 60 L/min, but the Mimic Breathing Monitor equipment supplied for the study fitted with a 22 mm pneumotachograph rather than the alternative 30 mm pneumotachograph could not record flows in excess of 35 L/min.

In addition, it was found that a tidal breathing pattern at 10 L/min resulted in an inhalation duration that was too short for the acoustic pharyngometer to record a stable reading. For these reasons the acoustic pharyngometer measurements D and E were limited to 20 and 30 L/min.

3.6.3 Acoustic pharyngometer data

For each subject each of the 7 measurements where investigated both without and with a nose clip resulting in 2 × 7 = 14 measurements, and for each measurement 4 acoustic pharyngograms were recorded. Thus a total of 126 (9 subjects × 14) measurements were made comprising 504 (126 × 4) acoustic pharyngograms. One measurement was performed during exhalation without a nose clip and 1 measurement was performed during exhalation with a nose clip (check 3.3.2.1), and the rest during inhalation (check 3.3.2.3). Thus 108 (9 subjects × 12) measurements were made during inhalation.

The measurements were coded A, B, C, D20, D30, E20 and E30 for the descriptive presentations of the data and for the statistical analyses. The addition of “NC” was included when the measurement was performed with a nose clip (ANC, BNC, CNC, D20NC, D30NC, E20NC and E30NC), Table 3.2.

Table 3.2: Description of the coding system for the measurements. For each subject 7 acoustic pharyngometer measurements were first performed without a nose clip, and then 7 measurements were performed with a nose clip (to prevent inhalation/exhalation via the nose).

Code	Description
A	Baseline acoustic pharyngometer measurement during **exhalation** with the subject breathing through the pharyngometer wavetube without additional attachments to the end of the wavetube (without and with nose clip).
B	Acoustic pharyngometer measurements during **inhalation** with the subject breathing through the pharyngometer wavetube with the I-neb nebuliser TBM mouthpiece attached to the end of the wavetube (without and with nose clip).
C	Acoustic pharyngometer measurements during **inhalation** with the subject breathing through the pharyngometer wavetube with the I-neb nebuliser TIM mouthpiece attached to the end of the wavetube (without and with nose clip).
D20, D30	Acoustic pharyngometer measurements (4 at each flow) during **inhalation** with the subject breathing through the pharyngometer wavetube during tidal breathing with inspiratory flows of 20 and 30 L/min without attachments to the wavetube (without and with nose clip).
E20, E30	Acoustic pharyngometer measurements (4 at each flow) during **inhalation** with the subject breathing through the pharyngometer wavetube with the I-neb nebuliser **TBM** mouthpiece attached to the end of the wavetube during tidal breathing with inspiratory flows of 20 and 30 L/min (without and with nose clip).

The raw acoustic pharyngometer data from each measurement was imported into Microsoft Excel as space delimited data and then converted to SAS data sets for the statistical analysis. All pharyngometer measurements were first performed "without nose clip" and then "with nose clip".

3.6.4 Goodness of Fit (GOF) analysis of pharyngograms

An initial review of all pharyngograms recorded during inhalation indicated that some of these deviated from the rest of the pharyngograms as illustrated in Figure 3.10 (subject 3) in which pharyngograms 1-3 follow a similar pattern, whereas the 4th pharyngogram ("Test No. 4") shows a deviating pattern. For the purpose of the analysis it was important to remove all such deviating pharyngograms as retaining these would decrease the chances to detect effects and correlations.

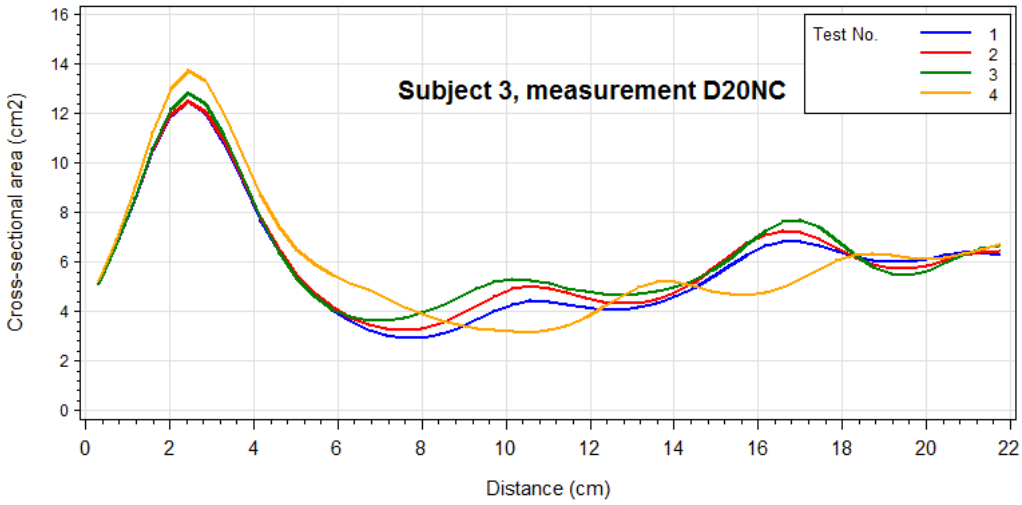


Figure 3.10: Subject 3, measurement D20NC (measurement D, at 20 L/min, with nose clip).

As noted above in section 3.6.3 there were 108 graphs like the one shown in Figure 3.10 each presenting the results of an acoustic pharyngometer measurement during inhalation and comprising 4 pharyngograms. It was therefore not practical to review and manually remove pharyngograms which did not fit with the general trend. An automatic procedure was required as this would create an objective tool for exclusion of pharyngograms. To identify deviating curves a measure of the "goodness of fit" (GOF) was required. For the process each of the 4 pharyngograms was compared to the median pharyngogram and those deviating too much were removed. The GOF was calculated as the square root of the average squared vertical distance between the median curve and the curve under study. The region over which the GOF-calculation was performed was limited to the region covering the pharyngogram from the start of the pharyngogram to the GL. Three maxima at approximately x = 2.5, 10 and 17 cm and three minima at approximately x = 7, 13 and 19 cm can be detected (Figure 3.10). The 3 minima should correspond with landmarks (L) equal to the OPJ (L1), the EG (L2) and the GL (L3) as highlighted in Figure 3.12. The positions of the maxima and minima were slightly different for different

pharyngograms and very different for deviating pharyngograms. Taking this into account the 1st maxima (oral cavity) and the 3rd minima (GL) were determined for each subject and for each of the 8 pharyngograms collected under measurements A and ANC (exhalation). Based on the 16 obtained estimates, the median 1st maxima and 3rd landmark (last minima, GL) were calculated for each subject. Using these cut-offs, the GOF value was calculated for B, C, D20, D30, E20 and E30, and BNC, CNC, D20NC, D30NC, E20NC and E30NC) (Figure 3.11).

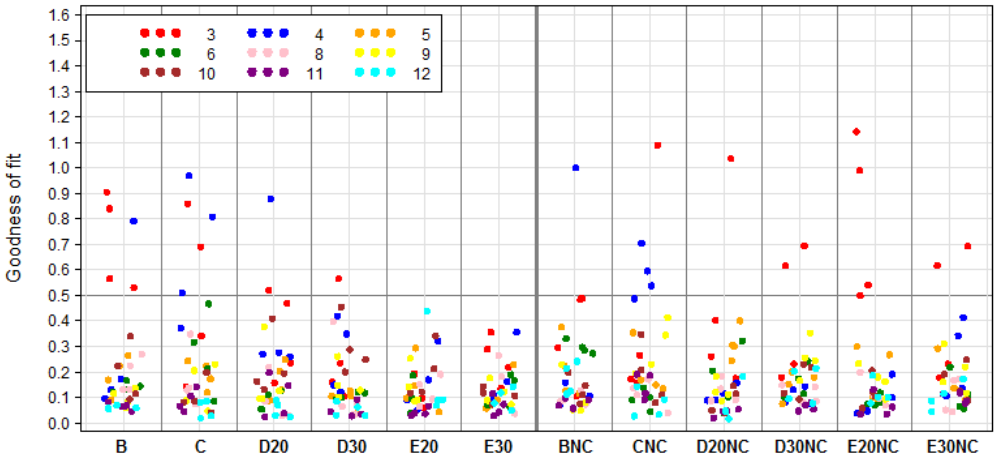


Figure 3.11: GOF analysis for the pharyngograms of each of the 9 subjects (colour codes). The measurements without (left; B, C, D20, D30, E20 and E30), and with nose clip (right; BNC, CNC, D20NC, D30NC, E20NC and E30NC) are highlighted on the x-axis.

As shown in Figure 3.11 most high GOF values occurred with subjects 3, 4 and 10. The GOF value was typically below 0.5 but a number of cases with higher values existed and the associated pharyngograms were removed from further analysis. The choice of a GOF value of 0.5 as cut-off between accepted or not was subjectively made based on the results in Figure 3.11.

3.6.5 Analysis of pharyngograms

The typical pharyngogram showed 3 landmarks (minima) along the pharyngogram and these corresponded to the OPJ, the EG and the GL. The CSA at each landmark was of interest as the

contribution to deposition of aerosol in the upper airways and the lungs could be related to the CSAs of the different landmarks. The volume of the upper airway from the end of the wavetube mouthpiece (teeth) to the 1st landmark, and between the 2 consecutive landmarks was also of interest as the contribution to deposition of aerosol in the upper airways and the lungs could also be related to the volume of the upper airway between the different landmarks (Figure 3.12). The volume of the portion of the airway between 2 landmarks (for example a, and b), where a, and b were distances from the mouthpiece was defined as the AUC between these points. A natural choice for a, and b, and thus for AUCs, was to use the points defined by the 3 landmarks as illustrated in Figure 3.12.

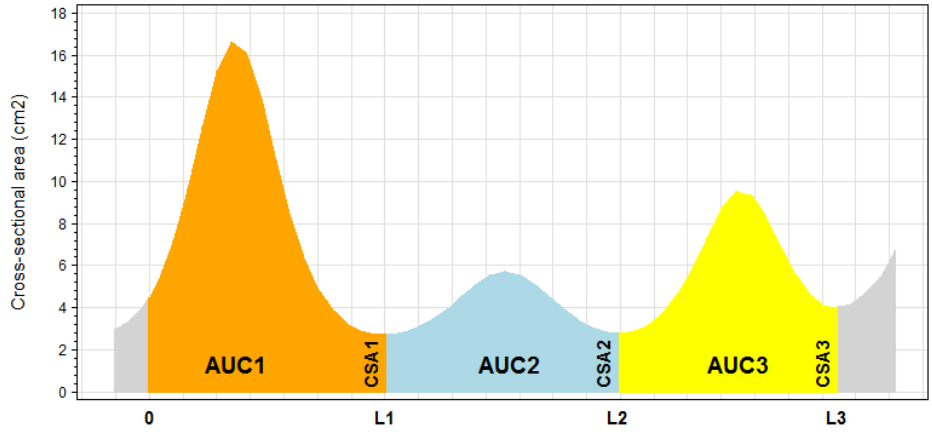


Figure 3.12: Illustration of AUCs defined by the wavetube mouthpiece (0) and 3 landmarks (L1, L2 and L3). The pharyngogram represents a CSA of the upper airway from the oral cavity (0 to L1) caudal to the GL (L3). The AUC represents volume over a given length of airway, and landmarks along the pharyngogram relate to specific anatomical landmarks (OPJ, EG, GL). The AUCs (AUC1, AUC2, AUC3) have been highlighted as have the CSAs for each landmark (CSA1, CSA2 and CSA3).

The definition of the landmarks required experience with acoustic pharyngometry and the 3 landmarks were for measurements A and ANC (baseline measurement during exhalation) for each subject therefore defined by an expert in the field (Dr John Viviano, Mississauga, Ontario, Canada)

(Viviano, 2002a, Viviano, 2002b, Viviano, 2004). The distances between the landmarks for each subject are presented in Table 3.3.

Table 3.3: Position of the individual landmarks (minima) on the x-axis of the pharyngogram (in cm from 0, check Figure 3.12).

Subject number	**L1 (cm from 0)**	**L2 (cm from 0)**	**L3 (cm from 0)**
3	7.16	13.59	20.02
4	7.81	14.88	21.96
5	9.09	18.31	23.45
6	8.67	15.74	20.24
8	7.81	15.74	20.45
9	7.81	14.88	22.60
10	8.45	14.45	20.88
11	7.16	15.31	20.02
12	7.81	15.10	19.60
Mean	8.0	15.3	21.0
SD	0.7	1.3	1.3

3.6.6 Analysis of CSAs

For each subject and measurement the mean CSA per landmark was determined and the results summarised descriptively. An ANOVA was performed per CSA (CSA1-3) for factors “Nose clip”, “Inhalation mode” (TBM or TIM) and “Flow rate” (20 or 30 L/min).

3.6.7 Analysis of the areas under the curve (AUCs)

For each subject and measurement the mean AUC between consecutive landmarks was determined and the results summarised descriptively. An ANOVA was performed per AUC1-3 for factors “Nose clip”, “Inhalation mode” (TBM or TIM)” and “Flow rate” (20 or 30 L/min).

3.6.8 Correlation analysis

Based on the results from the past lung deposition study and the present acoustic pharyngometer study, the correlation (Pearson correlation coefficient) between the 6 acoustic pharyngometer endpoints (CSA1-3, AUC1-3) and the lung, the upper airway and other depositions (12 endpoints)

was determined. The correlation analysis was performed with data from measurements BNC and CNC in the present study since nose clips were used in the past lung deposition study (Nikander et al., 2010c).

3.6.9 Analysis of secondary variables

The secondary variables have been listed per subject. The possible effect on the lung, the upper airway and other depositions was explored using correlation analysis for continuous variables and by ANOVA for discrete variables.

3.7 Results

3.7.1 Subject demographics

Nine of the original 12 subjects agreed to participate in the present study. No AEs were reported. In Table 3.4 the demographic data including age, height, weight, BMI and collar diameter are presented.

Table 3.4: Demographic data for the 9 study subjects (mean ± SD).

	All	**Male**	**Female**
Number of subjects	9	2	7
Age (years, mean ± SD)	38.2 (15.8)	31.0 (9.9)	40.3 (17.2)
Height (m, mean ± SD)	1.69 (0.05)	1.73 (0.04)	1.68 (0.05)
Weight (kilograms, mean ± SD)	77.5 (13.1)	84.8 (24.0)	75.4 (10.5)
BMI (kilograms/(height in m^2, mean ± SD)	27.1 (4.0)	28.2 (6.6)	26.7 (3.7)
Collar diameter (cm, mean ± SD)	35.3 (3.7)	40.3 (2.5)	33.9 (2.7)

For comparative purposes information on lung function has been included both from the previous lung deposition study (Nikander et al., 2010c) and from the present study (Table 3.5 and Table 3.6). There were no major differences in the two sets of lung function data for the 9 subjects.

Table 3.5: Lung function data for the 9 subjects from the previous lung deposition study (Nikander et al., 2010c).

Subject Number	FEV1 (L)	FEV1 Predicted (%)	FVC (L)	FVC Predicted (%)
3	2.92	105	3.87	120
4	4.42	110	5.6	115
5	3.82	112	4.8	122
6	2.79	116	3.59	126
8	3.37	97	3.96	99
9	4.04	99	4.77	99
10	3.59	104	4.37	111
11	2.29	92	3.3	113
12	3.36	101	3.78	99
Mean	**3.4**	**104**	**4.23**	**112**
SD	**0.66**	**7.68**	**0.72**	**10.47**

Table 3.6: Lung function data for the same 9 subjects from the present study.

Subject Number	FEV1 (L)	FEV1 Predicted (%)	FVC (L)	FVC Predicted (%)
3	2.78	102	3.88	122
4	4.58	115	5.59	116
5	3.43	103	4.71	122
6	2.76	117	3.36	120
8	3.49	100	4.26	107
9	4.09	98	4.91	101
10	3.53	103	4.24	108
11	2.14	87	3.16	108
12	3.26	98	3.82	100
Mean	**3.34**	**102**	**4.21**	**112**
SD	**0.73**	**9.04**	**0.77**	**8.66**

The individual deposition of ^{99m}Tc-DTPA in the lung, the oropharynx, the stomach and the mouth (emitted doses ex-mouthpiece) has been included in Table 3.7 (I-neb nebuliser; TBM) and Table 3.8 (I-neb nebuliser; TIM).

Table 3.7: Individual deposition data for each subject when using the I-neb nebuliser in TBM (Nikander et al., 2010c).

Subject Number	Total Lung (%)	Right Lung Central (%)	Right Lung Peripheral (%)	Left Lung Central (%)	Left Lung Peripheral (%)	Total Central (%)	Total Peripheral (%)	Oropharyngeal (%)	Stomach (%)	Total Oropharyngeal (%)	Mouth –wash (%)
3	50.54	6.30	13.37	5.87	10.71	12.17	24.08	8.06	39.74	49.41	1.62
4	61.24	8.09	13.46	9.36	11.79	17.44	25.25	4.34	32.18	37.82	1.30
5	58.64	6.74	17.39	6.88	12.55	13.62	29.94	4.08	33.49	39.88	2.31
6	89.89	9.90	23.69	14.50	17.51	24.40	41.20	2.96	3.72	8.34	1.66
8	92.26	11.69	27.80	10.20	18.39	21.89	46.19	4.01	2.05	6.71	0.65
9	54.95	9.48	12.31	7.93	8.71	17.42	21.02	4.30	38.89	44.49	1.29
10	38.81	3.96	11.27	4.43	9.28	8.40	20.55	6.93	44.26	60.18	8.98
11	53.50	7.18	12.52	10.57	9.87	17.76	22.39	5.06	40.12	45.79	0.61
12	80.64	11.62	17.73	10.82	17.17	22.44	34.90	5.00	12.60	18.10	0.50
Mean	**64.50**	**8.33**	**16.62**	**8.95**	**12.89**	**17.28**	**29.50**	**4.97**	**27.45**	**34.52**	**2.10**
SD	**18.67**	**2.57**	**5.72**	**3.04**	**3.80**	**5.21**	**9.32**	**1.58**	**16.63**	**18.96**	**2.65**

Table 3.8: Individual deposition data for each subject when using the I-neb nebuliser in TIM (Nikander et al., 2010c).

Subject Number	Total Lung (%)	Right Lung Central (%)	Right Lung Peripheral (%)	Left Lung Central (%)	Left Lung Peripheral (%)	Total Central (%)	Total Peripheral (%)	Oropharyngeal (%)	Stomach (%)	Total Oropharyngeal (%)	Mouth –wash (%)
3	70.07	7.82	18.88	8.15	15.04	15.97	33.92	3.09	25.95	29.82	0.78
4	89.91	11.88	18.28	11.36	19.27	23.24	37.55	2.52	7.07	9.98	0.39
5	70.01	7.46	20.62	7.48	15.37	14.94	35.99	2.82	26.40	29.86	0.64
6	94.48	9.64	25.75	12.49	19.15	22.13	44.90	1.47	2.84	4.97	0.66
8	91.93	11.21	26.55	11.84	19.75	23.05	46.30	4.03	3.75	7.81	0.04
9	70.67	9.04	21.13	8.21	13.95	17.25	35.07	2.80	25.83	29.17	0.53
10	57.19	6.34	15.20	5.79	13.91	12.13	29.11	2.99	38.74	42.54	0.80
11	66.20	9.44	14.55	10.93	12.75	20.37	27.29	6.81	26.18	33.55	0.56
12	81.84	11.34	19.37	9.19	20.68	20.53	40.05	4.74	12.84	17.99	0.42
Mean	**76.92**	**9.35**	**20.04**	**9.49**	**16.65**	**18.85**	**36.69**	**3.47**	**18.84**	**22.85**	**0.54**
SD	**13.06**	**1.90**	**4.11**	**2.27**	**3.02**	**3.94**	**6.41**	**1.55**	**12.57**	**13.11**	**0.23**

The results for “Total Lung”, “Total oropharyngeal” and “Stomach” depositions with the I-neb nebuliser used in TBM was in percent of the ex-mouthpiece dose 64.50%, 34.52% and 27.45%,

whereas the corresponding depositions with the I-neb nebuliser used in TIM were 76.92%, 22.85% and 18.84%, respectively. The inter-subject variability was large and especially large in the "Total oropharyngeal" and "Stomach" depositions.

3.7.2 Individual pharyngograms

The individual mean pharyngograms are presented in APPENDIX A.2. As an example, the results for subject 3 are shown in Figure 3.13. The plot covers 7 measurements each including 4 pharyngograms (check 3.6.3) with legend "Measurement": A, B, C, D20, D30, E20, E30 for the pharyngometer measurements without a nose clip, and 7 mean measurements with legend "Measurement": ANC, BNC, CNC, D20NC, D30NC, E20NC, E30NC for the pharyngometer measurements with a nose clip. In the plots the y-axis presents the CSA (in cm^2) of the upper airway, and the x-axis presents the length of the upper airway from end of the pharyngometer wavetube (0) to the GL (in cm).

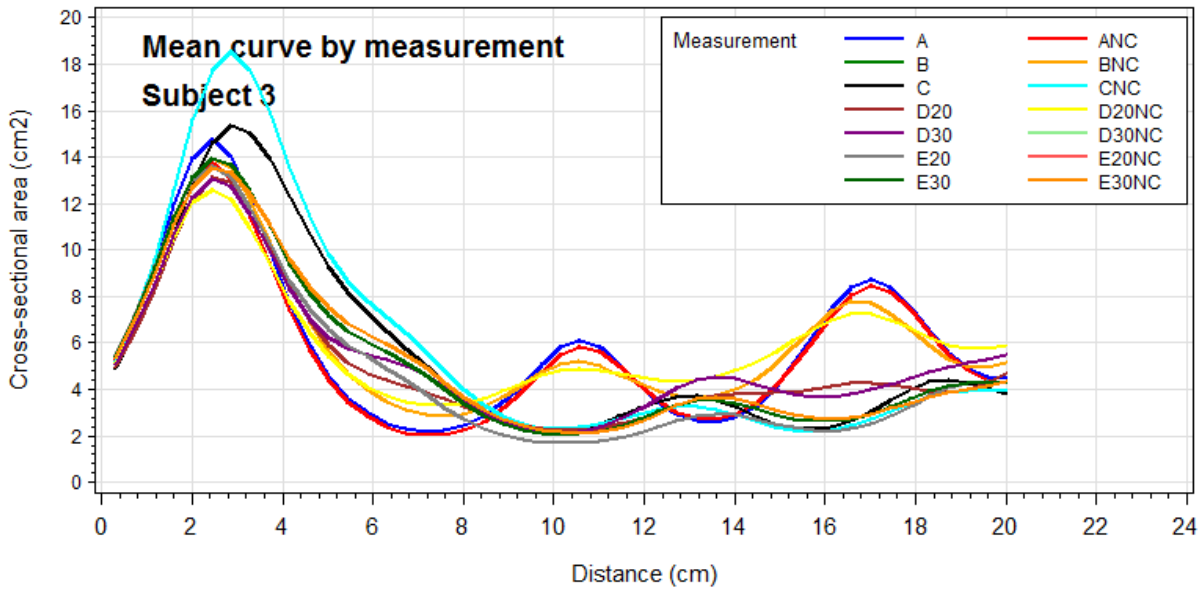


Figure 3.13: Subject 3, mean curve by measurement (measurements A to E30 without nose clip, and ANC to E30NC with nose clip). Each measurement consisted of 4 pharyngograms.

The position of the GL is obviously different for different subjects and therefore not fixed at a certain point, for example 20 cm (subject 3), as shown in Table 3.3.

3.7.3 CSAs at landmarks

For each subject and measurement the mean CSA (cm^2) at each of the 3 landmarks was determined (check and compare 3.6.5 and Figure 3.12). In Table 3.9 summary statistics for CSA1, CSA2 and CSA3 are presented for each of the 14 measurements (without and with nose clip).

Table 3.9: Summary statistics (mean ± SD) for CSA1-3 (cm^2) for each of the 14 study measurements (without and with nose clip).

Measurement	**Without nose clip (CSAs in cm^2)**			**With nose clip (CSAs in cm^2)**		
	CSA1	CSA2	CSA3	CSA1	CSA2	CSA3
A	3.2 ± 2.0	3.5 ± 1.3	5.0 ± 1.4	3.3 ± 1.6	4.0 ± 1.1	4.3 ± 1.3
B	3.8 ± 1.6	3.0 ± 0.7	4.9 ± 1.2	3.7 ± 1.4	3.9 ± 1.5	5.4 ± 1.8
C	4.4 ± 2.8	2.9 ± 0.7	4.5 ± 1.2	3.9 ± 1.3	3.8 ± 1.6	5.0 ± 1.4
D 20	4.0 ± 2.0	3.0 ± 0.7	4.7 ± 1.3	3.9 ± 1.5	4.1 ± 1.5	5.1 ± 0.9
D 30	4.5 ± 1.5	3.3 ± 1.1	5.0 ± 1.6	3.9 ± 1.5	4.0 ± 1.5	4.6 ± 0.7
E 20	3.8 ± 1.6	2.9 ± 0.7	4.5 ± 1.2	3.5 ± 1.5	3.9 ± 1.7	4.7 ± 0.5
E 30	4.1 ± 1.7	3.0 ± 0.8	4.6 ± 1.2	3.9 ± 1.2	3.9 ± 1.5	5.3 ± 1.8

The effect of nose clip was most obvious for CSA2 (EG, Figure 3.12) for which the use of nose clip increased the CSA for all measurements in comparison with the measurements without a nose clip. The effect of nose clip was smaller for CSA3 (GL), and did not include A and D30 for which the effect was the opposite. For CSA1 (OPJ) the results without nose clip were somewhat larger than those with nose clip (but for A), but the differences were small.

3.7.4 Statistical analysis of CSAs

The possible effects by “Inhalation mode” and “Nose clip” on CSAs have been investigated using measurements B and C (without nose clip) and BNC and CNC (with nose clip) as outlined in 3.6.3.

Measurements B and BNC were performed during tidal breathing through the pharyngometer wavetube with the I-neb nebuliser TBM mouthpiece attached to the end of the wavetube. Measurements C and CNC were performed during slow and deep breathing through the pharyngometer wavetube with the I-neb nebuliser TIM mouthpiece attached to the end of the wavetube. The data has been assessed using a main effects ANOVA including subject as a factor. Inhalation mode (TBM or TIM) had no statistically significant effect on the CSAs, whereas measurement without or with nose clip had a statistically significant effect for CSA2 ($p = 0.0108$). The use of a nose clip increased CSA2 by 26%. Although measurements without or with nose clip did not have a statistically significant effect for CSA3, the effect was an increase by 11%.

In order to investigate the effect of the two specific inspiratory flow rates (20 L/min or 30 L/min) and use of nose clip or not on the CSAs, data from measurements D and DNC or E and ENC - as outlined in 3.6.3 - could be used in 2 separate analyses. Measurements D and DNC (without and with nose clip) were performed during tidal breathing through the pharyngometer wavetube, with inspiratory flows of 20 and 30 L/min without the I-neb nebuliser mouthpiece attached to the back end of the wavetube. Measurements E and ENC (without and with nose clip) were performed with inspiratory flows of 20 and 30 L/min through the pharyngometer wavetube with the I-neb nebuliser TBM mouthpiece attached to the back end of the wavetube. As above subject was included as a factor in the statistical model.

There was no statistically significant effect of flow rate in measurements D and DNC, and E and ENC as expected as inhalation mode was shown to be non-significant in the previous analysis. The use of nose clip had, however, a statistically significant effect on CSA2 in both measurements D and DNC ($p = 0.0034$) and E and ENC ($p = 0.0005$). The effect was 25% (D *versus* DNC) and 28% (E *versus* ENC) higher for CSA2 when nose clip was used. This was in agreement with the previous analysis.

3.7.5 AUCs between landmarks

As highlighted in Figure 3.12, the acoustic pharyngogram represents a CSA of the upper airway from the end of the wavetube caudal to the GL. The AUC represents volume (in cm^3) over a given length of airway between landmarks along the pharyngogram.

For each subject and measurement, the mean AUCs between the end of the wavetube and the first landmark, and the consecutive 2 landmarks was determined. In Table 3.10 summary statistics for AUC1-3 are shown for each of the 14 measurements (without and with nose clip).

Table 3.10: Summary statistics (mean ± SD) for AUC1-3 (cm^3) for each of the 14 study measurements (without and with nose clip).

Measurement	**Without nose clip (AUCs in cm^3)**			**With nose clip (AUCs in cm^3)**		
	AUC1	AUC2	AUC3	AUC1	AUC2	AUC3
A	55 ± 13	31 ± 7	28 ± 10	52 ± 9	33 ± 10	29 ± 8
B	56 ± 12	23 ± 3	21 ± 8	54 ± 10	30 ± 6	29 ± 11
C	59 ± 15	24 ± 5	21 ± 8	55 ± 13	29 ± 7	28 ± 11
D 20	57 ± 12	24 ± 5	22 ± 8	53 ± 10	31 ± 6	30 ± 11
D 30	56 ± 12	27 ± 4	24 ± 10	54 ± 12	30 ± 7	27 ± 10
E 20	56 ± 12	23 ± 5	21 ± 7	52 ± 10	30 ± 7	28 ± 11
E 30	57 ± 13	25 ± 5	20 ± 7	54 ± 11	31 ± 7	28 ± 11

The effect of nose clip was obvious for AUC2 (OPJ to EG) and AUC3 (EG to GLs) for which the use of nose clip increased the upper airway volume. For AUC1 the effect was the opposite although considerably smaller.

3.7.6 Statistical analysis of AUCs

The possible effects by “Inhalation mode” and “Nose clip” on AUCs have been investigated using measurements B and C (without nose clip) and BNC and CNC (with nose clip). The data has been assessed using a main effects ANOVA including subject as one factor. As for the CSAs, inhalation

mode (TBM or TIM) had no statistically significant effect on any of the 3 AUCs, whereas use of nose clip or not had a statistically significant effect for AUC2 (P = 0.0006) and AUC3 (p = 0.0002) and increased the airway volume with 23% (AUC2) and 31% (AUC3). For AUC1 the effect was the opposite and considerably smaller (6.8%).

In order to investigate the effect of flow rates on AUCs, data from measurements D and DNC or E and ENC could be used. The possible effect of use of a nose clip (measurements DNC and ENC) could be explored in the same analysis. Data has been assessed using a main effects ANOVA including subject as a factor.

There was no statistically significant effect of flow rate in measurements D, DNC, E and ENC as expected as inhalation mode was shown to be non-significant in the previous analysis. The use of nose clip had, however, a statistically significant effect on AUCs in both measurements (D and DNC; E and ENC). The effects were 18% (AUC2; D *versus* DNC; p = 0.0014), 23% (AUC3; D *versus* DNC; p = 0.0012), 22% (AUC2; E *versus* ENC; p = 0.0002) and 30% (AUC2; E *versus* ENC; p <0.0001) higher when nose clip was used. The effects were opposite and smaller for AUC1 (4.7%). These results were in agreement with the previous analysis of the CSAs. Overall, 5 of the 9 subjects had clear effects of the use of nose clips.

3.7.7 Correlations between CSAs and deposition endpoints

As nose clips were used in the past lung deposition study (Nikander et al., 2010), the correlation analysis between CSAs and deposition endpoints has been performed for measurements with a nose clip (BNC and CNC). The acoustic pharyngometer data for CSA1-3 from measurements BNC and CNC were correlated to each of the 12 deposition endpoints from the lung deposition study: mouthwash, oropharyngeal, stomach, total oropharyngeal, right lung – central, left lung – central, total central lung, right lung – peripheral, left lung – peripheral, total peripheral lung, total lung

and right lung C/P ratio. The obtained Pearson correlation coefficients between CSAs and deposition endpoints are presented in Table 3.11. The corresponding p-values for correlations between CSA1-3 (with nose clip) and deposition endpoints are presented in Table 3.12. In this analysis results from TBM (BNC) and TIM (CNC) measurements were pooled.

Table 3.11: Pearson correlation coefficients for correlations between CSA1-3 (with nose clip) and deposition endpoints.

Deposition endpoints	**#**	**CSA1**	**CSA2**	**CSA3**
Mouth wash	1	0.25	0.06	0.76
Oropharyngeal	2	-0.36	-0.02	0.22
Stomach	3	-0.19	-0.21	0.40
Total oropharyngeal	4	-0.18	-0.18	0.47
Right lung - central	5	-0.14	0.14	-0.65
Left lung - central	6	0.21	0.25	-0.50
Total central lung	7	0.05	0.21	-0.61
Right lung - peripheral	8	0.25	0.06	-0.37
Left lung - peripheral	9	0.11	0.22	-0.27
Total peripheral lung	10	0.20	0.13	-0.34
Total lung	11	0.17	0.17	-0.48
Right lung C/P ratio	12	-0.42	0.06	-0.39

Table 3.12: The p-values for correlations between CSA1-3 (with nose clip) and deposition endpoints.

Deposition endpoints	**No**	**CSA1**	**CSA2**	**CSA3**
Mouth wash	1	0.31	0.82	0.0003
Oropharyngeal	2	0.14	0.92	0.39
Stomach	3	0.44	0.40	0.10
Total oropharyngeal	4	0.48	0.47	0.0495
Right lung - central	5	0.59	0.58	0.0037
Left lung - central	6	0.40	0.31	0.0342
Total central lung	7	0.83	0.39	0.0073
Right lung - peripheral	8	0.31	0.81	0.13
Left lung - peripheral	9	0.67	0.38	0.27
Total peripheral lung	10	0.43	0.59	0.16
Total lung	11	0.49	0.49	0.0454
Right lung C/P ratio	12	0.09	0.82	0.11

None of the correlation coefficients showed a strong correlation between CSA1-2 and deposition endpoints, whereas for CSA3 several relatively strong correlations (mouth wash, lung deposition parameters) were found (Table 3.11). There were several statistically significant correlations between CSA3 and mouth wash, total oropharyngeal and lung deposition endpoints (Table 3.12).

3.7.8 Correlations between AUCs and deposition endpoints

The acoustic pharyngometer data for AUC1-3 from measurements BNC and CNC were correlated to each of the 12 deposition endpoints from the lung deposition study. The obtained Pearson correlation coefficients between AUCs and depositions endpoints are presented in Table 3.13. The corresponding p-values for correlations between AUC1-3 (with nose clip) and deposition endpoints are presented in Table 3.14. As in the analysis for the CSAs and deposition endpoints the results from TBM (BNC) and TIM (CNC) measurements were pooled.

Table 3.13: Pearson correlation coefficients for correlations between AUC1-3 (with nose clip) and deposition endpoints.

Deposition endpoints	**#**	**AUC1**	**AUC2**	**AUC3**
Mouth wash	1	0.27	-0.18	0.33
Oropharyngeal	2	-0.25	-0.20	0.18
Stomach	3	0.40	-0.27	0.60
Total oropharyngeal	4	0.36	-0.28	0.59
Right lung – central	5	-0.53	0.30	-0.43
Left lung – central	6	-0.45	0.36	-0.61
Total central lung	7	-0.52	0.36	-0.56
Right lung - peripheral	8	-0.15	0.31	-0.52
Left lung - peripheral	9	-0.38	0.15	-0.54
Total peripheral lung	10	-0.26	0.25	-0.55
Total lung	11	-0.36	0.28	-0.58
Right lung C/P ratio	12	-0.36	0.06	0.11

Table 3.14: The p-values for correlations between AUC1-3 (with nose clip) and deposition endpoints.

Deposition endpoints	**#**	**AUC1**	**AUC2**	**AUC3**
Mouth wash	1	0.29	0.47	0.18
Oropharyngeal	2	0.32	0.43	0.48
Stomach	3	0.10	0.27	0.0086
Total oropharyngeal	4	0.14	0.25	0.0103
Right lung - central	5	0.0251	0.22	0.07
Left lung - central	6	0.06	0.15	0.0077
Total central lung	7	0.0270	0.15	0.0151
Right lung - peripheral	8	0.55	0.21	0.0277
Left lung - peripheral	9	0.12	0.56	0.0199
Total peripheral lung	10	0.30	0.31	0.0172
Total lung	11	0.14	0.27	0.0113
Right lung C/P ratio	12	0.14	0.82	0.67

None of the correlation coefficients showed a strong correlation between AUC1-2 and depositions endpoints, whereas for AUC3 several relatively strong correlations (stomach, total oropharyngeal and lung depositions related parameters) were found.

The p-values in Table 3.14 show statistically significant correlations between AUC3 and stomach, total oropharyngeal and lung depositions related endpoints. The results matched the stronger correlation coefficients for AUC3 shown in Table 3.13.

The correlations between AUCs and deposition endpoints - one for each AUC – are presented graphically in Figures 3.14 to 3.16, as follows:

- Figure 3.14, correlation between AUC1 and right lung - central deposition.
- Figure 3.15, correlation between AUC2 and total central lung deposition.
- Figure 3.16, correlation between AUC3 and stomach deposition.

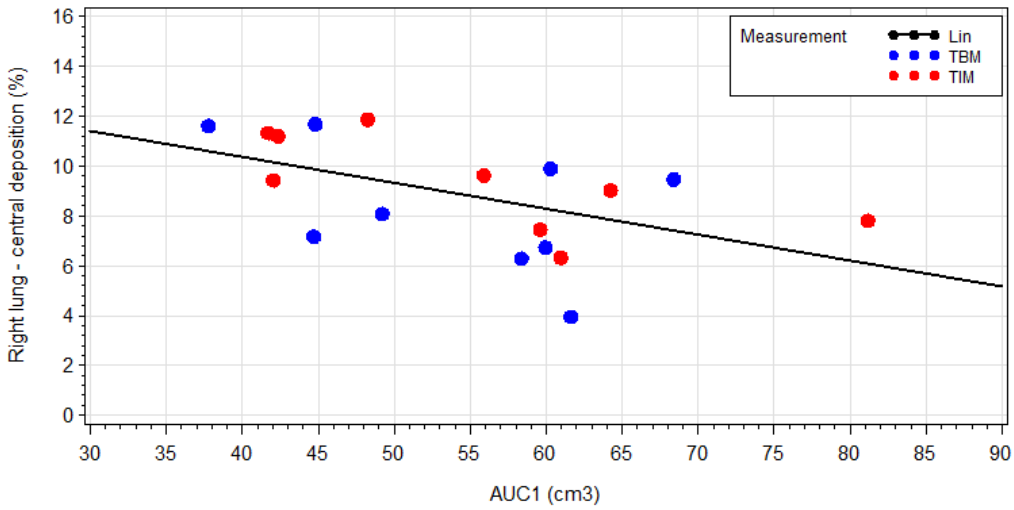


Figure 3.14: Correlation between AUC1 and right lung - central deposition. TBM (blue) and TIM (red) data were pooled.

The plot in Figure 3.14 shows that the right lung central deposition was negatively correlated with AUC1 and that TIM and TBM data followed the same trend.

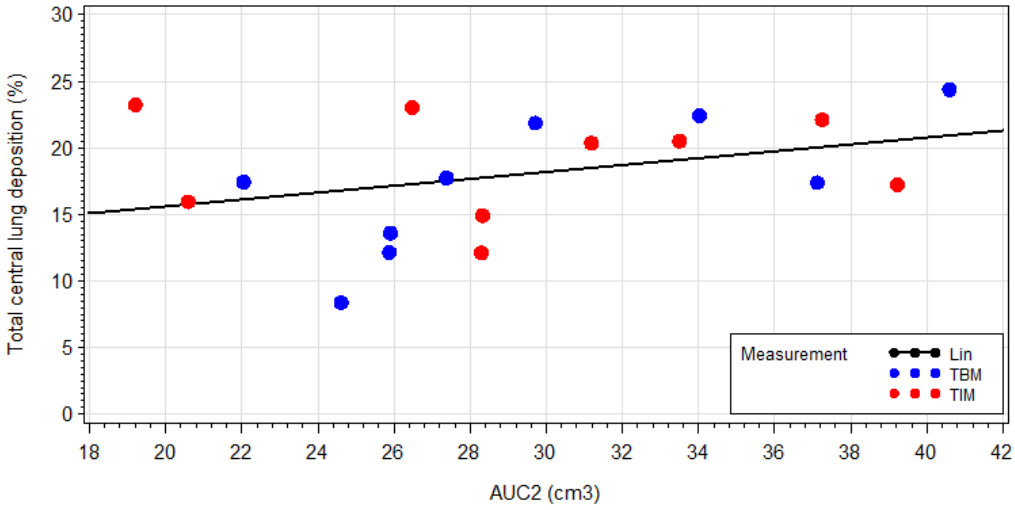


Figure 3.15: Correlation between AUC2 and total central lung deposition. TBM (blue) and TIM (red) data were pooled.

The plot in Figure 3.15 shows a weak correlation between the total central lung deposition and AUC2, and that the trend appears somewhat stronger for TBM data. The plot in Figure 3.16 shows that the stomach deposition was positively correlated with AUC3.

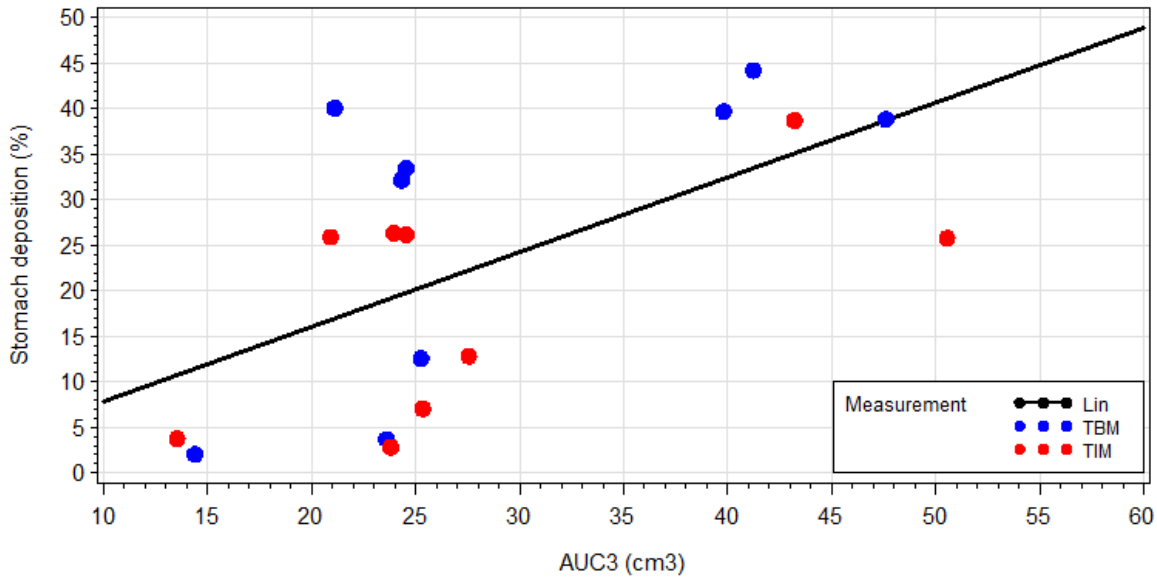


Figure 3.16: Correlation between AUC3 and stomach deposition. TBM (blue) and TIM (red) data were pooled.

3.7.9 Analysis of secondary variables

The parameters included in the secondary objectives were as follows:

- Assessment of the pharyngeal space using the pharyngeal grading system.
- Assessment of the tongue size using a modified Mallampati score.
- Measurement of the cricomental space.
- Measurement of tongue scalloping.
- Measurements with the Oral Mez device.

The results of the analysis of the secondary variables are presented in Table 3.15.

Table 3.15: Results of secondary parameters for each subject.

Subject		**3**	**4**	**5**	**6**	**8**	**9**	**10**	**11**	**12**
Pharyngeal Space (class)[1]		I	II	III	I	I	I	III	I	I
Mallampati score (class)[2]		II	IV	IV	II	I	I	I	II	II
Cricomental space (value)[3]		2	0	11	2	7	15	0	8	5
Tongue scalloping (class)[4]		A	C	B	A	A	A	D	B	A
Oral Mez	Palatal height[5]	14	14	15	14	16	25	9	12	8
	Maxillary intermolar distance[5]	R: 4 L: 2	R: 5 L:4.5	R: 3 L: 4	R: 7.5 L: 2.5	R: 2 L: 4	R: 5 L: 6	R: 3 L: 4	R: 3 L: 2	R: 2 L: 4
	Mandibular intermolar distance[5]	R: 3.5 L: 2	R: 5 L: 5	R: 2 L: 4	R: 6 L: 3	R: 2 L: 6	R: 6 L: 5	R: 3 L: 4	R: 3 L: 4	R: 3 L: 6
	Incisor overjet[5]	8	7.5	8	12.5	7	9	8	11	8
	Tongue length[5]	30	38	28	29	42	28	40	38	42
	Comments[5]				*					

Keys:

[1] Individual patient results for the assessment of pharyngeal space using the pharyngeal grading system:

- I = Palatopharyngeal arch intersects at the edge of the tongue
- II = Palatopharyngeal arch intersects at 25% or more of the tongue diameter
- III = Palatopharyngeal arch intersects at 50% or more of the tongue diameter

[2] Individual results for the assessment of tongue size using a modified Mallampati score

[3] Individual results for the measurement of cricomental space

[4] Individual results for the measurement of tongue scalloping:

A = Complete absence of scalloping

B = Scalloping evident but not pronounced

C = Scalloping pronounced and unresolved with tongue protrusion

D = Scalloping pronounced but resolved with tongue protrusion

[5] Individual subject results for parameters measured using the Oral Mez:

R = Right

L = Left

* Partial dentures

The pharyngeal space and tongue scalloping measurements followed the same trend with low (6 out of 9, I *versus* A or B), and high values for the same subjects, whereas this was less obvious for the Mallampati scores, and the cricomental space measurements. For the Oral Mez measurements the palatal height and the incisor overjet measurements (8 and 7 out of 9) followed the same trend with low values for the same subjects.

3.7.10 Correlation between secondary variables and deposition

The 8 numeric secondary parameters were correlated to each of the 12 deposition endpoints, and the results are shown in Table 3.16.

Table 3.16: Pearson correlation coefficients for correlations between numeric secondary parameters and deposition endpoints.

Deposition endpoint	**Crico-mental Space**	**Palatal Height**	**Maxillary Intermolar Distance**		**Mandibular Intermolar Distance**		**Incisor Overjet**	**Tongue Length**
			R	L	R	L		
Mouth wash	-0.26	-0.22	-0.03	0.04	-0.07	-0.19	-0.07	0.05
Oropharyngeal	-0.06	-0.29	-0.45	-0.27	-0.36	-0.07	-0.16	0.29
Stomach	0.12	0.03	-0.17	0.04	-0.11	-0.34	-0.13	-0.19
Total Oroharyngeal	0.07	-0.03	-0.20	0.02	-0.14	-0.33	-0.14	-0.13
Right lung - central	0.13	0.13	0.01	0.19	0.16	0.60	-0.01	0.29
Left lung - central	-0.06	0.02	0.38	-0.24	0.32	0.19	0.52	0.10
Total central lung	0.03	0.07	0.22	-0.04	0.26	0.40	0.30	0.20
Right lung - peripheral	0.08	0.17	0.13	-0.00	-0.02	0.22	0.07	-0.02
Left lung - peripheral	-0.21	-0.21	0.01	-0.03	-0.05	0.34	-0.04	0.27
Total peripheral lung	-0.05	0.01	0.08	-0.01	-0.03	0.28	0.02	0.11
Total lung	-0.07	0.03	0.20	-0.02	0.14	0.33	0.13	0.13
Right lung C/P ratio	0.16	0.09	-0.05	0.22	0.26	0.39	0.00	0.24

None of the Pearson correlation coefficients were strong and only two were larger than 0.50; mandibular intermolar distance L *versus* right lung – central (0.60), and incisor overjet *versus* left lung – central (0.52). These were statistically significant, $p = 0.0090$ and $p = 0.0267$. For the 3

character secondary endpoints the effect of these was explored using ANOVA; the associated effects (p-values) are presented in Table 3.17.

Table 3.17: p-values for correlations between Mallampati scores, pharyngeal spaces, tongue scalloping and deposition endpoints.

Deposition endpoint	**Mallampati score**	**Pharyngeal space**	**Tongue scalloping**
Mouth Wash	0.5584	0.0995	0.0448
Oropharyngeal	0.5377	0.7948	0.7822
Stomach	0.8150	0.1652	0.0892
Total oroharyngeal	0.8312	0.1548	0.0729
Right lung - central	0.8821	0.0128	0.0159
Left lung - central	0.2760	0.0170	0.0947
Total central lung	0.5197	0.0062	0.0253
Right lung - peripheral	0.8992	0.4343	0.1588
Left lung - peripheral	0.8259	0.5302	0.2920
Total peripheral lung	0.9686	0.5021	0.1963
Total lung	0.8329	0.1482	0.0705
Right lung C/P ratio	0.7911	0.0458	0.3227

No statistically significant results for the Mallampati score were found, whereas for pharyngeal space and tongue scalloping some significant results for the central lung endpoints were found. There was also a significant effect by tongue scalloping on the mouth wash.

3.8 Discussion

The primary objective of the present study was to use an AR technique for measurements of the upper airways of healthy subjects that had previously been enrolled in a lung deposition study in which the I-neb nebuliser had been used in both TBM and TIM breathing modes (Nikander et al., 2010c). The purpose of the characterisation of the subjects' upper airways was to perform correlation analyses between the upper airway characteristics, and the deposition of nebulised ^{99m}Tc-DTPA in the upper airways and lungs from the previous lung deposition study.

Acoustic pharyngometry was chosen in favour of other techniques as the subjects could be seated during the measurements in the same position as in the previous lung deposition study. The fact that acoustic pharyngometry is a non-invasive, inexpensive, simple and fast technique allowing numerous measurements, were additional reasons for the choice. Nine out of the 12 subjects that participated in the previous lung deposition study agreed to participate in the present study. The measurements were standardised such that the subjects would be able to mimic the inspiratory flows of their breathing patterns in the previous lung deposition study. The pharyngograms were analysed in terms of CSAs for the OPJ (CSA1), the EG (CSA2) and the GL (CSA3), and in terms of AUCs for the oral cavity (AUC1), the oropharynx (AUC2) and the hypopharynx (AUC3). The correlation of CSAs and AUCs with the oropharyngeal and lung deposition results of the 9 subjects from the previous lung deposition study was investigated using an exploratory analysis. No AEs were recorded.

In the present study the timing of the pharyngometer measurement to the subject's breathing cycle was important as the pharyngometer measurements were planned to be performed both during exhalation and inhalation. The use of a pneumotachograph connected to the back end of the pharyngometer wavetube made it possible to follow the subjects breathing cycle, and based on the cycle shown on a laptop monitor, decide when to start and when to stop the measurement. The upper airway is a dynamic structure and changes in the CSAs of this structure has been shown to occur during breathing with the maximal increase occurring during exhalation (Schwab et al., 1993a; Schwab et al., 1993b; Schwab, 1998). The pharyngometer measurement during exhalation was therefore started at mid-inhalation and stopped at end of exhalation with the pharyngogram from mid to end of exhalation recorded. The measurements during inhalation - with and without the addition of an I-neb nebuliser TBM mouthpiece - were started at mid-exhalation and stopped at end of inhalation with the pharyngogram from mid to end of inhalation recorded. When the I-

neb nebuliser TIM mouthpiece was used, the measurements were started at the start of inhalation, and stopped at end of the same inhalation with the pharyngogram from the mid to end of inhalation recorded. The standardisation of the pharyngometer measurement was novel and should have minimised variability of the acoustic pharyngometer measurements.

The aim was to record during inhalation 4 pharyngograms per each measurement and these should be as similar as possible. An initial review of all recorded acoustic pharyngograms indicated that some of the 4 pharyngograms per measurement deviated from the rest. To identify deviating pharyngograms, a measure of the "goodness of fit" (GOF) was developed by which each of the 4 pharyngograms was compared to the median pharyngogram and pharyngograms deviating too much were excluded from further statistical analysis. GOF was defined as the square root of the average squared vertical distance between the median pharyngogram and the pharyngogram under study. The GOF-calculation was performed from the first maxima to CSA3 and defined for each subject individually. The choice of a cut-off of 0.5 between accepted or not was subjectively made based on the plot as there were no published references to base the decision upon. There was considerable inter-subject variability between the pharyngograms. The addition of a nose clip caused a change in the pharyngograms with larger CSAs and AUCs for 5 of the 9 subjects.

The mean CSAs in the present study ranged from 3.2 cm^2 to 5.0 cm^2. Data on CSAs from pharyngograms in adult healthy subjects from measurements with the Eccovision ARP have been published by a number of authors (Kamal, 2001; Kamal, 2002; Jung et al., 2004; Kamal, 2004a; Kamal, 2004b; Monahan et al., 2005; Shiota et al., 2007; Allen et al., 2014; Oliver et al., 2014). In some of these studies the criteria for the acoustic pharyngometer measurements differ from those used in the present study (no information on position during measurements in Allen et al (2014) and Oliver et al (2014); supine position during measurements in Shiota et al (2007); different definition of CSAs in Monahan et al (2005). However, in the studies by Jung et al (2004), Kamal

(2001), Kamal (2002), Kamal (2004a), and Kamal (2004b), the CSAs can be compared with those measured in the present study (Table 2.1; section 2.2.9). In the studies by Kamal only mean CSAs for the whole pharyngograms are presented and these range from 2.4 cm^2 to 3.2 cm^2, whereas in the study by Jung the mean CSAs for the OPJ and GL are reported (1.6 cm^2 and 1.8 cm^2). As Kamal did not define when in the breathing cycle the pharyngograms were measured it seems reasonable to assume that the mean CSAs are similar to those measured in the present study. The CSAs reported by Jung are somewhat smaller than CSA2 and CSA3 measured in the present study which most probably is due to different populations.

The analysis of the pharyngograms for CSAs and AUCs showed that the TBM and TIM breathing modes ("inhalation mode"), and the different inspiratory flows had no statistically significant effect on any of the 3 CSAs or any of the 3 AUCs. This is novel information and interesting as lung deposition following slow and deep breathing has been shown to increase with this breathing pattern. The effect seems therefore to be related to droplet behaviour during inhalation and diminished impaction of the inhaled droplets.

The correlation analysis - when using "nose clip" data - between the CSAs and oropharyngeal and lung depositions, showed statistically significant correlations between CSA3 and total oropharyngeal and total lung deposition including some of the subdivisions. The correlation coefficients ranged from 0.47 (total oropharyngeal deposition) to -0.61 (total central lung). The same analysis between the AUCs and oropharyngeal and lung depositions, showed statistically significant correlations between AUC3 and total oropharyngeal and total lung deposition including the subdivisions with stronger correlation coefficients (-0.59 and 0.58). The results indicated that the volume of the lower parts of the upper airways between the EG and GL had the strongest correlation with the oropharyngeal and lung deposition data from the previous study. The reason for the lack of correlation between AUC1 and the oropharyngeal and lung depositions might be

due to the different study setups. In the lung deposition study the subjects had the chance to move their tongues – although they were instructed not to do that - as the I-neb nebuliser mouthpiece has a relatively short piece held between the teeth. In the present study the acoustic pharyngometer mouthpiece had a tongue depressor preventing major tongue movements. The small number of subjects in the study was most probably the main reason for the lack of more significant correlations between the acoustic pharyngometer derived upper airway dimensions, and oropharyngeal and lung depositions of ^{99m}Tc-DTPA in the 9 subjects.

The impact of inhalation through different inhalation devices on the upper airway has been the focus of a number of clinical studies in which the upper airways have been measured during inhalation or tidal breathing with MRI equipment with the subjects in a supine position (Ehtezazi et al., 2004; Pritchard et al., 2004; Ehtezazi et al., 2005; McRobbie et al., 2005). In some of these studies different CSA measures of the upper airway were shown to be prone to significant variations dependent on whether a DPI or a pMDI (with or without spacer or VHC) was used (Ehtezazi et al., 2004; Ehtezazi et al., 2005). Expansion of the oropharynx and the laryngo-pharynx was shown following forced breathing manoeuvres compared with tidal breathing when testing high resistance dummy inhalation devices (McRobbie et al., 2005). The CSAs of the oral cavity, the oropharynx and the larynx was shown to have considerable variability mainly due to the variability of the tongue position (Ehtezazi et al., 2004). The CSAs of these studies were only reported by Ehtezazi et al (2004) and only for the oropharynx instead of the OPJ as in the present study, and the CSAs were smaller than those found in the present study (Table 3.18).

Table 3.18: The CSAs and volumes of the upper airways in adult healthy subjects in published studies compared with CSAs and AUCs in the present study. In the published studies MRI with the subjects in supine position was used.

1st author, year published	**Subjects (male)**	**CSA (cm^2)**	**Volume (cm^3)**
Ehtezazi et al., 2004	10 (6)	Oropharynx CSA: pMDI = 1.5 cm^2 spacer = 2.1 cm^2 DPI = 2.8 cm^2	Mean total upper airway volume: pMDI = 56 cm^3 spacer = 59 cm^3 DPI = 70 cm^3
Ehtezazi et al., 2005	7 (5)	Not reported	Mean total upper airway volume: orifice 1 = 72 cm^3 orifice 6 = 101 cm^3
McRobbie et al., 2005	5 (3)	Not reported	Mean total upper airway volume, tidal breathing = 38 cm^3
Pritchard et al., 2004	20 (10)	Not reported	Mean total upper airway volume, tidal breathing: males = 47 cm^3 females = 43 cm^3
Present study	9 (2)	CSA1 = 3.2 cm^2 CSA2 = 3.5 cm^2 CSA3 = 5.0 cm^2	AUC1 = 56 cm^3 AUC2 = 23 cm^3 AUC3 = 21 cm^3 Mean total AUC = 100 cm^3

The volumes of the upper airways were reported in these studies but are again somewhat difficult to compare with the present results due to different definitions of the volumes measured. As shown in Table 3.18 the volumes of the upper airways ranged from 38 cm^3 (McRobbie et al., 2005) to 101 cm^3 (Ehtezazi et al., 2005). The reasons for the differences in CSA and upper airway volume could probably be found in the somewhat different definitions of the upper airways, in the individual differences between subjects, and in the difference in position (seated position *versus* supine position) when measuring the CSAs and the volumes of the upper airways. The impact of the supine position in comparison with the seated position on the size of the upper airways has

been measured in healthy subjects using AR (Fouke et al., 1987), acoustic pharyngometry (Jan et al., 1994; Jung et al, 2004) and CT)/CBCT scans (Van Holsbeke et al., 2014a) (section 2.8). The CSAs were shown to be 23% (Fouke et al., 1987), 21% (OPJ; Jan et al., 1994), 19% (OPJ) and 24% (GL; Jung et al., 2004), and ~50% (hard palate and the bottom of the uvula; Van Holsbeke et al., 2014a) smaller in the supine position.

Nose clips have been used in lung deposition studies in order to ensure oral breathing. Köhler et al (2004) investigated in 10 subjects with CF whether the use of nose clips would improve the relative lung deposition of nebulised sodium cromoglycate. The urinary excretion of sodium cromoglycate was used as a marker of lung deposition and the results did not show a statistically significant difference between inhalation without and with nose clips (Köhler et al., 2004). There are, however, other results that indicate that the use of a nose clip might increase the amount of drug inhaled when using nose clips (Meier et al., 2001). In the study by Meier et al inspiratory and expiratory filters were added to the nebuliser in order to catch the amount of nebulised salbutamol that could have been inhaled by the 13 subjects that participated in the study. The authors concluded that: "Wearing a noseclip leads to an increase of 113% (SEM 23.5) in drug delivery and improves the inspiratory *versus* expiratory ratio (ratio 2.07 *versus* 0.75)." Thus the use of nose clips during lung deposition studies could have an impact on the deposition of the inhaled aerosol as the nose clip would eliminate air entrainment through the nose. The use of a nose clip during inhalation might therefore increase inhalation effectiveness.

To ensure oral breathing during acoustic pharyngometer measurements, nose clips are widely used although there is limited information on the possible impact of these on the pharyngogram. As nose clips were used in the previous lung deposition study these were also included in the present study in which the acoustic pharyngometer measurements were performed both without and with nose clips. The correlation analyses were also performed with the nose clip data as an impact of

the nose clips could not be ruled out. The statistical analysis of the pharyngograms for the different CSAs showed, however, that the use of nose clips had a statistically significant effect on CSA2 increasing it by 26%. The same analysis for the AUCs showed that the use of nose clips had a statistically significant effect on both AUC2 and AUC3 increasing these by 23% (AUC2) and 31% (AUC3), respectively. There are few references on the possible impact of the use of nose clips on the pharyngograms. In the letter to the editor by Molfino et al (1990) the authors discussed possible artefacts during acoustic pharyngometer measurements and mentioned that: "Opening of the velum frequently occurs when the subject is wearing noseclips during measurements; removal of the noseclips may result in the closure of the velum." Molfino et al (1990) also showed an example of two pharyngograms performed with the subject either wearing a nose clip or not, and stated: "Probably the most important and frequent artifact that results in overestimation of glottis and tracheal areas is opening of the nasopharyngeal velum." The artefact discussed by Molfino et al has also been reported by Marshall et al (1993) who used a later prototype version of the acoustic pharyngometer although not the Eccovision ARP, and mentioned by authors using the Eccovision ARP (Monahan et al., 2005; Patel et al., 2008).

So why would an open velum create an artefact during acoustic pharyngometry measurements? According to Molfino et al (1990) an open nasopharyngeal velum during acoustic pharyngometry leads to an over-estimation of the lower upper airway (distal pharynx, GL and trachea) as the acoustic pulses will propagate from the mouth to the nasopharynx and the paranasal sinuses where they are reflected in order to propagate along the rest of the upper airway. The pharyngogram presented by Marshall et al (1993) indicated that the artefact might occur at ~10-14 cm from the end of the wavetube which should correspond with CSA2. This might be a reasonable explanation to the increases found in CSA2, AUC2 and AUC3 in this study. It does not, however, explain why this did not occur in all subjects in the present study or why the increases were very modest in

comparison with the increases shown by Molfino et al. It also indicates that based on the present results nose clips should be avoided when performing acoustic pharyngometer measurements during inhalation.

The secondary objectives of the study included several assessments and measurements of the oral cavity including a grading of the pharyngeal space with focus on the palatopharyngeal arch of the tongue, a modified Mallampati classification with focus on the visibility of the faucial pillars, the soft palate and the uvula, an assessment of the cricomental space using a ruler to connect the cricoid cartilage to the inner mentum, a measurement of the tongue scalloping, and a measurement of the oral cavity with the Oral Mez. The pharyngeal space and tongue scalloping measurements followed the same trend with high values for the same subjects, whereas this was less obvious for the Mallampati scores and the cricomental space measurements. For the Oral Mez measurements the palatal height and the incisor overjet measurements followed the same trend with low values for the same subjects. The correlation analysis between these oral cavity focused endpoints and lung depositions showed statistically significant correlations between mandibular intermolar distance L *versus* right lung – central, incisor overjet *versus* left lung – central, and pharyngeal space and tongue scalloping *versus* central lung endpoints.

3.9 Conclusions

The study hypothesis: “the anatomy of the upper airway determines subsequent deposition of aerosol in the upper airway and therefore the deposition of aerosol in the lung” was based on the results of the previous lung deposition study (Nikander et al., 2010c), and published results supporting the hypothesis (Svartengren et al., 1996; Borgström et al., 2006). In the review by Borgström et al (2006), the authors found 71 studies with relevant information on lung deposition and its variability. The authors concluded that: “Using a published throat deposition model, the

observed correlation of lung deposition variability to mean lung deposition could be explained as being determined largely by the extent of and variability in throat deposition". Borgström et al hypothesised that: "throat deposition is the major determinant for lung deposition of an inhaled aerosol, and its absolute variability will largely be determined by the absolute variability in throat deposition". Their conclusion and hypothesis support the present study hypothesis.

The correlation analyses between the acoustic pharyngometry measurements and the oropharyngeal and lung depositions showed statistically significant correlations. The correlations between AUC3, and total oropharyngeal and total lung deposition showed the strongest correlation coefficients. These correlations indicated that the volume of the lower parts of the upper airways between the EG and the GL had the strongest correlation with the oropharyngeal and lung deposition data from the previous study. Thus the study confirmed the study hypothesis that: "the anatomy of the upper airway determines subsequent deposition of aerosol in the upper airway and therefore the deposition of aerosol in the lung".

Chapter 4 Mandibular advancement achieved through a stepped mouthpiece design and the size of the upper airways – a proof-of-concept study

4.1 Introduction

4.1.1 Study background

The background to this proof-of-concept study can be found in the analysis of the acoustic pharyngometer data presented in Chapter 3 "Assessment of the upper airways in healthy subjects using acoustic pharyngometry". The analysis of the pharyngograms showed large differences in upper airway size and the differences were related to both the CSAs and the AUCs of the upper airways. This raised questions related to the expansion and contraction of the upper airways and whether it would be possible to increase the size of the CSAs and AUCs of the upper airways. The movement of the mandible or the tongue through the use of different oral appliances in order to increase the CSAs and AUCs of the upper airways in subjects diagnosed with OSA has been well documented (Chapter 2, Table 2.6). The question was whether the upper airways could be expanded with a mouthpiece that advanced the mandible during inhalation. The new mouthpiece was labelled a "stepped mouthpiece". The assumption was that as mandibular advancement could expand the size of the upper airways in subjects with OSA both during wakefulness and sleep, the same might be achieved during wakefulness in subjects not diagnosed with OSA. The stepped mouthpiece would be a device that could be adapted to different inhaler designs including nebulisers, pMDIs (with or without spacers and VHCs) and DPIs. A patent application was subsequently submitted in 2009 for a stepped mouthpiece and was published in October 2011 (US 2001/0240015 A1; Chapter 2, section 2.10 of this thesis).

4.1.2 Oral appliances for the treatment of OSA

As described in Chapter 2, sections 2.9.1 to 2.9.5 of this thesis.

Oral appliances for the treatment of OSA differ in terms of design, material, location of coupling mechanism and amount of possible horizontal (advancement) and vertical jaw movement (Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Ferguson et al., 2006; Hoffstein, 2007; Fleetham et al., 2010; Wee, 2012; Sutherland et al., 2014). An increase of the size of the upper airway (Ryan et al., 1999) and a decrease of the collapsibility of the upper airway during sleep are the two main proposed actions of oral appliances in subjects with OSA (Ng et al., 2003; Hoekema et al., 2004; Viviano, 2004; Bailey, 2005; Hoffstein, 2007; Fleetham et al., 2010; Wee, 2012; Sutherland et al., 2014).

A number of airway-imaging studies have been performed in both healthy subjects and in subjects with OSA using oral appliances including cephalometry, CT, MRI and videoendoscopy (Fleetham et al., 2010). Mandibular advancement and tongue protrusion have been shown to increase the size of the upper airway and alter the shape of the upper airways – particularly in the velopharynx in healthy subjects and in subjects with OSA (Ferguson et al., 1997a; Johal et al., 1999). The use of oral appliances have in other studies been shown to increase the anteroposterior diameter of the upper airway (oropharynx and hypopharynx; Ng et al., 2003), to increase the total volume of the upper airway and CSAs of the retropalatal and retroglossal regions (Sam et al., 2006; Kyung et al., 2005) and to increase the lateral dimensions of the velopharynx (Zhao et al., 2008; Chan et al., 2010a). These results indicate that mandibular advancement achieved with a new stepped mouthpiece might expand the upper airway during inhalation.

4.1.3 Acoustic pharyngometry

As described in Chapter 2, section 2.7 of this thesis.

The Eccovision ARP (Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com) was used in the study and the pharyngograms obtained from the acoustic pharyngometer measurements were analysed in terms of CSAs and AUCs of the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx and the GL.

4.1.4 A new stepped mouthpiece – the patent

As described in Chapter 2, section 2.10 of this thesis.

The possibility to enlarge the upper airway through mandibular advancement led to the development of a new stepped mouthpiece as a tool to achieve mandibular advancement when using an inhaler such as a nebuliser, a pMDI or a DPI. The stepped mouthpiece (without tongue depressor) is shown in Chapter 1, Figure 1.1 (patent US 2011/0240015 A1; published 6 October, 2011; PCT filed 23 November, 2009). The new stepped mouthpiece is described in the abstract of the patent US 2011/0240015 A1 as follows:

"The invention of the present application relates to an apparatus to aid in administering inhaled pharmaceutical aerosol to a patient. The apparatus is used in conjunction with an aerosol delivery device. The apparatus comprises steps on the top and bottom of the apparatus, which when used aid the patient causes mandibular advancement, and opening of the mouth, causing opening of patient's airway, resulting in improved aerosol lung deposition. The invention also relates to a method of using such apparatus in a combination with an aerosol delivery device or a system, and to the mouthpiece of said apparatus."

A proof-of-concept study of a stepped mouthpiece design was discussed in order to gain information regarding the potential effects of mandibular advancement achieved with the stepped mouthpiece on the upper airway CSAs and AUCs during inhalation.

4.1.5 Study hypothesis

Horizontal mandibular advancement and incisal opening following use of a stepped mouthpiece can increase the CSAs and AUCs of the upper airways - including the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx and the GL - in healthy subjects.

4.2 Methods

4.2.1 Study design and study variables

The study was designed as a proof-of-concept study including 4 healthy subjects. The analysis of the upper airways included the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx and the GL. The following acoustic pharyngometry recordings were made:

- Two baseline acoustic pharyngometer recordings during exhalation using the standard mouthpiece attached to the wavetube. The recordings were made at FRC during tidal breathing without nose clips.
- Two baseline acoustic pharyngometer recordings during inhalation using the standard mouthpiece attached to the wavetube. The recordings were made at mid-inhalation during tidal breathing without nose clips.
- Two acoustic pharyngometer recordings during exhalation per each of the 12 stepped mouthpiece options. The recordings were made at FRC during tidal breathing without nose clips.
- Two acoustic pharyngometer recordings during inhalation per each of the 12 stepped mouthpiece options. The recordings were made at mid-inhalation during tidal breathing without nose clips.

In the study the Eccovision ARP was used. The measurements with the acoustic pharyngometer were performed and analysed by a dentist, Dr John Viviano at his office in Mississauga, Ontario, Canada and the raw data was saved onto the hard disk of the Eccovision ARP in his office. The pharyngograms were further analysed in terms of mean CSAs and AUCs through the acoustic pharyngometer software.

4.2.2 The new stepped mouthpieces

A set of 12 stepped mouthpieces were designed with a round back orifice to be connected to the pharyngometer wavetube and an oval front orifice to be kept between the incisors (Figure 4.1).

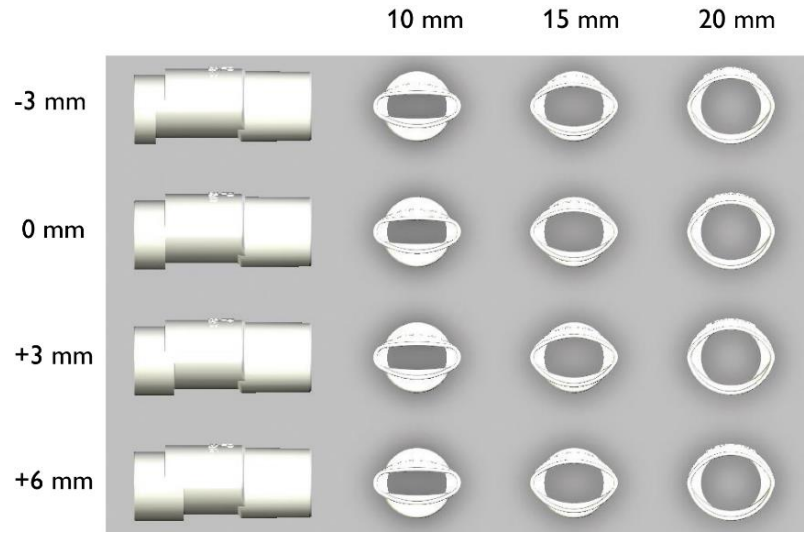


Figure 4.1: The stepped mouthpieces used in the study. The oval orifices to be kept between the incisors were designed with 10 mm, 15 mm and 20 mm vertical distances (right 3 columns). The protrusion on the lower side (left column) was made to achieve 4 horizontal advancements of the mandible of either -3 mm, ±0 mm, +3 mm or +6 mm in relation to the protrusion on the upper side.

The oval orifices of the mouthpiece to be kept between the incisors were designed with 10 mm, 15 mm and 20 mm orifices (vertical diameters). These orifices were also designed with a single protrusion on the upper side and 4 protrusions on the lower side at different distances (-3 mm, ±0 mm, +3 mm and +6 mm) in relation to the protrusion on the upper side for horizontal movement of the mandible. The horizontal offsets were -3 mm (lower jaw moved back from an incisal edge-to-edge position), ±0 (incisal edge-to-edge position), + 3 mm and +6 mm (mandible moved forward from an incisal edge-to-edge position). The stepped mouthpiece was 40 mm long and the CSAs of the orifices with 10 mm, 15 mm and 20 mm vertical diameters were 161 mm^2, 232 mm^2 and 278 mm^2, respectively. The stepped mouthpieces were manufactured on a 3D prototyping

machine (Stratasys Dimension BST 768; Eden Prairie, MN, USA) from an acrylonitrile butadiene styrene copolymer.

In Figure 4.2 a stepped mouthpiece is shown with the medium (15 mm) sized orifice kept between the incisors. The round back orifice was designed to fit the acoustic pharyngometer wavetube. In the figure the area of the upper airways that might be expanded covers the upper airways from the oral cavity to the GL.

Figure 4.2: The medium (15 mm orifice) sized stepped mouthpiece shown between the incisors. The upper incisors are set against the protrusion on the upper side of the stepped mouthpiece, whereas the lower incisors are extended over a similar protrusion on the lower side of the stepped mouthpiece. The area of the upper airways that might show a change in dimensions is highlighted in dark violet.

The stepped mouthpiece (to the left in Figure 4.3) was connected with the acoustic pharyngometer wavetube through a green elastomeric-lipped ISO connector (Intersurgical Ltd, Wokingham, UK).

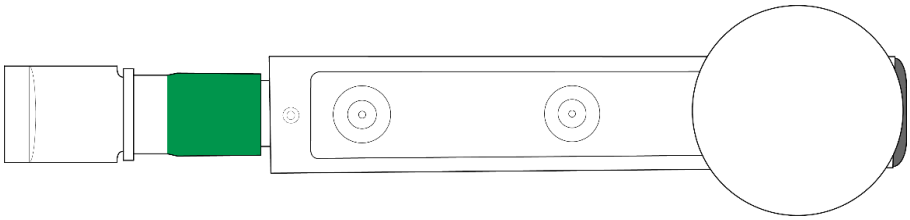


Figure 4.3: The stepped mouthpiece (to the left) attached to the pharyngometer wavetube through a green connector as used in the study.

4.2.3 Study subjects

Four healthy male subjects (A, B, C and D), age range 45-65, were included. The acoustic pharyngometer measurements were performed with the subjects seated on a straight-backed chair. The aim was to keep the wavetube horizontally parallel to the floor and prevent head, neck and shoulder movement by instructing the subjects to keep their gaze fixed at a point on the wall. A comfortable position was important in order to avoid any increase in muscle tonus through heavy occlusion on the mouthpieces (Viviano, 2002a). The measurements followed the instructions to a subject in the Eccovision Acoustic Pharyngometry Operator Manual (Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com) and as outlined in Chapter 3, section 3.3.2.1.

4.3 Statistical analysis

4.3.1 Data analysis

The analysis of the data was descriptive due to the proof-of-concept study design with only 4 subjects included. The primary analysis of the pharyngograms was focused on the mean CSAs and AUCs of the upper airways measured with the stepped mouthpieces during exhalation and inhalation.

The addition of the stepped mouthpiece to the end of the pharyngometer wavetube created a displacement of the pharyngograms of ~4 cm to the right (x-axis) on the screen in comparison with pharyngograms performed without a stepped mouthpiece. The actual displacement was defined by the dentist Dr John Viviano (Mississauga, Ontario, Canada) who performed all the pharyngometry measurements in his office.

The raw acoustic pharyngometer data of each measurement was imported into Microsoft Excel as space delimited data and then converted to SAS data sets for the descriptive analyses and graphic

presentations. The measurements were coded separately for baseline measurements performed during exhalation and inhalation without the stepped mouthpieces, for stepped mouthpiece measurements performed during exhalation (2 recordings per stepped mouthpiece) and inhalation (2 recordings per stepped mouthpiece) based on the stepped mouthpiece orifice size (10 mm, 15 mm or 20 mm orifice, vertical distance) and based on the 4 protrusions on the lower side of the mouthpiece (-3 mm, ±0 mm, +3 mm and +6 mm).

A secondary analysis was focused on CSA1-3 and AUC1-3. This was based on the identification of three landmarks along the pharyngograms from the exhalation measurements with the standard mouthpiece. This was performed for each subject and these corresponded to the OPJ, the EG and the GL. The displacement of the pharyngograms due to the addition of the steppe mouthpiece to the wavetube is shown for each subject in Table 4.1 together with data on the positions of the OPJ, the EG and the GL, and the length of the upper airway analysed.

Table 4.1: Position of landmarks (OPJ, EG, GL) on the pharyngograms per subject after correction for the displacement caused by the stepped mouthpiece on the pharyngogram (x-axis) including the length of the upper airway analysed. The position is given in cm from the y-axis.

Subject	Stepped mouthpiece ended at (cm)	OPJ (cm)	EG (cm)	GL (cm)	Airway length analysed (cm)
A	4.16	9.31	12.31	20.45	18.00
B	4.59	9.31	12.31	20.45	18.43
C	4.59	8.88	12.74	21.74	18.87
D	4.16	7.59	12.74	20.88	18.43

4.4 Results

4.4.1 Baseline - individual pharyngograms

The mean CSAs and AUCs of the individual baseline acoustic pharyngograms are presented per subject in Table 4.2. The measurements were performed without stepped mouthpieces during exhalation (2 pharyngograms) and inhalation (2 pharyngograms).

Table 4.2: Baseline acoustic pharyngometer mean CSAs (cm^2) and mean AUCs (cm^3) recorded with the standard pharyngometer mouthpiece during exhalation (E) and inhalation (I). The segment (cm) of the upper airways included in the analysis is highlighted for each subject.

Subject A	Mean CSA, segment 2.5 cm to 20.5 cm					
Baseline	E	E	Mean	I	I	Mean
	3.82	3.75	3.79	3.79	3.79	3.79

Subject B	Mean CSA, segment 2.0 cm to 20.5 cm					
Baseline	E	E	Mean	I	I	Mean
	3.08	3.15	3.12	3.25	3.38	3.32

Subject C	Mean CSA, segment 2.9 cm to 21.7 cm					
Baseline	E	E	Mean	I	I	Mean
	3.44	3.27	3.36	3.39	3.29	3.34

Subject D	Mean CSA, segment 2.5 cm to 20.9 cm					
Baseline	E	E	Mean	I	I	Mean
	3.15	3.26	3.21	3.30	3.33	3.32

Subject A	Mean AUC, segment 2.5 cm to 20.5 cm					
Baseline	E	E	Mean	I	I	Mean
	68.67	67.44	68.06	68.22	68.15	68.19

Subject B	Mean AUC, segment 2.0 cm to 20.5 cm					
Baseline	E	E	Mean	I	I	Mean
	56.94	58.29	57.62	60.20	62.47	61.34

Subject C	Mean AUC, segment 2.9 cm to 21.7 cm					
Baseline	E	E	Mean	I	I	Mean
	64.75	61.50	63.13	63.78	61.76	62.77

Subject D	Mean AUC, segment 2.5 cm to 20.9 cm					
Baseline	E	E	Mean	I	I	Mean
	58.01	59.94	58.98	60.64	61.27	60.96

4.4.2 Stepped mouthpiece - individual measurements

The individual pharyngograms from the different measurements are included in APPENDIX B.1. As examples the measurements (2 pharyngograms per measurement) for subject A recorded during exhalation (Figure 4.4) and inhalation (Figure 4.5) are shown. The graphs in each figure cover 12 measurements with the stepped mouthpieces (2 pharyngograms per measurement) with separate legends, as follows:

- small (S; 10 mm orifice; -3 mm, 0 mm, 3 mm, 6 mm protrusions),
- medium (M; 15 mm orifice; -3 mm, 0 mm, 3 mm, 6 mm protrusions).
- large (L; 20 mm orifice; -3 mm, 0 mm, 3 mm, 6 mm protrusions).

In the figures the y-axis presents the CSA (cm^2) of the upper airway and the x-axis presents the length of the upper airway from the end of the pharyngometer wavetube (0; check Table 4.1 for clarification) somewhat past the GL (cm).

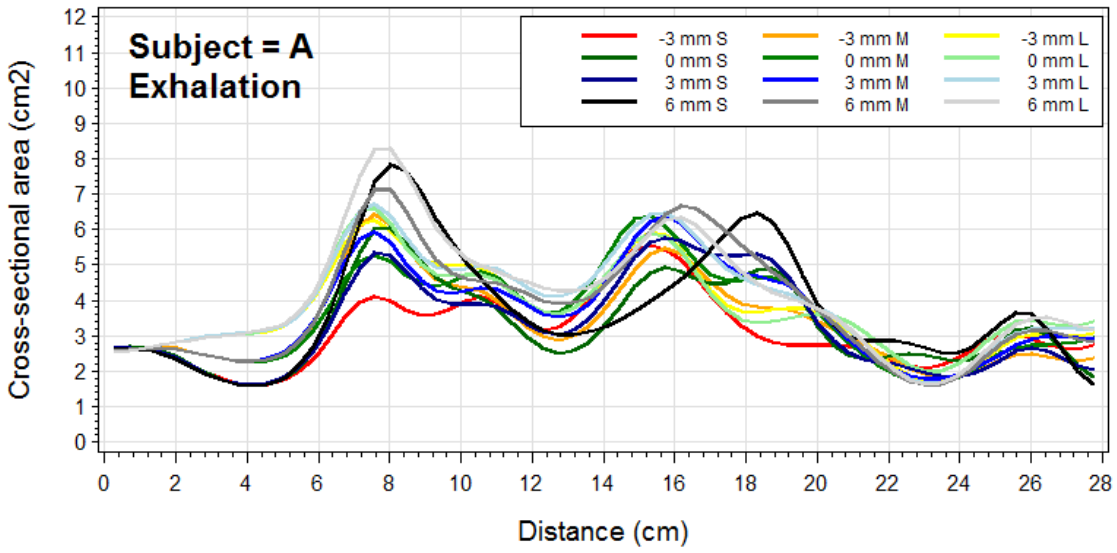


Figure 4.4: The measurements (2 pharyngograms per measurement) performed with the stepped mouthpieces connected to the acoustic pharyngometer wavetube during exhalation have been plotted for subject A for each of the 12 stepped mouthpiece configurations.

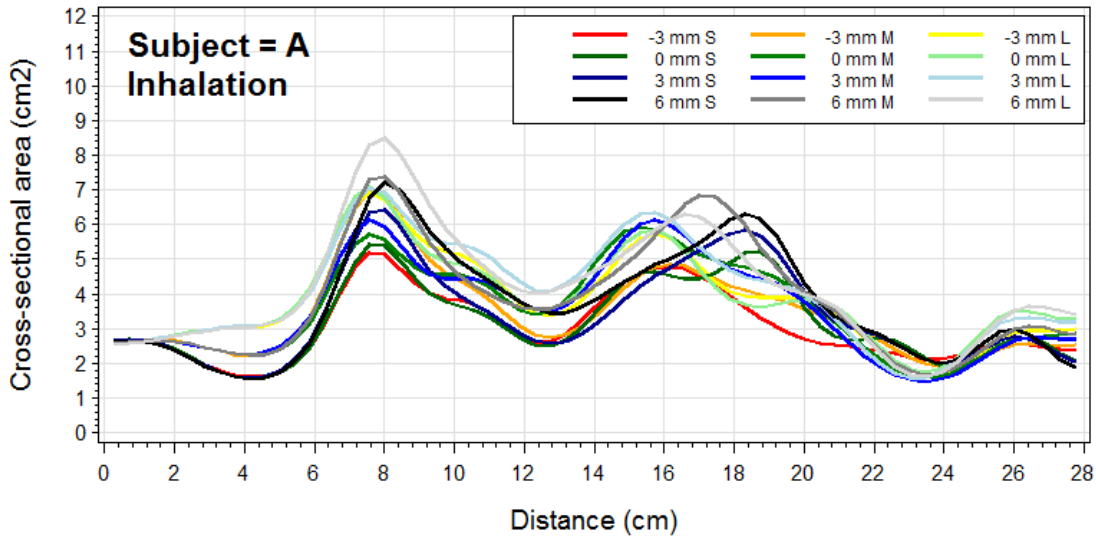


Figure 4.5: The measurements (2 pharyngograms per measurement) performed with the stepped mouthpieces connected to the acoustic pharyngometer wavetube during inhalation have been plotted for subject A for each of the 12 stepped mouthpiece configurations.

4.4.3 Individual mean CSAs

The individual mean upper airway CSAs are presented in Tables 4.3 to 4.6 per subject for the segment on the x-axis ranging from the end of the stepped mouthpiece to the GL. The CSAs are presented per stepped mouthpiece size (small, medium, large) and per protrusion (-3 mm, ±0 mm, +3 mm, +6 mm). Two acoustic pharyngometer recordings were performed during exhalation (E) and 2 during inhalation (I) for each stepped mouthpiece configuration.

Table 4.3: Subject A, CSAs (cm^2) for segment 4.16 cm to 22.16 cm on the x-axis with 2 acoustic pharyngometer recordings performed during exhalation (E) and 2 during inhalation (I).

Subject A	Small mouthpiece				Medium mouthpiece				Large mouthpiece			
	E	E	I	I	E	E	I	I	E	E	I	I
-3 mm	3.55	3.46	3.52	3.45	3.97	4.08	4.09	4.01	4.36	4.41	4.47	4.54
±0 mm	3.83	3.76	3.69	3.69	4.35	4.21	4.30	4.31	4.34	4.41	4.50	4.55
+3 mm	4.00	3.71	3.73	3.69	4.32	4.31	4.34	4.31	4.70	4.75	4.83	4.86
+6 mm	4.39	4.08	4.15	4.09	4.63	4.69	4.71	4.70	5.03	4.96	5.05	5.11

Table 4.4: Subject B, CSAs (cm^2) for segment 4.59 cm to 23.02 cm on the x-axis with 2 acoustic pharyngometer recordings performed during exhalation (E) and 2 during inhalation (I).

Subject B	Small mouthpiece				Medium mouthpiece				Large mouthpiece			
	E	E	I	I	E	E	I	I	E	E	I	I
-3 mm	2.69	2.51	2.66	2.65	3.34	3.19	3.37	3.36	3.81	3.82	3.88	3.67
±0 mm	2.77	2.78	2.72	2.62	3.15	3.06	3.16	3.35	3.71	3.48	3.65	3.82
+3 mm	3.20	2.97	3.13	3.11	3.44	3.50	3.59	3.53	3.82	3.68	3.72	3.73
+6 mm	3.25	3.16	3.15	3.13	3.61	3.44	3.56	3.52	3.60	3.65	3.85	4.01

Table 4.5: Subject C, CSAs (cm^2) for segment 4.59 cm to 23.46 cm on the x-axis with 2 acoustic pharyngometer recordings performed during exhalation (E) and 2 during inhalation (I).

Subject C	Small mouthpiece				Medium mouthpiece				Large mouthpiece			
	E	E	I	I	E	E	I	I	E	E	I	I
-3 mm	4.91	5.02	4.89	4.85	4.53	4.56	4.59	4.66	4.74	4.96	4.82	4.71
±0 mm	4.85	4.80	4.78	4.75	4.82	4.83	4.92	4.96	4.65	4.79	4.89	4.99
+3 mm	4.95	4.88	4.92	4.91	5.22	5.30	5.45	5.55	4.94	4.94	5.05	5.17
+6 mm	5.23	5.24	5.20	5.25	5.37	5.38	5.61	5.53	5.81	5.86	5.92	5.98

Table 4.6: Subject D, CSAs (cm^2) for segment 4.16 cm to 22.59 cm on the x-axis with 2 acoustic pharyngometer recordings performed during exhalation (E) and 2 during inhalation (I).

Subject D	Small mouthpiece				Medium mouthpiece				Large mouthpiece			
	E	E	I	I	E	E	I	I	E	E	I	I
-3 mm	3.36	3.29	3.51	3.33	3.76	3.97	4.06	4.03	4.38	4.69	4.41	4.36
±0 mm	3.62	3.67	3.89	3.60	4.32	4.35	4.41	4.14	4.71	4.82	4.70	4.64
+3 mm	4.08	3.91	4.11	3.89	3.88	4.35	4.45	3.96	4.83	4.82	4.88	5.01
+6 mm	4.31	4.61	4.33	4.54	4.94	4.75	4.67	4.63	4.95	4.63	4.91	4.71

Some observations regarding the effects of the stepped mouthpieces on the mean CSAs can be made from the results in Tables 4.3 to 4.6:

- For Subject A the change in mean CSA during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the "Small mouthpiece" ~9%, with the "Medium mouthpiece" ~24% and with the "Large mouthpiece" ~34%. The change in mean CSA during inhalation following the introduction of the

mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~-8%, to the “Medium mouthpiece” ~7% and to the “Large mouthpiece” ~19%.

- For Subject B the change in mean CSA during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~-5%, with the “Medium mouthpiece” ~7% and with the “Large mouthpiece” ~18%. The change in mean CSA during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~-20%, to the “Medium mouthpiece” ~1% and to the “Large mouthpiece” ~14%.
- For Subject C the change in mean CSA during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~56%, with the “Medium mouthpiece” ~67% and with the “Large mouthpiece” ~78%. The change in mean CSA during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~46%, to the “Medium mouthpiece” ~38% and to the “Large mouthpiece” ~43%.
- For Subject D the change in mean CSA during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~34%, with the “Medium mouthpiece” ~41% and with the “Large mouthpiece” ~45%. The change in mean CSA during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~3%, to the “Medium mouthpiece” ~22% and to the “Large mouthpiece” ~33%.
- The trends were relatively similar for changes during exhalation and inhalation.

4.4.4 Individual mean AUCs

The individual mean upper airway AUCs are presented per subject for the segment on the x-axis ranging from the end of the stepped mouthpiece to the GL in Tables 4.7 to 4.10. The mean AUCs are presented per stepped mouthpiece size (small, medium, large) and per protrusion (-3 mm, ±0 mm, +3 mm, +6 mm). Two acoustic pharyngometer recordings were performed during exhalation (E) and 2 during inhalation (I).

Table 4.7: Subject A, AUCs (cm^3) for segment 4.16 cm to 22.16 cm on the x-axis with two acoustic pharyngometry recordings performed during exhalation (E) and 2 during inhalation (I).

Subject A	Small mouthpiece			
	E	E	I	I
-3 mm	63.81	62.23	63.28	62.05
±0 mm	68.97	67.71	66.38	66.36
+3 mm	71.96	74.21	74.66	73.82
+6 mm	78.94	81.54	82.93	81.90

Subject A	Medium mouthpiece			
	E	E	I	I
-3 mm	71.41	73.45	73.67	72.22
±0 mm	78.31	75.71	77.35	77.59
+3 mm	77.69	77.59	78.21	77.65
+6 mm	83.30	84.41	84.71	84.59

Subject A	Large mouthpiece			
	E	E	I	I
-3 mm	78.48	79.44	80.45	81.63
±0 mm	78.21	79.43	81.03	81.93
+3 mm	84.66	85.46	86.89	87.55
+6 mm	89.53	88.27	89.80	90.99

Table 4.8: Subject B, AUCs (cm^3) for segment 4.59 cm to 23.02 cm on the x-axis with two acoustic pharyngometry recordings performed during exhalation (E) and 2 during inhalation (I).

Subject B	Small mouthpiece			
	E	E	I	I
-3 mm	46.76	43.66	46.34	46.11
±0 mm	51.06	51.09	49.97	48.30
+3 mm	58.96	54.68	57.60	57.15
+6 mm	59.72	58.14	58.03	57.53

Subject B	Medium mouthpiece			
	E	E	I	I
-3 mm	61.50	58.69	62.08	61.80
±0 mm	58.03	56.32	58.06	61.65
+3 mm	63.38	64.34	66.13	64.93
+6 mm	66.40	63.38	65.59	64.69

Subject B	Large mouthpiece			
	E	E	I	I
-3 mm	70.12	70.30	71.46	67.53
±0 mm	68.33	64.11	67.16	70.22
+3 mm	70.20	67.79	68.40	68.64
+6 mm	66.26	67.24	70.85	73.87

Table 4.9: Subject C, AUCs (cm^3) for segment 4.59 cm to 23.46 cm on the x-axis with two acoustic pharyngometry recordings performed during exhalation (E) and 2 during inhalation (I).

Subject C	Small mouthpiece			
	E	E	I	I
-3 mm	92.73	94.79	92.39	91.71
±0 mm	91.58	90.80	90.43	90.30
+3 mm	93.52	92.30	93.04	92.85
+6 mm	98.77	99.13	98.28	99.26

Subject C	Medium mouthpiece			
	E	E	I	I
-3 mm	85.71	86.13	86.83	87.98
±0 mm	91.13	91.32	93.07	93.82
+3 mm	98.59	100.17	102.94	104.86
+6 mm	101.43	101.70	105.96	104.55

Subject C	Large mouthpiece			
	E	E	I	I
-3 mm	89.56	93.76	91.19	89.04
±0 mm	87.97	90.55	92.39	94.23
+3 mm	93.41	93.45	95.44	97.73
+6 mm	109.80	110.69	111.87	113.00

Table 4.10: Subject D, AUCs (cm^3) for segment 4.16 cm to 22.59 cm on the x-axis with two acoustic pharyngometry recordings performed during exhalation (E) and 2 during inhalation (I).

Subject D	Small mouthpiece			
	E	E	I	I
-3 mm	61.81	60.48	64.54	61.32
±0 mm	66.54	67.55	71.57	66.18
+3 mm	75.12	71.93	75.54	71.56
+6 mm	79.24	84.85	79.66	83.61

Subject D	Medium mouthpiece			
	E	E	I	I
-3 mm	69.15	73.02	74.64	74.23
±0 mm	79.46	80.03	81.17	76.18
+3 mm	71.35	80.06	81.97	72.88
+6 mm	90.97	87.40	85.95	85.22

Subject D	Large mouthpiece			
	E	E	I	I
-3 mm	80.55	86.27	81.09	80.29
±0 mm	86.75	88.72	86.39	85.34
+3 mm	88.82	88.78	89.79	92.20
+6 mm	91.15	85.20	90.31	86.62

Again, some observations regarding the effects of the stepped mouthpiece on the mean AUCs can be made from the results in Tables 4.7 to 4.10:

- For Subject A the change in mean AUC during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~21%, with the “Medium mouthpiece” ~24% and with the “Large mouthpiece” ~33%. The change in mean AUC during inhalation following the introduction

of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~-8%, to the “Medium mouthpiece” ~7% and to the “Large mouthpiece” ~19%.

- For Subject B the change in mean AUC during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~-6%, with the “Medium mouthpiece” ~6% and with the “Large mouthpiece” ~18%. The change in mean AUC during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~-25%, to the “Medium mouthpiece” ~1% and to the “Large mouthpiece” ~13%.
- For Subject C the change in mean AUC during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~57%, with the “Medium mouthpiece” ~68% and with the “Large mouthpiece” ~79%. The change in mean AUC during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~47%, to the “Medium mouthpiece” ~39% and to the “Large mouthpiece” ~44%.
- For Subject D the change in mean AUC during inhalation following mandibular advancement was from baseline to maximal advancement (+6 mm) with the “Small mouthpiece” ~34%, with the “Medium mouthpiece” ~40% and with the “Large mouthpiece” ~45%. The change in mean AUC during inhalation following the introduction of the mouthpieces (impact of vertical size) was from baseline to the “Small mouthpiece” (-3 mm) ~3%, to the “Medium mouthpiece” ~22% and to the “Large mouthpiece” ~32%.
- The trends were relatively similar for changes measured during exhalation and inhalation.

4.4.5 Individual mean CSA1-3 and AUC1-3

The individual mean CSA1-3 measured at the OPJ, the EG and the GL are presented per subject, stepped mouthpiece size and protrusion in Table 4.11.

Table 4.11: Mean CSAs (cm^2) presented per subject, stepped mouthpiece size (small, medium, large) and per protrusion (-3 mm, ±0 mm, +3 mm, +6 mm). Two acoustic pharyngometer recordings were performed during exhalation and 2 during inhalation.

End-point	Subject	Mouth-piece size	Exhalation				Inhalation			
			-3 mm	*±0 mm*	*3 mm*	*6 mm*	*-3 mm*	*±0 mm*	*3 mm*	*6 mm*
CSA1	A	Small	3.60	4.64	4.04	6.30	4.02	4.02	4.66	5.82
CSA1	A	Medium	4.60	4.41	4.26	5.16	4.97	4.58	4.57	5.49
CSA1	A	Large	5.01	4.73	4.92	6.08	5.40	5.14	5.61	6.54
CSA1	B	Small	3.77	4.15	4.87	4.91	3.55	3.80	4.36	4.64
CSA1	B	Medium	4.66	3.78	5.58	5.63	4.54	4.18	6.08	5.71
CSA1	B	Large	5.26	5.13	6.73	6.43	5.80	5.31	6.79	7.32
CSA1	C	Small	7.05	7.48	7.91	7.11	7.17	7.73	8.17	7.51
CSA1	C	Medium	7.37	8.05	7.08	7.46	7.92	8.67	8.19	8.55
CSA1	C	Large	9.15	8.65	9.06	8.19	9.16	9.37	9.53	8.69
CSA1	D	Small	6.97	5.26	6.86	6.85	5.46	5.43	6.92	7.16
CSA1	D	Medium	7.64	7.20	8.32	8.30	7.86	7.36	7.71	8.86
CSA1	D	Large	9.09	8.96	9.87	10.12	9.08	9.59	10.02	9.87
CSA2	A	Small	3.17	2.65	3.11	3.16	2.62	2.55	2.66	3.56
CSA2	A	Medium	2.98	3.67	3.61	4.01	2.82	3.40	3.56	3.55
CSA2	A	Large	3.69	3.68	4.16	4.36	3.46	3.59	4.12	4.03
CSA2	B	Small	2.23	2.60	2.64	2.70	2.01	2.11	2.16	2.18
CSA2	B	Medium	2.79	2.86	2.81	2.66	2.59	2.54	2.25	2.19
CSA2	B	Large	2.95	2.73	2.67	2.61	2.44	2.38	2.22	2.10
CSA2	C	Small	3.99	5.96	6.29	7.02	4.35	6.14	6.70	7.22
CSA2	C	Medium	5.64	6.34	4.52	5.42	6.15	6.83	5.09	5.63
CSA2	C	Large	5.66	6.27	6.80	5.39	5.52	6.80	7.42	5.70
CSA2	D	Small	2.78	3.39	3.44	3.39	3.83	3.70	3.57	3.79
CSA2	D	Medium	3.66	3.50	4.39	4.95	3.74	4.74	4.65	4.42
CSA2	D	Large	4.11	3.47	5.10	4.11	4.08	4.84	4.99	5.11
CSA3	A	Small	2.74	2.76	2.86	3.32	2.60	3.07	3.57	3.74
CSA3	A	Medium	3.21	3.25	3.32	3.38	3.40	3.55	3.40	3.80
CSA3	A	Large	3.43	3.57	3.56	3.54	3.69	3.80	3.72	3.87
CSA3	B	Small	2.59	3.07	3.33	2.74	2.79	3.24	3.59	3.19
CSA3	B	Medium	3.21	2.80	3.41	3.59	3.67	3.54	3.66	3.88
CSA3	B	Large	3.58	3.34	3.65	3.48	3.76	3.78	3.75	3.46
CSA3	C	Small	2.96	2.47	2.35	2.17	3.34	2.71	2.44	2.34
CSA3	C	Medium	2.24	2.13	3.36	3.28	2.23	2.30	3.85	3.67
CSA3	C	Large	2.62	2.22	2.23	3.23	2.56	2.35	2.32	3.71
CSA3	D	Small	1.77	2.25	2.25	3.17	2.11	2.30	2.53	3.25
CSA3	D	Medium	2.11	2.85	2.37	2.67	2.32	2.44	2.48	2.46
CSA3	D	Large	2.65	3.32	2.25	2.72	2.36	2.54	2.46	2.38

The individual mean AUC1-3 measured between the end of the wavetube and the OPJ, and between the following landmarks are presented per subject, stepped mouthpiece size and protrusion in Table 4.12.

Table 4.12: Mean AUCs (cm^3) presented per subject, stepped mouthpiece size (small, medium, large) and per protrusion (-3 mm, ±0 mm, +3 mm, +6 mm). Two acoustic pharyngometer recordings performed during exhalation and 2 during inhalation.

End-point	Subject	Mouthpiece size	Exhalation				Inhalation			
			-3 mm	*±0 mm*	*3 mm*	*6 mm*	*-3 mm*	*±0 mm*	*3 mm*	*6 mm*
AUC1	A	Small	16.7	21.6	19.8	26.1	19.3	19.7	22.4	24.4
AUC1	A	Medium	24.3	21.7	23.2	26.3	25.7	22.7	23.7	26.8
AUC1	A	Large	26.5	27.0	27.3	31.7	28.0	28.3	28.5	32.0
AUC1	B	Small	13.5	15.4	17.6	21.3	15.0	14.7	18.0	21.0
AUC1	B	Medium	22.3	21.8	23.8	24.1	23.0	22.0	24.2	23.2
AUC1	B	Large	28.2	28.8	28.6	28.4	27.7	28.8	27.6	31.6
AUC1	C	Small	19.1	19.8	21.3	20.1	18.8	19.7	21.0	20.3
AUC1	C	Medium	22.8	24.3	22.6	23.0	22.9	24.7	24.2	24.6
AUC1	C	Large	29.2	28.4	28.8	28.0	28.6	29.4	28.8	27.9
AUC1	D	Small	13.3	11.6	13.2	13.0	11.7	11.4	13.0	13.1
AUC1	D	Medium	16.2	15.8	17.1	16.7	16.0	15.8	16.0	17.0
AUC1	D	Large	19.9	19.8	21.0	21.2	19.7	20.4	20.7	20.8
AUC2	A	Small	12.7	12.9	12.7	15.5	11.7	11.4	12.3	15.7
AUC2	A	Medium	13.6	15.0	14.1	15.5	13.5	14.2	14.5	14.7
AUC2	A	Large	15.7	15.1	16.2	17.3	15.8	15.3	17.2	17.2
AUC2	B	Small	11.0	12.2	13.4	12.9	10.2	10.6	11.7	11.3
AUC2	B	Medium	14.3	12.2	14.6	14.2	13.2	12.5	14.8	13.5
AUC2	B	Large	15.5	14.2	16.2	15.4	16.0	14.0	15.6	15.5
AUC2	C	Small	27.6	31.7	32.0	31.2	28.0	32.1	33.0	31.8
AUC2	C	Medium	30.5	32.9	26.2	27.8	33.3	34.1	29.1	29.8
AUC2	C	Large	33.8	34.1	35.6	28.2	32.6	35.4	37.7	30.1
AUC2	D	Small	30.7	24.1	29.7	29.5	29.5	27.5	30.9	33.0
AUC2	D	Medium	32.2	33.6	36.7	38.0	36.7	35.4	34.9	38.6
AUC2	D	Large	39.2	39.0	42.1	40.6	40.2	42.1	44.0	43.3
AUC3	A	Small	33.4	34.1	38.9	38.0	31.5	34.7	36.7	40.7
AUC3	A	Medium	34.5	40.8	40.6	42.9	33.4	40.6	40.1	43.2
AUC3	A	Large	37.2	36.6	42.1	41.6	37.3	37.6	42.3	42.4
AUC3	B	Small	18.2	19.5	21.9	21.4	17.6	19.2	22.3	20.7
AUC3	B	Medium	19.8	19.0	21.9	22.8	21.0	20.6	22.8	24.6
AUC3	B	Large	22.0	19.3	21.1	19.9	21.5	21.2	21.8	21.9
AUC3	C	Small	71.4	76.2	77.6	76.0	71.4	76.2	78.1	76.4
AUC3	C	Medium	79.0	82.6	74.6	76.4	81.5	84.0	78.6	79.5
AUC3	C	Large	89.2	88.9	90.6	82.4	87.3	90.7	92.4	83.9
AUC3	D	Small	19.7	32.5	33.1	40.6	23.5	31.5	31.8	36.9
AUC3	D	Medium	25.4	32.3	25.1	37.4	24.4	30.4	29.5	33.1
AUC3	D	Large	27.4	30.9	30.2	29.8	24.0	27.1	30.6	28.6

There were some differences in the three CSAs (CSA1, CSA2, CSA3) between acoustic pharyngometer measurements made during exhalation *versus* inhalation. For measurements made during inhalation, the largest CSA1-3 per subject was in only 4 out of 12 cases found for the combination "Large" orifice and the "+6 mm" protrusion. Focusing on the acoustic pharyngometer measurements made during inhalation, the largest AUC1-3 per subject was in only 5 out of 12 cases found for the combination "Large" orifice and the "+6 mm" protrusion. The largest AUC1-3 matched in most cases the largest CSA1-3.

4.4.6 Graphical presentation of the changes in mean CSAs

The changes in mean CSAs (measured from end of stepped mouthpiece to GL; cm^2) during exhalation (Figure 4.6) and inhalation (Figure 4.7) following the use of the stepped mouthpieces have been plotted for the 4 subjects (A, B, C and D).

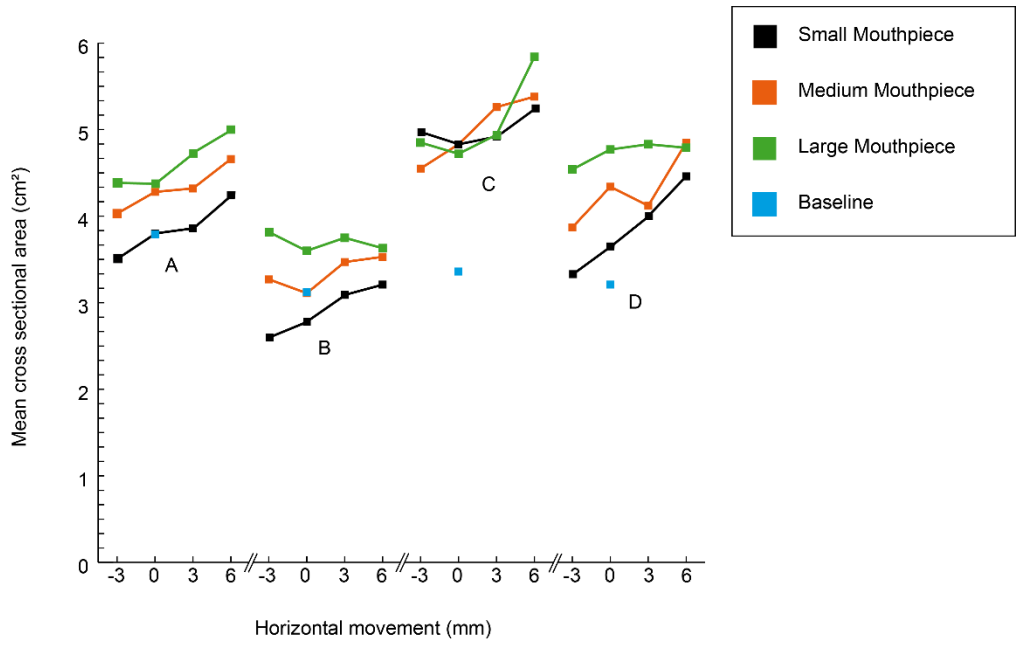


Figure 4.6: The changes in mean CSA during exhalation are shown for the 4 subjects (A, B, C and D). The baseline CSA data per subject are shown as a dot in blue colour. The impact of the vertical movement of the lower jaw is shown through the three colour codes for the 10 mm (Small), the 15 mm (Medium) and the 20 mm (Large) diameter mouthpiece orifices. The impact of the horizontal movement of -3, ±0, +3 and +6 mm of the lower jaw is shown as a function of the X-axis scale.

The plotted mean CSA data in Figure 4.6 (exhalation) highlights the impact of the vertical movement in subjects A, B and D, the impact of the mandibular advancement in subject C and the effect of the combination of the vertical movement and the mandibular advancement in subjects A and D following use of the stepped mouthpieces. The medium and large mouthpieces had partly a negative effect in subjects B and D.

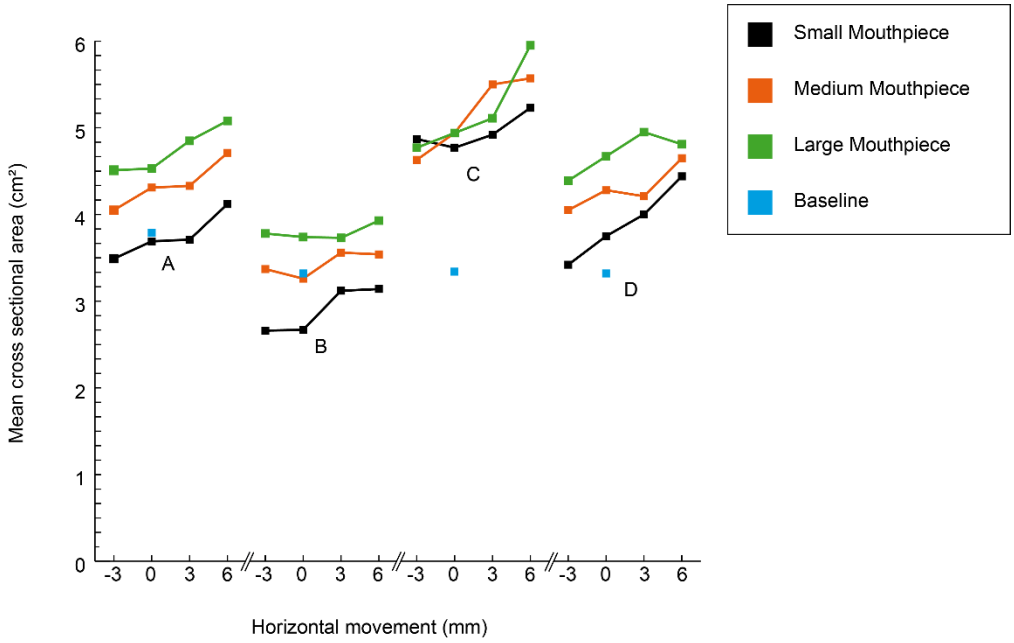


Figure 4.7: The changes in mean CSA during inhalation are shown for the 4 subjects A, B, C and D. The baseline CSA data per subject are shown as a dot in blue colour. The impact of the vertical movement of the lower jaw is shown through the three colour codes for the 10 mm (Small), the 15 mm (Medium) and the 20 mm (Large) diameter mouthpiece orifices. The impact of the horizontal movement of -3, ±0, +3 and +6 mm of the lower jaw is shown as a function of the X-axis scale.

The plotted mean CSA data in Figure 4.7 (inhalation) follows the trend in Figure 4.6 (exhalation) and highlights the impact of the vertical movement in subjects A, B and D, the impact of the mandibular advancement in subject C and the effect of the combination of the vertical movement and the mandibular advancement in subjects A and D following use of the stepped mouthpieces.

The large mouthpiece with the +6 mm protrusion had in contrast to the measurements during exhalation a positive effect in subject B and a somewhat more negative effect in subject D.

4.4.7 Graphical presentation of the changes in mean AUCs

The changes in mean AUCs measured (from end of stepped mouthpiece to GL, cm^3) during exhalation (Figure 4.8) and inhalation (Figure 4.9) following the use of the stepped mouthpieces has been plotted for the 4 subjects (A, B, C and D).

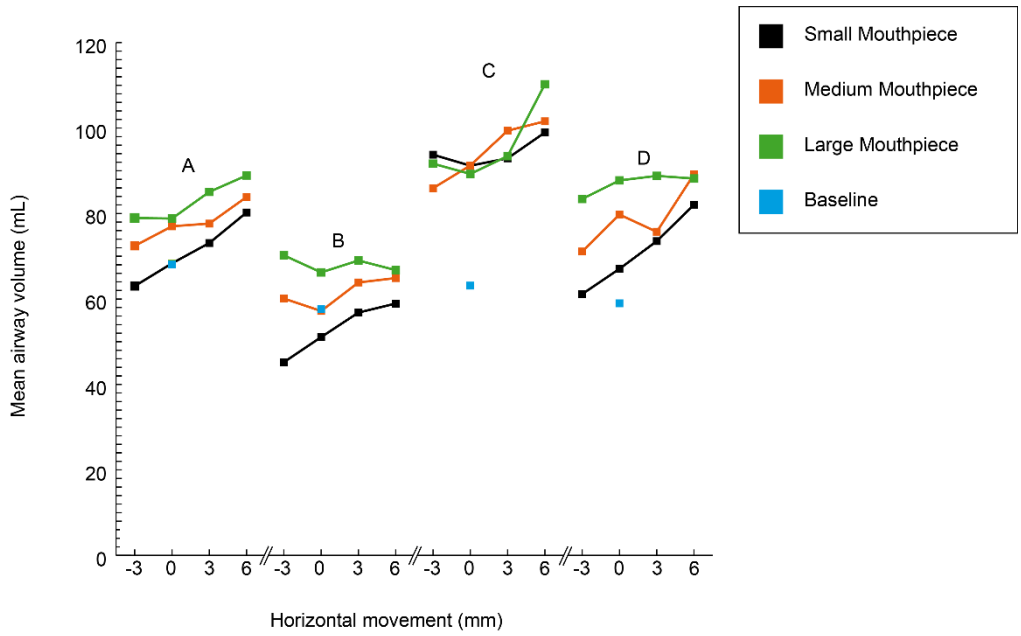


Figure 4.8: The changes in mean AUCs (mL) during exhalation are shown for the 4 subjects A, B, C and D. The baseline AUC data per subject are shown as a dot in blue colour. The impact of the vertical movement of the lower jaw is shown through the three colour codes for the 10 mm (Small), the 15 mm (Medium) and the 20 mm (Large) diameter mouthpiece orifices. The impact of the horizontal movement of -3, ±0, +3 and +6 mm of the lower jaw is shown as a function of the X-axis scale.

The plotted mean AUC data in Figure 4.8 (exhalation) follows the trend in Figure 4.6 (CSA, exhalation) and highlights the impact of the vertical movement especially in subjects B and D, the impact of the mandibular advancement in subject C and the effect of the combination of the vertical movement and the mandibular advancement in subjects A, B and D following use of the stepped

mouthpieces. The large mouthpiece with the +6 mm protrusion had a somewhat negative effect in subjects B and D.

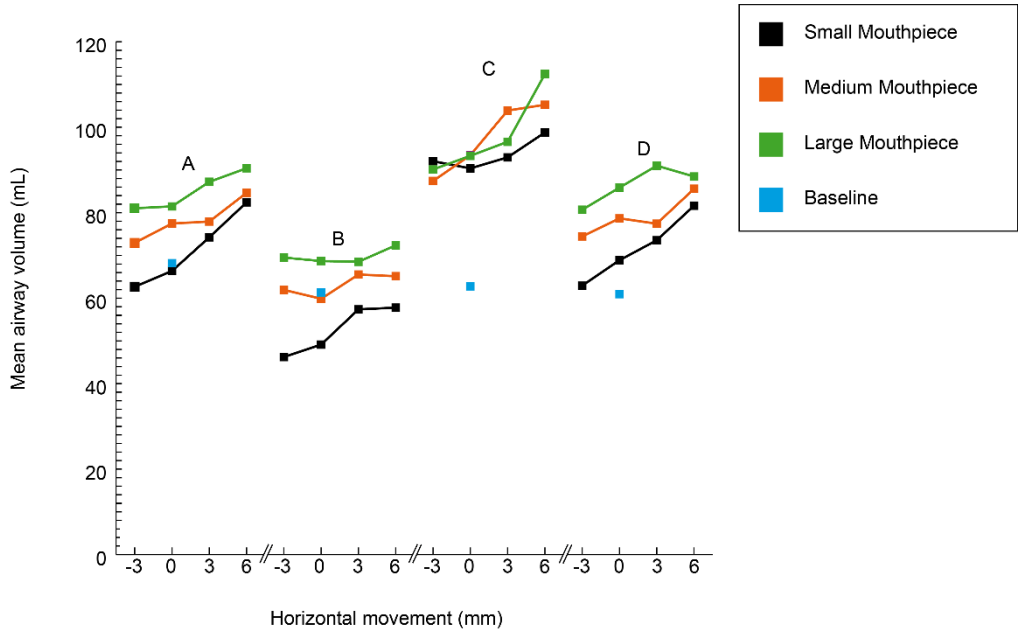


Figure 4.9: The changes in mean AUCs (mL) during inhalation are shown for the four subjects A, B, C and D. The baseline AUC data per subject are shown as a dot in blue colour. The impact of the vertical movement of the lower jaw is shown through the three colour codes for the 10 mm (Small), the 15 mm (Medium) and the 20 mm (Large) diameter mouthpiece orifices. The impact of the horizontal movement of -3, ±0, +3 and +6 mm of the lower jaw is shown as a function of the X-axis scale.

The plotted mean AUC data in Figure 4.9 (inhalation) follows the trend in Figures 4.6 to 4.8 and highlights the impact of the vertical movement in subjects A, B and D, the impact of the mandibular advancement in subject C and the effect of the combination of the vertical movement and the mandibular advancement in subjects A and D following use of the stepped mouthpieces. The large mouthpiece with the +6 mm protrusion had as in Figure 4.7 (CSA, inhalation) a negative effect in subject D.

4.4.8 Graphical presentation of the changes in mean CSA1-3

The changes in mean CSA1-3 during exhalation (Figure 4.10) and inhalation (Figure 4.11) following the use of the stepped mouthpieces are shown for the four subjects (A, B, C and D). The CSA1, CSA2 and CSA3 present the OPJ, the EG and the GL. The data has been plotted on the X-axis for S0 (small mouthpiece with 0 mm protrusion), L0 (large mouthpiece with 0 mm protrusion), L3 (large mouthpiece with 3 mm protrusion), and L6 (large mouthpiece with 6 mm protrusion). This presents the impact of the movement of the incisors (S0 to L0) and the mandibular advancement (L0 to L3 and L6).

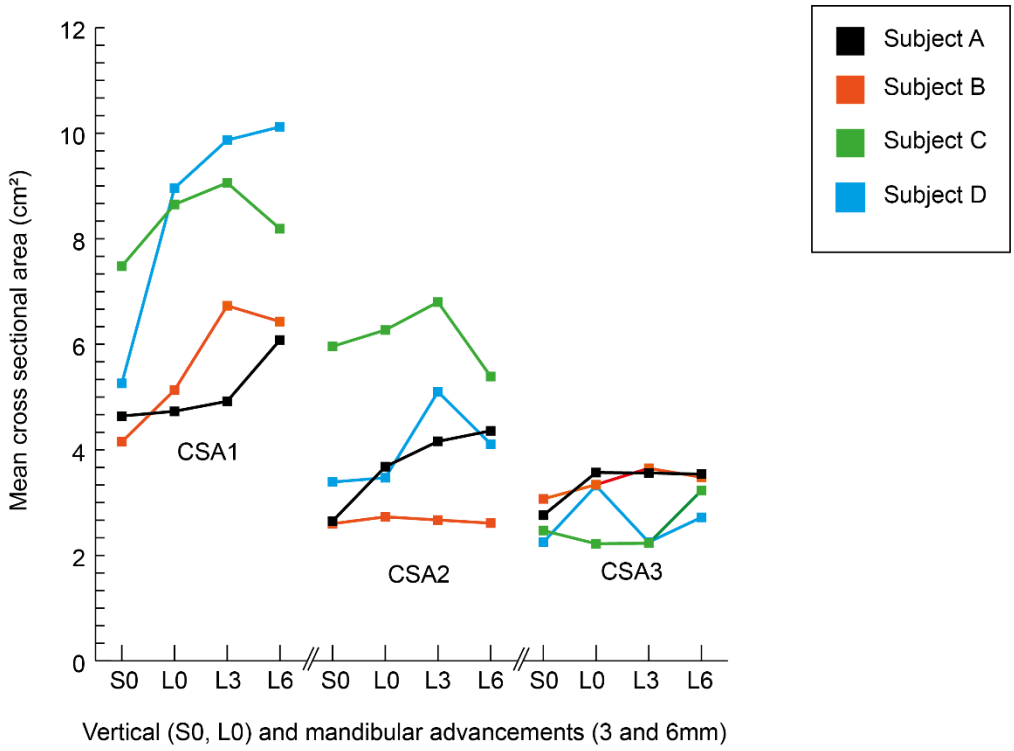


Figure 4.10: The changes in mean CSA1-3 during exhalation when testing the stepped mouthpieces are presented. The data has been plotted on the X-axis for S0 (small mouthpiece with 0 mm protrusion), L0 (large mouthpiece with 0 mm protrusion), L3 (large mouthpiece with 3 mm protrusion), and L6 (large mouthpiece with 6 mm protrusion).

The plotted specific landmarks (mean CSA1-3) in Figure 4.10 (exhalation) at the OPJ, the EG and the GL highlight the individual differences in response to the use of the stepped mouthpieces. The

change in CSA1 due to the change from small (S0) to large (L0) stepped mouthpiece for subject D differs from the changes seen in the other subjects. The use of the large mouthpiece with the +6 mm protrusion had in subject C a negative effect at CSA1 and CSA2 whereas the effect at CSA3 was the opposite.

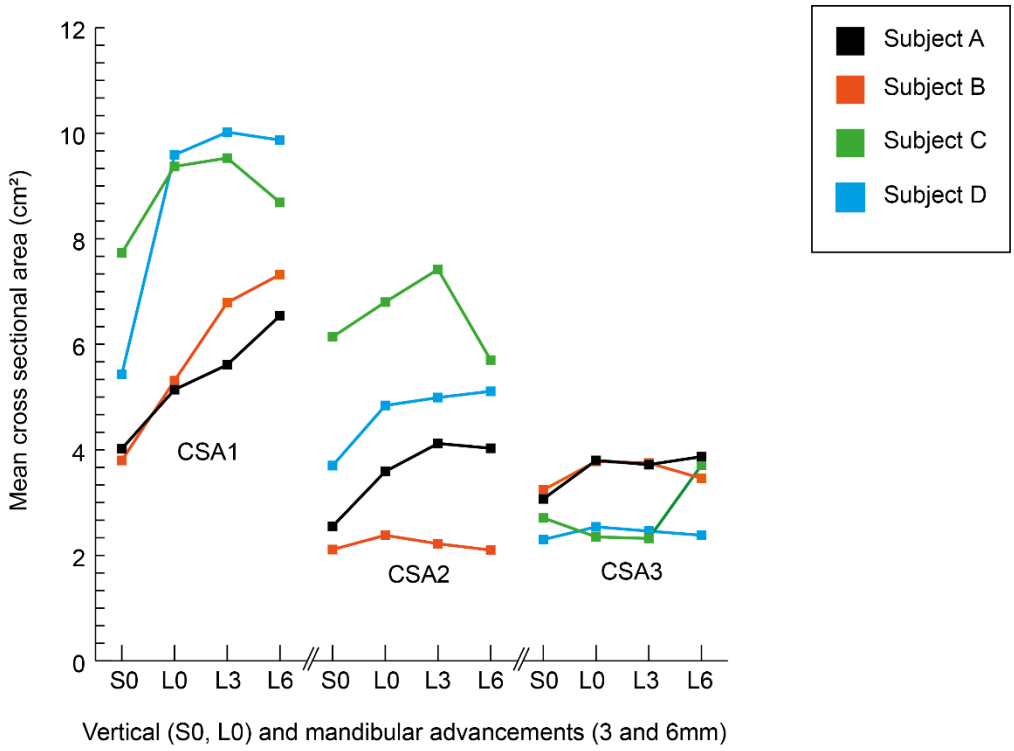


Figure 4.11: The changes in CSA1-3 during inhalation when testing the stepped mouthpieces are presented. The data has been plotted on the X-axis for S0 (small mouthpiece with 0 mm protrusion), L0 (large mouthpiece with 0 mm protrusion), L3 (large mouthpiece with 3 mm protrusion), and L6 (large mouthpiece with 6 mm protrusion).

The plotted mean CSA1-3 in Figure 4.11 (inhalation) highlight as in Figure 4.10 (exhalation) the individual differences in response to the use of the stepped mouthpieces. The change in CSA1 due to the change from small (S0) to large (L0) stepped mouthpiece for subject D differs from the changes seen in the other subjects. The use of the large mouthpiece with the +6 mm protrusion had in subject C a negative effect at CSA1 and CSA2 whereas the effect at CSA3 was the opposite.

4.4.9 Graphical presentation of the changes in mean AUC1-3

The changes in AUC1-3 during exhalation (Figure 4.12) and inhalation (Figure 4.13) following the use of the stepped mouthpieces are shown for the four subjects (A, B, C and D). AUC1 covers the volume for the oral cavity, AUC2 the volume from the OPJ to the EG and AUC3 the volume from the Eg to the GL.

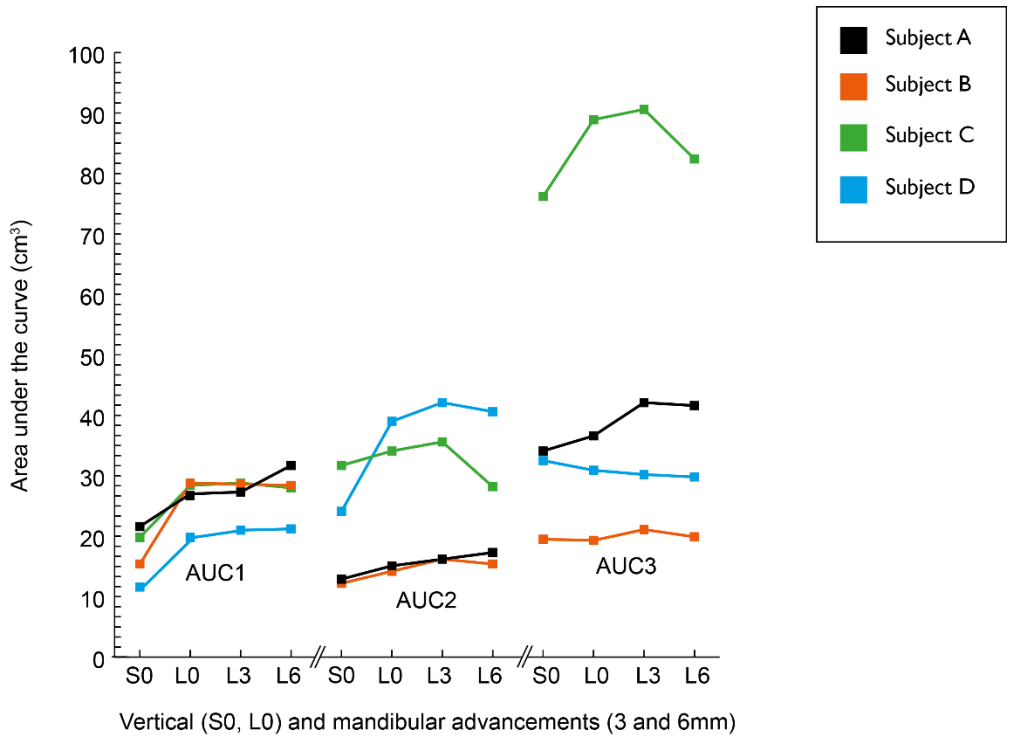


Figure 4.12: The changes in AUC1-3 during exhalation when testing the stepped mouthpieces. The data has been plotted on the X-axis for S0 (small mouthpiece with 0 mm protrusion), L0 = (large mouthpiece with 0 mm protrusion), L3 (large mouthpiece with 3 mm protrusion), and L6 (large mouthpiece with 6 mm protrusion).

The plotted mean AUC1-3 in Figure 4.12 (exhalation) highlight the individual differences in response to the use of the stepped mouthpieces. The changes were relatively similar for all 4 subjects with the exception of the magnitude of AUC3 in subject C.

The plotted mean AUC1-3 in Figure 4.13 (inhalation) are quite similar to the AUC1-3 in Figure 4.12 (exhalation), and again the changes were relatively similar for all 4 subjects with the exception of the magnitude of AUC3 in subject C.

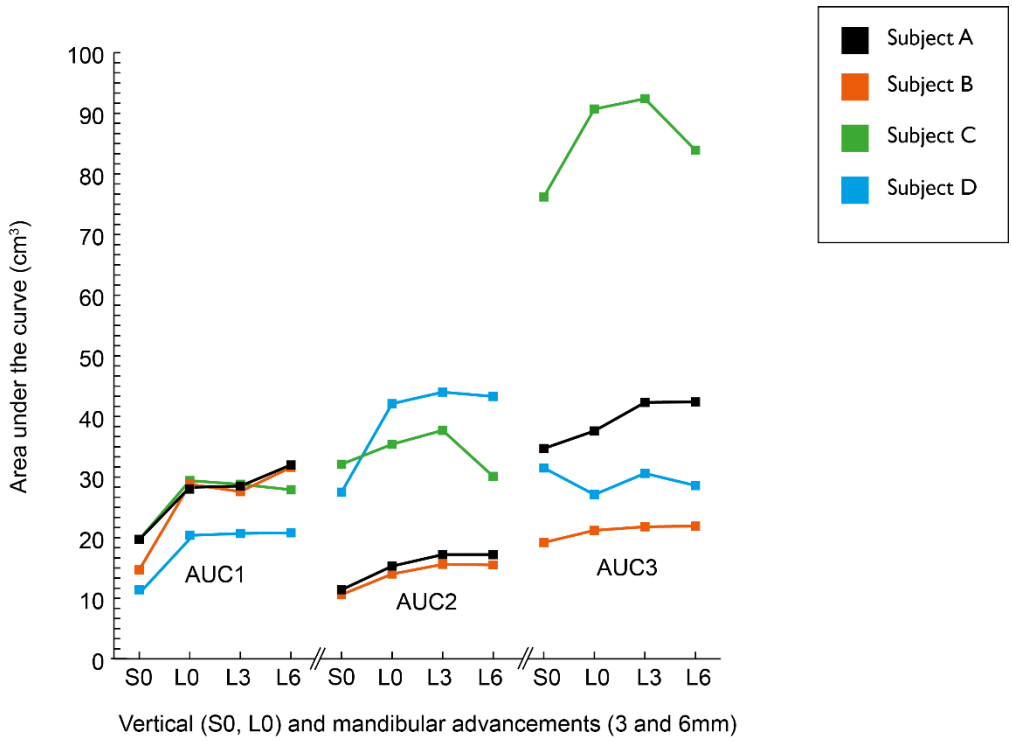


Figure 4.13: The changes in AUC1-3 during inhalation when testing the stepped mouthpieces. The data has been plotted on the X-axis for S0 (small mouthpiece with 0 mm protrusion), L0 = (large mouthpiece with 0 mm protrusion), L3 (large mouthpiece with 3 mm protrusion), and L6 (large mouthpiece with 6 mm protrusion).

4.4.10 Observations made during the performance of the measurements

The stepped mouthpieces were designed with front end orifices with four protrusions on the lower side at different distances (-3 mm, ±0 mm, +3 mm and +6 mm) in relation to the protrusion on the upper side for horizontal movement of the mandible. Some of the subjects found it difficult to use the stepped mouthpiece with the +6 mm protrusion. In these cases it was a struggle to advance the mandible that far without bending the head backwards which would have deviated from the

standard position for the acoustic pharyngometer measurements. Hypersalivation did also occur as the subjects could not swallow during the acoustic pharyngometer measurements.

The standard acoustic pharyngometer mouthpiece has been designed with a short tongue depressor. The lack of a tongue depressor on the stepped mouthpieces tested could be observed initially when going from baseline measurements to the measurements with the stepped mouthpieces but could be dealt with through instructions from the dentist.

4.5 Discussion

The primary objective of this proof-of-concept study was to measure with an acoustic pharyngometer changes in the upper airways of 4 healthy subjects while these were using a set of stepped mouthpieces. The mouthpieces were designed to facilitate mandibular advancements and incisal opening in order to increase the mean CSA and the volume (AUCs) of the upper airways. The acoustic pharyngometer software was used for the analysis of the pharyngograms in terms of mean CSAs and mean AUCs. The stepped mouthpieces were attached to the pharyngometer wavetube for the measurements. Due to the addition of the 40 mm long stepped mouthpieces to the wavetube the pharyngograms were extended ~4 cm on the x-axis. In order to avoid inclusion of the area of the pharyngogram covered by the stepped mouthpieces in the analyses of the pharyngograms, these were set to cover only the area from the incisors to the GL.

During inhalation through the stepped mouthpieces the mean CSAs were in 3 of 4 subjects affected by both the horizontal advancement of the mandible and the incisal opening. The changes in the CSAs showed a large variability between the 4 subjects and were far from linear. The change in the CSA following mandibular advancement ranged for subject A from ~9% to ~34%, for subject B from ~-5% to ~18%, for subject C from ~56% to ~78% and for subject D from ~34% to ~45%. The impact of the incisal opening on these changes was in subjects A, B and D considerable

especially when testing the large mouthpiece whereas this effect was almost the opposite when testing the small mouthpiece. The 10 mm incisal opening created by the small mouthpiece (-3 mm) had a surprisingly negative effect in subjects A and B. The changes in the mean AUCs during inhalation followed the changes in the mean CSAs.

There were a few negative changes in the mean CSAs and AUCs in response to the mandibular advancements and the incisal opening. The negative changes were surprising but in accordance with the results published by Gao et al (2004) who in 14 healthy subjects investigated through MRI changes in the CSAs of the upper airways following mandibular advancement and incisal opening. A custom made oral appliance was used to keep the mandible at 0%, 50%, 75% and 100% of maximal mandibular advancement, and at 50%, 75% and 100% of maximum incisal opening at 75% mandibular advancement. The incisal openings were 4 mm, 9.8 ± 4.1 mm, 14.7 ± 4.1 mm and 19.6 ± 4.1 mm and therefore similar in size to the vertical orifices of the stepped mouthpieces. Some of the individual changes were negative when compared with baseline further highlighting the large variability of the individual results especially in the hypopharynx. One of the reasons for the large variability might have been the lack of tongue depressor.

The changes in the CSAs highlighted the large variability in response to the vertical movements of the mouth and the mandibular advancements. The impact of the incisal opening during inhalation was obvious in CSA1 and less pronounced in CSA2 and CSA3. An increase in CSA1 might be more important than changes in CSA2-3 as the OPJ is a critical location from an impaction perspective as particles during inhalation change direction from horizontal to almost vertical. The impact of the mandibular advancement was rather variable with very positive results in CSA1 for subjects A and B and in CSA2 for subjects A and C. The changes in AUC1-3 followed partly those seen in the CSA1-3 but for the AUC3 result for subject C following mandibular advancement from +3 to +6 mm when the AUC3 was reduced. The question is whether this large

AUC3 represents an artefact due to leakage at the velum as discussed by Molfino et al (1990) and Marshall et al (1993). A check of the pharyngograms of subject C (APPENDIX B.1) shows that the increase in the pharyngogram from the measurement with the large stepped mouthpiece with the +6 mm protrusion is located within the hypopharynx. Thus the change could be a result of an artefact due to leakage at velum as discussed by Molfino et al and Marshall et al. Overall the results highlight large variability of the CSAs and the AUCs, and the need for a stepped mouthpiece with a smaller distance between each step, at least after +3 mm.

Following use of the stepped mouthpieces the changes in mean CSAs and AUCs during inhalation were similar to those measured during exhalation. This was surprising since results of several studies have shown that the upper airway is a dynamic structure and that changes in upper airway CSAs occur during breathing with maximal increase occurring during exhalation (Schwab et al., 1993a; Schwab et al., 1993b; Schwab, 1998). The reason for the lack of difference between the measurements performed during exhalation and inhalation is difficult to explain but might be a consequence of the seated position - in contrast with the supine position in the Schwab studies (Schwab et al., 1993a; Schwab et al., 1993b; Schwab, 1998) - and the use of the stepped mouthpieces.

As the stepped mouthpiece represents a new mouthpiece design in terms of mandibular advancement properties the results cannot directly be compared with any previously published results. The impact of the vertical diameters of the stepped mouthpieces (10-20 mm) on the upper airways can, however, be compared to the results of the study by Pritchard et al (2004) who investigated the impact of 4 dummy inhalation devices - essentially prototype mouthpieces with different diameters and resistances – on the size of the upper airways measured through an inhalation-gated MRI technique developed to allow data acquisition at a fixed point in the subject`s breathing cycle. The 20 healthy subjects were scanned in a supine position. Data from 2 of the

mouthpieces (Device A, mouthpiece diameter 25 mm; Device C, mouthpiece diameter 14 mm) were of interest as these mouthpieces had diameters resembling those used in the present study and had low resistances. The results indicated that the size of the buccal volume and total upper airway volume were statistically significantly larger with the large diameter (mean 33.2 cm^3) *versus* the small diameter mouthpiece (mean 22.4 cm^3). There were, however, no statistically significant effects on the CSAs. The authors concluded that the measured CSAs (naso-pharynx-soft palate, EG, vocal cords) seemed to be independent of mouthpiece design. The lack of statistically significant differences in the area from the OPJ to the vocal cords might have been due to the smaller size of the upper airways while in the supine position during scanning.

The Pritchard et al (2004) results can be compared with those of Van Holsbeke et al (2014b) who recently presented the results of a study in which the impact of mouthpiece design on the upper airway CSA was investigated. An ultrafast spoiled gradient echo sequence MRI was used in 12 healthy subjects who were supine during the scans. The influence of mouthpiece height (12-27 mm), width (19-32.1 mm), protrusion (4-40 mm into the mouth), orifice size (3-7 mm) and resistance to airflow were investigated. Mouthpiece protrusion and height had the most positive effect on CSA whereas the impact of width and orifice size was minimal. The changes in CSA were mainly found in the oral cavity whereas the changes in the oropharynx were small and inverse and did not affect the hypopharynx. The authors concluded that the influence of the mouthpiece protrusion on the CSAs of the oral cavity and the oropharynx was probably a consequence of the interaction between the mouthpiece and the tongue (Van Holsbeke et al., 2014b). Thus both Pritchard et al (2004) and Van Holsbeke et al (2014b) showed considerable increases in the oral cavity following use of the mouthpieces but less so in the rest of the upper airways.

As a stepped mouthpiece might eventually be used with an inhaler, the impact of different mouthpiece designs on aerosol passage through the mouthpiece would be of interest. Boyd et al

(2004) investigated the impact of mouthpiece cross-sectional shape, volume and taper on oropharyngeal and lung deposition of inhaled insulin using a prototype AERx inhaler (Aradigm Corporation, Hayward, CA, USA). The 3 tested mouthpieces were designed either as a cylindrical mouthpiece or as an elliptical mouthpiece, both with constant CSAs of 7.9 cm^2 and 7.5 cm^2, or as a tapered elliptical mouthpiece with an exit CSA equal (3.7 cm^2) to one half the entrance CSA (7.5 cm^2). The CSAs of these mouthpieces were quite large compared with the CSAs of the stepped mouthpieces (1.6 cm^2, 2.3 cm^2 and 2.8 cm^2) used in the present study. Fifteen healthy subjects participated in the gamma scintigraphy study in which each inhalation of the radiolabelled aerosol was followed by a 5-second breath-hold. The MMADs ranged from 2.2 to 2.3 μm. There were no statistically significant differences in oropharyngeal or lung depositions between males and females and the cross-sectional shapes of the mouthpieces had no significant effect on the oropharyngeal or lung depositions. The lack of effect of the cross-sectional shapes of the mouthpieces might have been related to the use of particles too small to be affected by the differences in mouthpiece designs.

Svartengren et al (1996) investigated whether the mouthpiece length, ~4 cm *versus* ~6.4 cm, would have an impact on oropharyngeal and lung depositions in 9 patients with obstructive airway diseases. The shorter mouthpiece was a standard mouthpiece whereas the longer mouthpiece was designed to bypass part of the oral cavity and thereby reduce oropharyngeal deposition and was cut off at the level of the hard palate for each subject. The subjects inhaled at 0.5 L/s an aerosol consisting of monodisperse radiolabelled Teflon particles with a mean aerodynamic diameter of 3.5 μm. There were, however, no statistically significant differences in oropharyngeal or lung depositions between the mouthpieces.

As a patent application may require some demonstration of functionality of a new invention like the stepped mouthpiece, a small proof-of-concept study is a reasonable means of providing the

early proof. As the stepped mouthpiece design was novel, some guidance for possible future clinical studies on the design of a more durable stepped mouthpiece was also required in terms of design, size and number of mandibular advancement steps. The results showed that increases in upper airway CSA and AUC could be achieved with mandibular advancement during both exhalation and inhalation through a tube design. The results also highlighted that the vertical diameter of a new stepped mouthpiece would be an important factor to consider. The difficulty some of the subjects had with the +6 mm protrusion highlighted the need for a stepped mouthpiece with a smaller distance between each advancement. The possible need for a tongue depressor was highlighted when moving from baseline measurements to the measurements with the stepped mouthpieces.

4.6 Conclusions

In the present study the hypothesis that horizontal mandibular advancement and incisal opening following use of a stepped mouthpiece would increase the mean CSAs and the volumes of the upper airways in 4 healthy subjects was tested. The results – although quite variable – indicated that the size of the upper airways could be increased following use of the stepped mouthpiece during both exhalation and inhalation. Considering the results and the design of the stepped mouthpieces a follow-up clinical study with a redesigned stepped mouthpiece would be warranted. The new stepped mouthpiece should be designed with a tongue depressor, ideally with 1 mm mandibular advancement steps and a vertical diameter of the mouthpiece close to the large mouthpiece used in the present study.

Chapter 5 Assessment of the impact of a stepped mouthpiece on the size of the upper airways of healthy subjects and subjects with OSA measured by acoustic pharyngometry

5.1 Introduction

5.1.1 Study background

A set of stepped mouthpieces were tested in a proof-of-concept study presented in Chapter 4. The stepped mouthpieces were designed with a round back orifice to be connected to the wavetube of the acoustic pharyngometer and an oval front orifice to be kept between the teeth. The front end of the stepped mouthpieces kept between the teeth was designed with 10 mm, 15 mm or 20 mm orifices (vertical diameters). These front end orifices were designed with a single protrusion on the upper side for the upper incisors, and 4 protrusions on the opposite side of the mouthpiece at distances of -3 mm, ±0 mm, +3 mm and +6 mm in relation to the protrusion on the upper side. The purpose of these was to facilitate a horizontal movement of the mandible as shown in Chapter 4, Figure 4.2.

The CSAs (mm^2) of the three different orifices with 10 mm, 15 mm and 20 mm vertical diameters were 161 mm^2, 232 mm^2 and 278 mm^2, and the length 40 mm. The stepped mouthpieces were not designed with a tongue depressor. The results reported in Chapter 4 indicated that a tongue depressor might be required, and that the vertical dimension of the stepped mouthpieces, the horizontal mandibular advancement and a combination of these increased the size of the upper airways defined as the area between the incisors and the GL.

5.1.2 A redesigned stepped mouthpiece

A new stepped mouthpiece with tongue depressor was designed for the purpose of a parallel group clinical study. The abstract of the patent of the stepped mouthpiece with tongue depressor (patent US 2012/0240922 A1; published 27 September, 2012; PCT filed 9 November, 2010) described the new invention, as follows:

"An apparatus and method to aid in administering inhaled pharmaceutical aerosol to a patient is configured to maintain a tongue in proper position and offset the patient's upper and lower jaws during aerosol delivery. An adjustable member is provided adjacent a mouthpiece and at least partially surrounds and moves with respect to the body of the apparatus. The adjustable member has a step structure to impart a selected amount of mandibular advancement to a patient during aerosol delivery. A tongue depressor which may be integrally formed with the adjustable member configured to prevent a tongue from occluding a flow of aerosol is also provided."

The new stepped mouthpiece was 81 mm long fully extended including tongue depressor and the external horizontal and vertical diameters were 34 mm and 24 mm, respectively (Figure 5.1). The tongue depressor and the related part of the mouthpiece to be held in the mouth were 33 mm long and the external horizontal and vertical diameters 34 mm and 18 mm, respectively. The 18 mm vertical external diameter was chosen partly based on the results of the previous proof-of-concept study in which the largest mouthpiece had an external vertical mouthpiece diameter of 20 mm and partly as this is a common vertical size of jet nebuliser mouthpieces. The length of the stepped mouthpiece from the round end (right in Figure 5.1) to the position for the upper incisors was 52 mm.

Figure 5.1: Picture of the stepped mouthpiece with the circular back end to be connected to the acoustic pharyngometer wavetube to the right and the tongue depressor with the mouthpiece slider to the left.

The round 22 mm end of the stepped mouthpiece was designed to be connected to the 22 mm end of the wavetube through a green elastomeric-lipped ISO connector (Intersurgical Ltd, Wokingham, UK) (Figure 5.2).

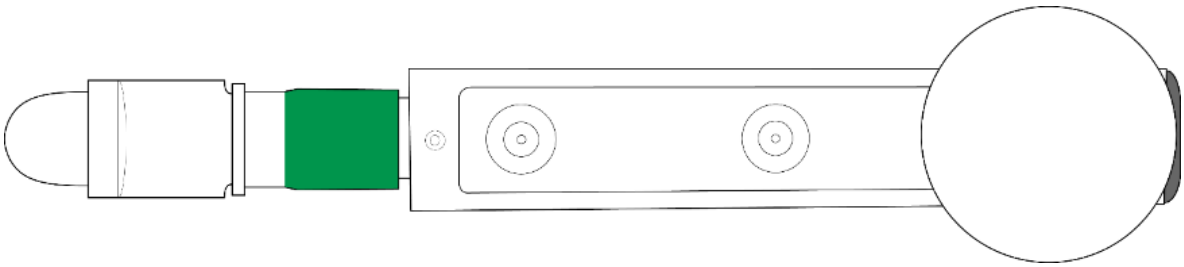


Figure 5.2: The stepped mouthpiece attached to the pharyngometer wavetube through a green elastomeric-lipped ISO connector (Intersurgical Ltd, Wokingham, UK) as used in the study.

The stepped mouthpiece was designed with a wall thickness of 1.1 mm, and with 6 steps of 1 mm pitch each providing a total horizontal movement of 6 mm. The new stepped mouthpiece was made of transparent Polycarbonate Makrolon 2858 resin (Bayer Material Science, Pittsburgh, PA, USA). The design of the stepped mouthpiece is shown in more detail in Figure 5.3 (body of stepped mouthpiece) and Figure 5.4 (slider with tongue depressor).

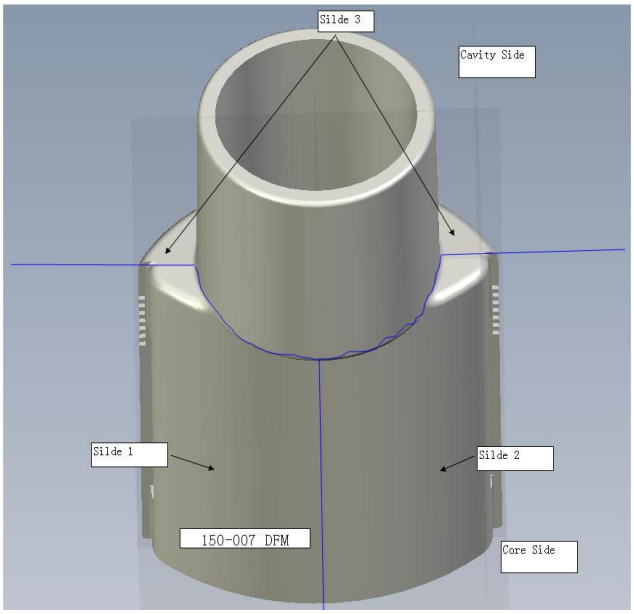


Figure 5.3: The body of the stepped mouthpiece shown with the circular back end to be connected to the acoustic pharyngometry wavetube (top) used in the study.

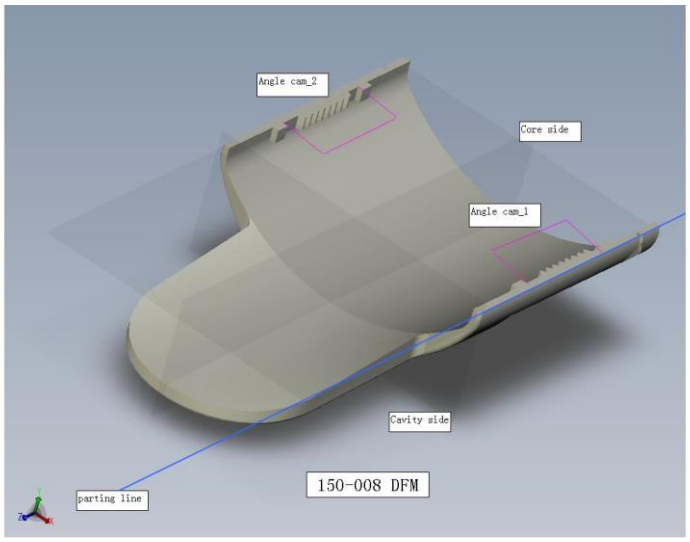


Figure 5.4: The stepped mouthpiece slider with tongue depressor shown from the cavity side. The pitches designed for 1 mm incremental movement are shown on both sides of the slider.

The CSAs (mm^2) of the three stepped mouthpieces used in the proof-of-concept study were 161 mm^2, 232 mm^2 and 278 mm^2. As the new stepped mouthpiece was designed to partly match the largest of the stepped mouthpieces, the CSAs of the new stepped mouthpiece were 266 mm^2 (front), 247 mm^2 (middle) and 298 mm^2 (rear) as shown in Figure 5.5.

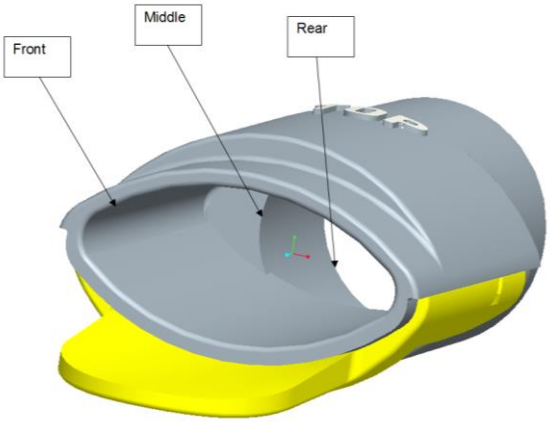


Figure 5.5: The stepped mouthpiece used in the study with CSAs of 266 mm^2 (front), 247 mm^2 (middle) and 298 mm^2 (rear).

The transparent stepped mouthpiece used in the study is shown in 2 photographs in Figure 5.6.

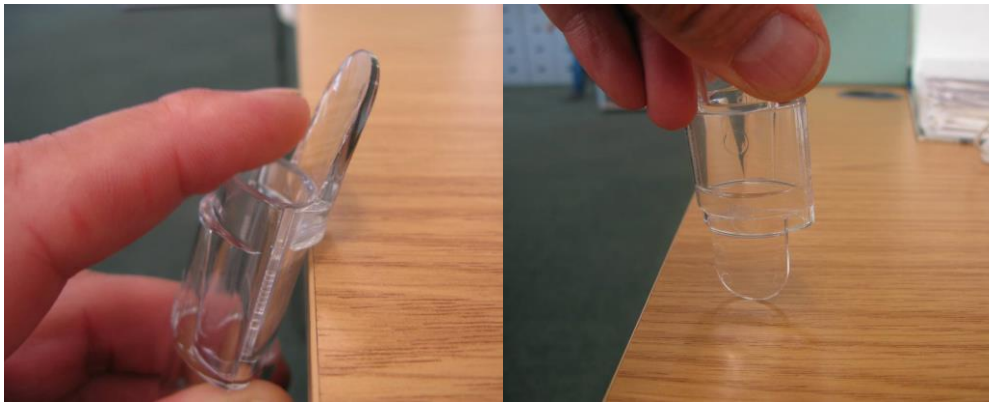


Figure 5.6: Two photographs of the new stepped mouthpiece are shown. In the left hand picture the stepped mouthpiece is shown with the slider in the 1st position with no horizontal movement. In the right hand picture the stepped mouthpiece is shown with the slider in the 6th position with 6 mm horizontal movement.

5.1.3 Acoustic pharyngometry

As described in Chapter 2, section 2.7 of this thesis.

The Eccovision ARP has been used in a large number of clinical studies of the upper airways (section 2.7.6-2.7.7). The pharyngogram obtained from a measurement with the acoustic

pharyngometer can be analysed in terms of the CSAs and AUCs of the oral cavity, the OPJ, the oropharynx, the EG, the hypopharynx and the GL.

5.1.4 Measurement of the range of motion of the mandible

A George Gauge (Great Lake Orthodontic Products, Tonawanda, NY, USA) is a tool for measurement of the protrusive and retrusive capacities of the mandible (Figure 5.7). The George Gauge was used in the present study for the measurement of the most protrusive (mm) and most retrusive positions (mm) of the mandible, each measured from an edge-to-edge position of the upper and lower incisors. The incisal edge-to-edge position was selected as basis for the measurements due to the limited adjustability of the mouthpiece. In addition to the above measurements, the anteroposterior range of motion (mm) of the mandible was measured.

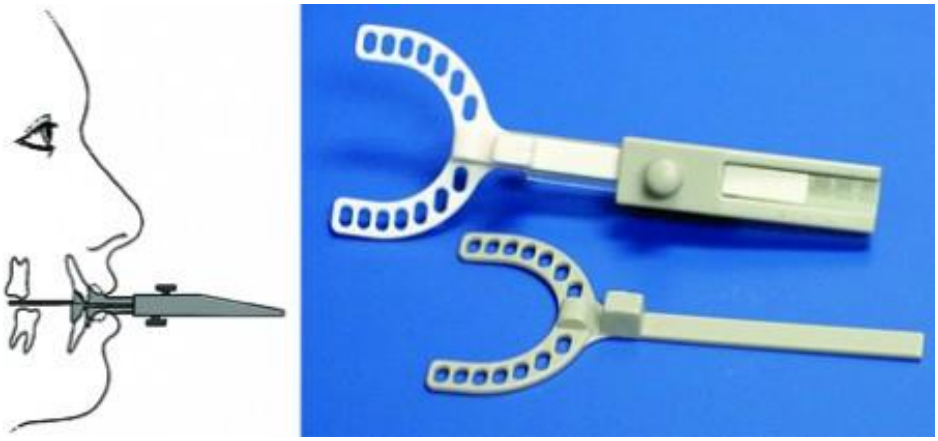


Figure 5.7: The George Gauge is shown in a subject's mouth (left), and with the bite fork connected to the body with the mm rule and the bite fork alone (right).

The George Gauge consists of a body and a bite fork and is available in either 2- or 5-mm incisal thickness and the 2-mm bite fork was used in the present study (Figure 5.7) (Wee, 2012).

5.1.5 The Epworth Sleepiness Scale

The Epworth Sleepiness Scale test is a subjective questionnaire for evaluation of the extent of daytime sleepiness in everyday situations (Johns, 1991; Johns, 1992). Through this questionnaire

a subject is asked to rate the likelihood of falling asleep in certain situations he/she would encounter in the course of a day. On the form the subject can choose the option that best reflects his/her recent experience. There are 8 questions, and answers are rated from 0 to 3 and the scale is 0-24. An answer of 0 means that the subject would never fall asleep in that situation, whereas answering 3 means it was very likely that the subject would fall asleep. The following scale was used: *0 = no chance of dozing*; *1 = slight chance of dozing*; *2 = moderate chance of dozing*; *3 = high chance of dozing.* A "Situation" table was used to clarify the examples (Table 5.1).

Table 5.1: The "Situation" table used in the Epworth questionnaire.

Situation	**Chance of dosing**
Sitting and reading	
Watching TV	
Sitting inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in traffic	

The Epworth Sleepiness Scale test was used during study inclusion to test subjects claiming no history of OSA.

5.1.6 Study hypothesis

Mandibular advancement together with incisal opening during tidal breathing achieved through a stepped mouthpiece design affects the size of the upper airways in subjects without and with OSA.

5.2 Study objectives

5.2.1 Primary objective

The primary objective of the study was to measure through acoustic pharyngometry the impact of different horizontal mandibular advancement positions - achieved with a stepped mouthpiece with a tongue depressor - on the size of the upper airways in subjects without and with OSA. Subjects with OSA were selected as a control group as mandibular advancement has been practised in in this group during several decades. Therefore a large number of publications on CSAs, AUCs and protrusive and retrusive data following mandibular advancement is available from studies in subjects with OSA. The definition of "upper airways" included the area from the incisors to the GL. The measurements were performed while the subjects were seated in a chair and inhaled room air during tidal breathing through the stepped mouthpiece. The acoustic pharyngometry measurement was performed at mid inhalation.

5.2.2 Secondary objectives

The secondary objectives included:

- Assessment of the most protrusive and most retrusive positions of the mandible - each measured from an incisal edge-to-edge position - and the anteroposterior range of motion of the mandible.
- Four baseline acoustic pharyngometry measurements during exhalation during tidal breathing with the standard wavetube mouthpiece which included a tongue depressor (without the stepped mouthpiece); 2 at FRC, 1 during nasal breathing and 1 with a Mueller or a Valsalva manoeuvre (Brown et al., 1986; www.sleepgroupsolutions.com).
- Four baseline acoustic pharyngometry measurements during inhalation during tidal breathing with the standard wavetube mouthpiece which included a tongue depressor (without the

stepped mouthpiece). The acoustic pharyngometry measurement was performed at mid inhalation.

- Measurement of the upper airways through acoustic pharyngometry during inhalation during slow and deep breathing while the subject used a stepped mouthpiece. The acoustic pharyngometry measurement was performed at mid inhalation.
- Assessment of the most comfortable mandibular protrusion position for the subject when using the stepped mouthpiece during tidal breathing. The scoring of the "comfortable position" was performed through a Likert-style questionnaire (Likert, 1932).
- Assessment of the most comfortable mandibular advancement position for the subject when using the stepped mouthpiece during slow and deep breathing. The scoring of the "comfortable position" was performed through a Likert-style questionnaire (Likert, 1932).
- When the most "comfortable position" with the stepped mouthpiece during both tidal, and slow and deep breathing had been established as outlined above, the subject was asked to hold that position for 3 minutes. After 3 minutes, the subject's level of comfort was re-evaluated through a Likert-style questionnaire (Likert, 1932).

5.3 Methods

5.3.1 Study design

The study was performed as an open investigation including 60 subjects. The subjects were enrolled from subjects visiting the dental practice of Dr John Viviano (Mississauga, ON, Canada). When enrolled, the subjects visited the dental practise once for study inclusion and measurements of their upper airways through acoustic pharyngometry which was performed by Dr Viviano. The 60 subjects were stratified such that 30 were healthy subjects and 30 had been diagnosed with OSA with equal numbers of male and female subjects. The acoustic pharyngometer measurements

were performed with the subjects seated on a straight-backed chair. The aim was to keep the wavetube horizontally parallel to the floor and prevent head, neck and shoulder movement by instructing the subjects to keep their gaze fixed at a point on the wall. A comfortable position was important in order to avoid any increase in muscle tonus through heavy occlusion on the mouthpieces (Viviano, 2002a). The measurements followed the instructions to a subject in the Eccovision Acoustic Pharyngometry Operator Manual Hood Laboratories, Pembroke, MA, USA; presently (www.sleepgroupsolutions.com):

- You will sit in a chair and hold a wand with a mouthpiece on it.
- You will place the mouthpiece in your mouth and do various breathing on the mouthpiece as instructed by the technologist.
- Breathing through the mouth normally for 10 to 12 seconds.
- Breathing through the nose for 10 to 12 seconds.
- Closing your glottis and exhaling.
- Closing your glottis and inhaling.
- A technologist will instruct you on how to perform the test and coach and encourage you to do your best.

5.3.2 Clinical assessments

Demographics included: age (years), height (cm), weight (kg), Body Mass Index (BMI; [weight in kg/(height in m^2)], gender, diagnosis of OSA (yes/no), neck circumference (cm), most protrusive (mm) and most retrusive positions (mm) of the mandible, and the range of motion of the mandible (mm).

Measurement of the upper airways by acoustic pharyngometry (as described in Chapter 2, section 2.7 of this thesis), as follows:

- Four baseline pharyngograms with the acoustic pharyngometer with the subject exhaling through the wavetube (without stepped mouthpiece and nose clip) were recorded. Two of

the pharyngograms were recorded at FRC, 1 during nasal breathing and 1 with a Mueller or a Valsalva manoeuvre (Brown et al., 1986). The Investigator highlighted and recorded the positions of the OPJ, the EG and the GL when saving the data onto the acoustic pharyngometer.

- Four baseline recordings made during mid-inhalation with the subject inhaling through the wavetube (without stepped mouthpiece and nose clip).
- During tidal breathing 4 pharyngograms were recorded at mid-inhalation per each mandibular protrusion achieved with the stepped mouthpiece. Five of the 6 possible protrusions of the stepped mouthpiece were used (1, 2, 3, 4, and 5 mm). The pharyngograms were recorded during tidal breathing (without nose clip) with the stepped mouthpiece connected to the wavetube. Due to the addition of the stepped mouthpiece to the wavetube a shift of the pharyngogram to the right occurred in comparison with the baseline pharyngograms. The Investigator highlighted and recorded the positions of the OPJ, EG and GL when saving the data onto the acoustic pharyngometer.
- During slow and deep breathing 4 pharyngograms were recorded at mid-inhalation per each mandibular protrusion achieved with the stepped mouthpiece. Five of the 6 possible advancements of the stepped mouthpiece were used (1, 2, 3, 4, and 5 mm). The pharyngograms were recorded during slow and deep breathing (without nose clip) with the stepped mouthpiece connected to the wavetube. Due to the addition of the stepped mouthpiece to the wavetube a shift of the pharyngogram to the right occurred in comparison with the baseline pharyngograms. The Investigator highlighted and recorded the positions of the OPJ, EG and GL when saving the data onto the acoustic pharyngometer.

5.3.3 Study subjects

5.3.3.1 Inclusion criteria

- Subjects provided written informed consent to participate in the study.
- Adult male or female subjects over 18 years of age who had not been or had been diagnosed with OSA.
- Subjects claiming no history of OSA were tested through the Epworth Sleepiness Scale test and obtained a result less than 10 (scale 0-24).
- Subjects satisfied the study investigator about their fitness to participate in the study and their availability to complete the study.

5.3.3.2 Exclusion criteria

- Subjects not compliant with the instructions for use of the stepped mouthpiece and the study procedures.
- Subjects who had participated in a clinical trial in the previous month.

5.3.3.3 Withdrawal criteria

- If the Investigator considered that the subject's health would be compromised by remaining in the study or the subject was not sufficiently cooperative.
- On request from the subject for any reason.

Verbal and written (information form) description of the study was given to the subjects who were given sufficient time to decide whether they would like to enter the study. Written consent would be obtained prior to commencement of any study procedures. If a subject withdrew from the study at any time either at his/her request or at the Investigator's discretion, the reason(s) for withdrawal was recorded on the relevant page of the CRF. Data from such subjects would be used in the

analyses of the study if appropriate data was available. Furthermore, it was vital to obtain follow up data on any subject withdrawn because of an adverse event.

5.3.4 Study variables

5.3.4.1 Primary study variables

The primary study variable was the size of the upper airways from the incisors to the GL defined in terms of CSA1-3 (for OPJ, EP and GL; in cm^2) and volume (area under the curve, AUC; AUC1-3 for the area between incisors and OPJ, between OPJ and EG, between EG and GL; in cm^3). The CSAs and the AUCs were derived through computer processing.

5.3.4.2 Secondary study variables

The secondary study variables included the demographics, the collar size and the most protusive and retrusive positions of the mandible - each measured from an incisal edge-to-edge position - and the anteroposterior range of motion of the mandible.

5.4 Adverse events

An AE was defined as any untoward medical occurrence in a subject undergoing an investigational procedure and which did not necessarily have a causal relationship with the device under investigation. An AE could therefore be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a device, whether or not considered related to the device under investigation. AEs were recorded on the CRFs.

5.5 Ethical considerations

The study was performed according to the principles of the Declaration of Helsinki (South Africa, 1996) and the ABPI Guidelines for Medical Experiments in Non-Patient Human Volunteers - 1988, amended May 1990 and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP). The study was registered at www.clinicaltrials.gov - a service of the U.S. National

Institutes of Health - with the identifier: NCT01069068. Ethics Committee approval was obtained (APPENDIX C-1) prior to the start of the study and prior to any communication with potential study subjects and no study related procedures were carried out before ethics committee approval had been granted.

5.6 Statistical analysis

Due to the exploratory nature of this study, the sample size employed in the study was not based on any previous results. The main statistical analysis was planned to be focused on the impact of the stepped mouthpiece on the upper airways and the possible differences in this aspect between the two study groups. The study data was analysed by SAS 9.2 for Windows (W32_VSPRO platform), running on a Lenovo L412 under Windows 7 Professional. The significance level was established at 0.05.

5.6.1 Acoustic pharyngometry data

The acoustic pharyngometry measurements were coded BFL (baseline, exhalation, in all BFL1-4), BMI0 (baseline, inhalation), SMI0-5 (tidal breathing) and SSI0-5 (slow and deep breathing), Table 5.2.

For each subject 4 pharyngograms were recorded for baseline measurements during **exhalation** and 4 for baseline measurements during **inhalation**. For measurements during either **tidal breathing** or **slow and deep breathing**, 4 pharyngograms were recorded for each subject per stepped mouthpiece position, in total 24 pharyngograms per breathing pattern. The 6 stepped mouthpiece positions tested included no advancement (0), and advancements of 1, 2, 3, 4, and 5 mm. Thus the last possible stepped mouthpiece advancement of 6 mm was not tested. The decision not to test the 6 mm advancement was based on the experience from the proof-of-concept study in which the subjects found the +6 mm protrusion to be difficult to achieve. The raw acoustic

pharyngometry data from each measurement was imported into Microsoft Excel as space delimited data for statistical analysis.

Table 5.2: Codes for the acoustic pharyngometry data.

Codes	Explanations
BFL1-4	BFL, in which B = Baseline, F = FRC and L = Landmarks. Each BFL file contained 4 acoustic pharyngometry readings as follows: • 1 – FRC • 2 - FRC • 3 - Nasal breathing • 4 - Coaching for glottal closure In the figures the following short forms were used: BFL = two measurements from FRC, BFL3 = one measurement during nasal breathing, and BFL4 = one measurement during glottal closure.
BMI0	BMI0, in which B = Baseline, M = Mid tidal inhalation and I = Inhalation. The BMI file contained 4 acoustic pharyngometry readings.
SMI0	SMI0, in which S = Stepped mouthpiece, M = Measurement at mid tidal inhalation, I = Inhalation and 0 = 0 mm, no advancement with stepped mouthpiece.
SMI1	SMI1, as above with 1 mm advancement with stepped mouthpiece.
SMI2	SMI2, as above with 2 mm advancement with stepped mouthpiece.
SMI3	SMI3, as above with 3 mm advancement with stepped mouthpiece.
SMI4	SMI4, as above with 4 mm advancement with stepped mouthpiece.
SMI5	SMI5, as above with 5 mm advancement with stepped mouthpiece.
SSI0	SSI0, in which S = Stepped mouthpiece, S = Measurement during slow and deep inhalation, I = Inhalation and 0 = 0 mm, no advancement with stepped mouthpiece.
SSI1	SSI1, as above with 1 mm advancement with stepped mouthpiece.
SSI2	SSI2, as above with 2 mm advancement with stepped mouthpiece.
SSI3	SSI3, as above with 3 mm advancement with stepped mouthpiece.
SSI4	SSI4, as above with 4 mm advancement with stepped mouthpiece.
SSI5	SSI5, as above with 5 mm advancement with stepped mouthpiece.

5.6.2 Pharyngograms – analysis of CSA

Three CSAs were identified, CSA1-3 as described in Chapter 3, section 3.6.5 in this thesis. For each subject and pharyngograms the CSA for each landmark (OPJ, EG and GL) was determined and the results summarised descriptively.

5.6.3 Pharyngograms – analysis of AUC

Three AUCs were identified, AUC1-3 as described in Chapter 3, section 3.6.5 in this thesis. For each subject and pharyngograms, the AUC between the incisors/wavetube mouthpiece and CSA1, and between CSA1 and CSA2, and between CSA2 and CSA3 was determined and the results summarised descriptively.

5.6.4 Statistical analyses of CSAs and AUCs

For each of the endpoints (CSAs, AUCs) the possible effect of the introduction of the stepped mouthpiece - and therefore the possible impact of the vertical diameter of 18 mm – and the possible effect of the mandibular advancement was investigated using a main fixed effect ANOVA analysis, using subject and displacement in mm as factors. Separate analyses were performed for the 2 study groups and the 2 breathing patterns. The same analysis was performed both with and without some extreme data (data outside ± 3 SD).

5.6.5 GOF analysis of the pharyngograms

As described in Chapter 3, section 3.6.4 in this thesis.

As noted in previous evaluations of pharyngograms in Chapter 3, outlier pharyngograms were occasionally recorded and were removed from the analysis. A similar approach was followed in the present study. For each subject and group of acoustic pharyngometry data (BMI0, SMI0-5, SSI0-5) the individual pharyngograms were compared to the median curve by calculating a GOF measure. GOF was defined as the square root of the average squared vertical distance between the median curve and the curve under study. Individual pharyngograms with too large GOF values were excluded from subsequent evaluations. However, a slightly different approach for the GOF evaluation compared to the one presented in Chapter 3 was adopted due to differences in study design:

- BFL pharyngograms were excluded from the GOF calculation.
- For the BMI0, SMI0-5 and SSI0-5 (13 measurements each consisting of 4 pharyngograms) measurements and each subject, the first maxima and last minima that coincided for at least 2 of the 4 pharyngograms was recorded (total of 13 measurements × 60 subjects = 780 measurements × 4 pharyngograms = 3120 pharyngograms).
- GOF was calculated between these two points.
- Using the calculated cut-offs, GOF was calculated for each of the 3,120 pharyngograms (Figure 5.8).

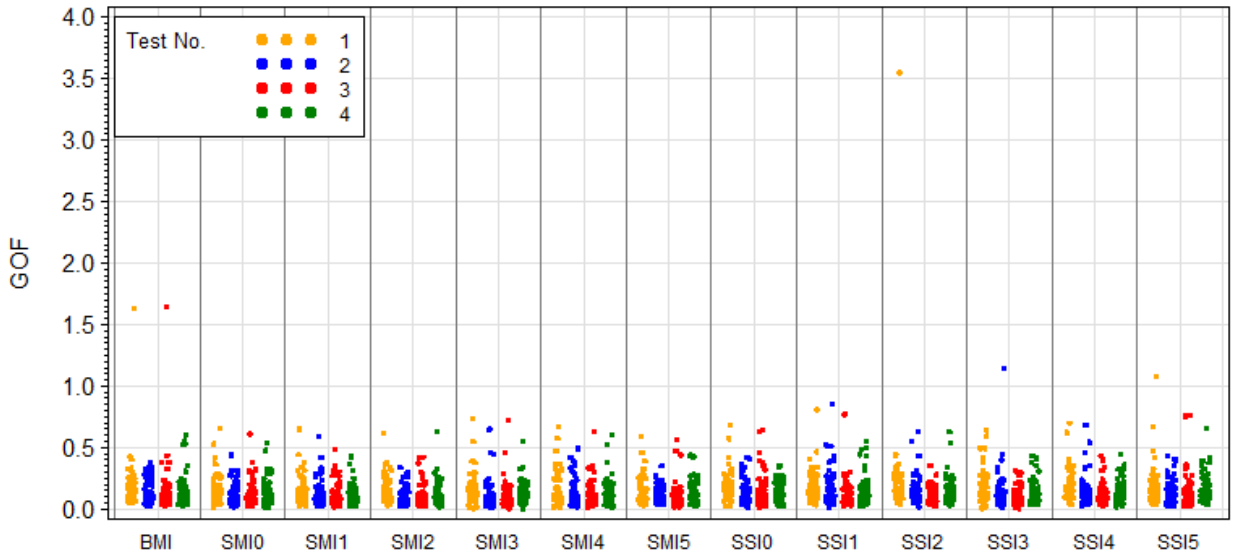


Figure 5.8: GOF analysis of the pharyngograms from the 13 measurements highlighted on the x-axis. The legend refers to the 4 pharyngograms recorded during each of the measurements for each of the 60 study subjects.

The GOF value was mainly below 0.5 but for a few higher values which were removed from further analysis. Five pharyngograms had GOF>1 (0.2%) and 1.7% had GOF>0.5. The choice of a GOF value of 0.5 as a cut-off between accepted or not was subjectively made based on Figure 5.8.

5.7 Results

5.7.1 Demographics

Sixty subjects were included and all met the inclusion and none the exclusion criteria. No AEs were reported. The majority of the subjects were Caucasian (57), with two Black but not Hispanic subjects and one Asian subject. The demographic data including age, height, weight, BMI, and neck circumference are presented in Tables 5.3 to 5.7, separately for the 30 healthy subjects (non-OSA group) and the 30 subjects diagnosed with OSA (OSA group).

Table 5.3: Eight demographic parameters compared statistically between groups.

Parameter	**Mean**		**Difference**		**p-value**
	Non-OSA	OSA	Absolute	Relative (%)	
Age (years)	40.2	50.6	-10.4	-22.9	0.0003
Height (cm)	171.3	170.1	1.24	0.7	0.57
Weight (kg)	76.8	86.1	-9.4	-11.5	0.0388
BMI	26.0	29.8	-3.8	-13.6	0.0048
Neck circumference (cm)	38.2	40.8	-2.7	-6.8	0.0100
Most protrusive (cm)	5.93	7.40	-1.47	-22.1	0.0306
Most retrusive (cm)	6.40	5.47	0.93	15.7	0.0763
Range (cm)	12.3	12.9	-0.5	-4.0	0.24

The two groups differed statistically significantly for age, weight, BMI, collar size and most protrusive mandibular position.

Table 5.4: Subject demographics – subject age (years) at time of study.

OSA	**Sex**	**Mean**	**Median**	**Min**	**Max**	**± SD**
N	F	43.8	41.0	26	62	11.0
	M	36.6	34.0	23	59	8.9
	All	40.2	36.5	23	62	10.5
Y	F	52.8	51.0	38	76	10.3
	M	48.3	50.0	31	66	9.9
	All	50.6	50.0	31	76	10.2
All		45.4	43.5	23	76	11.5

The group with OSA was statistically significantly (~10 years) older than the non-OSA group, females were ~5 years older than males (Table 5.4). Males were ~10 cm taller than females, but there were no apparent differences between groups (Table 5.5).

Table 5.5: Subject demographics – height (cm).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	164.9	164.0	158	173	4.8
	M	177.7	177.5	170	190	5.0
	All	171.3	172.3	158	190	8.1
Y	F	165.9	165.0	152	177	7.9
	M	174.2	174.0	160	188	7.3
	All	170.1	171.5	152	188	8.6
All		170.1	171.5	152	188	8.6

The group with OSA was statistically significantly heavier than the non-OSA group mainly because the females in the OSA group had higher mean weight than the females in the non-OSA group (Table 5.6).

Table 5.6: Subject demographics – weight (kg).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	67.8	63.0	55	97	12.0
	M	85.8	80.0	71	113	12.9
	All	76.8	75.0	55	113	15.3
Y	F	85.4	77.6	54	150	24.7
	M	86.8	90.8	68	104	11.0
	All	86.1	83.8	54	150	18.8
All		81.5	78.5	54	150	17.6

The group with OSA had a statistically significantly higher average BMI than the non-OSA group mainly due to the fact that the females in the OSA group had a higher mean BMI than the females in the non-OSA group (Table 5.7). The differences between the females of the OSA group in comparison with the non-OSA group were similar to the differences in weight**.**

Table 5.7: Subject demographics - BMI.

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	24.9	23.4	21	35	4.0
	M	27.1	26.0	24	33	3.0
	All	26.0	24.7	21	35	3.7
Y	F	30.8	29.3	20	48	7.3
	M	28.7	28.0	23	38	4.5
	All	29.8	28.5	20	48	6.0
All		27.9	27.3	20	48	5.3

The group with OSA had a statistically significantly larger average neck circumference than the non-OSA group mainly due to the fact that the females in the OSA group had a larger neck circumference than the females in the non-OSA group (Table 5.8). The differences between the females of the OSA group in comparison with the non-OSA group were similar to the differences in weight and BMI.

Table 5.8: Subject demographics – neck circumference (cm).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	34.7	34.0	32	39	2.2
	M	41.6	41.0	39	46	2.3
	All	38.2	39.0	32	46	4.1
Y	F	39.4	38.0	36	51	4.2
	M	42.3	42.5	38	46	2.4
	All	40.8	40.0	36	51	3.7
All		39.5	39.8	32	51	4.1

The demographic data included information about the subjects' ability to move the mandible. Data on the most protrusive and most retrusive positions of the mandible measured from an edge-to edge position of the incisors and the range of motion of the mandible are presented in Tables 5.9 to 5.11, separately for the non-OSA and the OSA groups.

Table 5.9: Subject demographics – most protrusive mandible position (mm).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	6.0	6	2	9	1.6
	M	5.9	6	2	9	1.8
	All	5.9	6	2	9	1.7
Y	F	6.1	7	0	10	3.1
	M	8.7	8	4	14	2.9
	All	7.4	8	0	14	3.2
All		6.7	6	0	14	2.6

The most protrusive mandible position was largest for males in the OSA group and mainly due to the difference between males in the two groups there was a statistically significantt difference of ~1.5 mm between the non-OSA and OSA groups (Table 5.9).

The most retrusive mandible position was smallest for males in the OSA group and mainly due to the difference between males in the two groups there was a nonsignificant difference of 1.1 mm between the non-OSA and OSA groups (Table 5.10).

Table 5.10: Subject demographics – most retrusive mandible position (mm).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	6.2	6	5	8	1.1
	M	6.6	7	4	9	1.4
	All	6.4	7	4	9	1.2
Y	F	6.5	6	2	12	2.6
	M	4.4	4	2	9	2.1
	All	5.5	5	2	12	2.5
All		5.9	6	2	12	2.0

The range of motion of the mandible is presented separately for the non-OSA and the OSA groups in Table 5.11.

Table 5.11: Subject demographics – range of motion of the mandible (mm).

OSA	Sex	Mean	Median	Min	Max	± SD
N	F	12.2	12	9	17	1.8
	M	12.5	13	9	15	1.7
	All	12.3	12	9	17	1.7
Y	F	12.6	12	10	16	1.6
	M	13.1	13	10	16	2.0
	All	12.9	13	10	16	1.8
All		12.6	12	9	17	1.8

5.7.2 Landmarks, CSA1-3

For each subject the position of the OPJ, EG and GL was determined by the Investigator during the recording of the four baseline pharyngograms (BFL, exhalation). The individual landmarks are included in APPENDIX C.2, whereas summary statistics for the OP, EG and GL for all 60 subjects are presented as distance (cm) from the incisors in Table 5.12.

Table 5.12: Summary statistics for landmark (OPJ, EG and GL) positions for the 60 subjects in relation to the incisors (cm from incisors; mean, minimum and maximum, median, ± SD).

Landmark	Mean	Median	Min	Max	± SD
OPJ	8.48	8.88	6.30	9.31	0.95
EG	12.22	12.53	9.31	13.59	1.08
GL	20.72	20.45	18.74	23.45	1.34

In Table 5.13 the mean position for each of the three landmarks is presented by gender and separately for the non-OSA and OSA groups. There was no apparent effect on the landmark positions by gender or OSA (Table 5.13) which was confirmed by ANOVA (Table 5.14).

Table 5.13: Mean landmark (OPJ, EG, GL) position (cm from incisors) by gender and OSA.

Gender	OPJ		EG		GL	
	OSA=No	OSA=Yes	OSA=No	OSA=Yes	OSA=No	OSA=Yes
Female	8.3	8.3	11.9	12.3	20.8	21.2
Male	8.6	8.7	12.4	12.3	20.7	20.2

Table 5.14: ANOVA for effects (p-values) by gender and OSA.

Factor	OPJ	EG	GL
Gender	0.20	0.37	0.10
OSA	0.73	0.54	0.80

For each landmark, the Pearson correlation coefficient was calculated to quantify the association between landmark position and each of the parameters age, height, weight, BMI and neck circumference (Table 5.15). All correlations were close to zero indicating weak associations.

Table 5.15: Correlation between landmarks and patient characteristics.

Landmark	Age	Height	Weight	BMI	Neck circumference
OPJ	0.07	0.11	-0.08	-0.20	0.10
EPI	0.11	0.09	-0.02	-0.11	0.13
GL	-0.13	-0.04	0.04	0.11	0.08

5.7.3 Individual pharyngograms

The individual mean pharyngometry data and individual mean pharyngograms are included in APPENDIX C-3. As an example the measurements of subject 1 are shown in Figure 5.9 showing the measurements (each mean of 4 pharyngograms but for BFL, BFL3, and BFL4; check Table 5.2, section 5.6.1) with legends. The y-axis presents the CSA (in cm^2) of the upper airway and the x-axis presents the length of the upper airway (in cm) from the end of the wavetube mouthpiece (BFL, BMI measurements) or the end of the stepped mouthpiece to the GL.

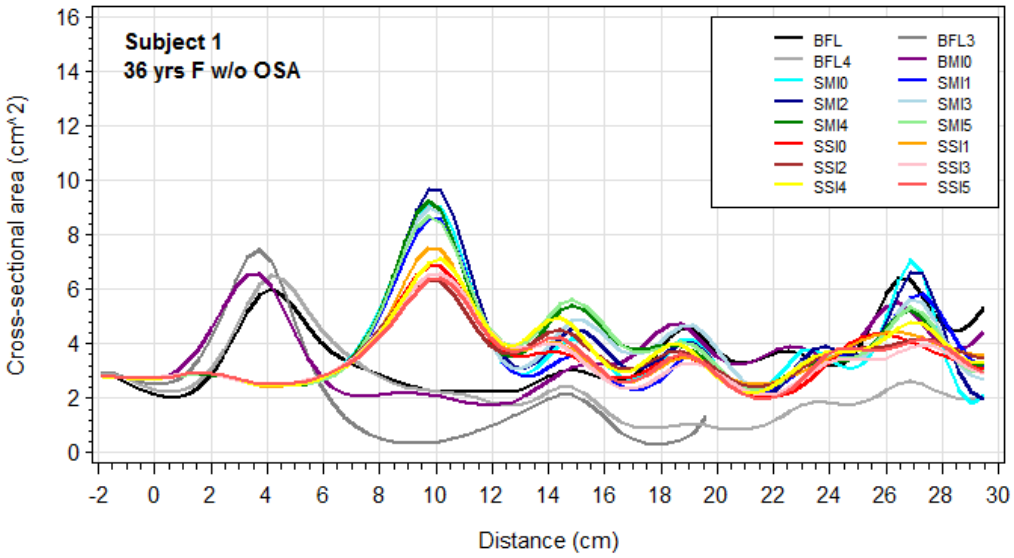


Figure 5.9: Pharyngograms (mean of 4 recordings) by measurement are shown for Subject 1 from the non-OSA group (w/o = without). The codes for the different measurements (BFL, BMI, SMI and SSI) are presented in Table 5.2, section 5.6.1. The baseline measurements differed from the stepped mouthpiece measurements due to the addition of the stepped mouthpiece to the acoustic pharyngometer wavetube which created a shift of the pharyngograms to the right on the x-axis.

The baseline pharyngograms differed from the pharyngograms recorded with the stepped mouthpiece as the addition of the stepped mouthpiece to the acoustic pharyngometer wavetube created a shift of the pharyngograms to the right. BFL and BMI pharyngograms agreed closely, whereas Mueller or Valsalva (Brown et al., 1986) and nasal exhalation pharyngograms deviated as expected.

5.7.4 Summary statistics for CSA1-3 for the non-OSA and OSA groups

Summary statistics for the CSAs are presented in subsections 5.7.4.1 to 5.7.4.3 and Tables 5.16-5.18 for the non-OSA and OSA groups.

5.7.4.1 Summary statistics for CSA1

Table 5.16: Summary statistics for the CSA1 (cm^2) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	2.13	1.98	0.89	4.25	0.71
	BMI0	30	2.18	2.14	0.98	3.92	0.71
	SMI0	30	3.73	3.33	1.86	6.91	1.38
	SMI1	30	3.79	3.04	2.03	7.42	1.49
	SMI2	30	3.82	3.58	1.80	7.27	1.46
	SMI3	30	3.75	3.28	2.03	6.98	1.47
	SMI4	30	4.07	3.86	2.18	7.31	1.54
	SMI5	29	3.89	3.26	1.85	6.63	1.45
	SSI0	30	3.84	3.72	1.50	7.41	1.56
	SSI1	30	3.77	3.55	1.58	6.75	1.44
	SSI2	30	3.90	3.93	1.84	6.59	1.27
	SSI3	30	3.78	3.42	1.49	7.24	1.49
	SSI4	30	4.07	4.07	2.13	6.54	1.38
	SSI5	29	3.98	3.83	1.97	5.93	1.23
Y	BFL1-2	30	1.58	1.56	0.84	2.68	0.39
	BMI0	30	1.60	1.51	0.85	3.03	0.48
	SMI0	30	2.92	2.78	1.18	8.79	1.41
	SMI1	30	2.97	2.71	1.21	10.91	1.68
	SMI2	30	2.99	2.89	1.07	9.31	1.40
	SMI3	29	3.25	2.83	1.13	10.90	1.80
	SMI4	29	3.21	3.03	1.20	8.67	1.40
	SMI5	29	3.10	2.70	1.14	9.06	1.51
	SSI0	30	2.86	2.54	1.18	10.06	1.56
	SSI1	30	3.21	2.78	1.34	9.94	1.68
	SSI2	30	3.24	2.68	1.26	9.40	1.66
	SSI3	29	3.22	3.04	1.07	8.55	1.41
	SSI4	29	3.14	3.21	1.12	7.60	1.17
	SSI5	29	3.27	2.88	1.05	8.32	1.48

5.7.4.2 Summary statistics for CSA2

Table 5.17: Summary statistics for CSA2 (cm^2) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	2.64	2.41	1.05	6.57	1.09
	BMI0	30	2.58	2.32	1.10	5.83	1.06
	SMI0	30	3.32	3.42	1.17	5.94	1.30
	SMI1	30	3.59	3.66	1.17	6.99	1.51
	SMI2	30	3.58	3.38	1.23	7.78	1.65
	SMI3	30	3.84	3.63	1.30	7.45	1.71
	SMI4	30	3.96	3.98	1.38	7.78	1.75
	SMI5	29	4.23	4.03	1.15	8.20	2.07
	SSI0	30	3.38	3.17	1.02	6.42	1.47
	SSI1	30	3.28	3.03	1.15	6.33	1.35
	SSI2	30	3.57	3.32	1.14	7.74	1.58
	SSI3	30	3.77	3.37	1.10	7.70	1.65
	SSI4	30	3.67	3.54	1.40	7.50	1.58
	SSI5	29	3.72	3.79	1.46	8.08	1.72
Y	BFL1-2	30	2.59	2.58	1.23	4.21	0.76
	BMI0	30	2.59	2.52	1.38	4.77	0.74
	SMI0	30	2.72	2.47	1.29	5.73	1.07
	SMI1	30	2.96	2.65	1.46	6.74	1.26
	SMI2	30	2.91	2.70	1.44	5.60	1.08
	SMI3	29	2.93	2.77	1.47	5.99	1.10
	SMI4	29	2.99	2.78	1.33	5.94	1.16
	SMI5	29	3.05	3.00	1.57	6.23	1.20
	SSI0	30	2.84	2.71	1.41	5.61	1.14
	SSI1	30	2.74	2.53	1.25	6.06	1.17
	SSI2	30	2.92	2.63	1.60	6.07	1.22
	SSI3	29	2.97	2.54	1.44	7.00	1.44
	SSI4	29	2.98	2.42	1.43	7.19	1.47
	SSI5	29	3.02	2.72	1.40	6.91	1.44

5.7.4.3 Summary statistics for CSA3

Table 5.18: Summary statistics for CSA3 (cm^2) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	2.88	2.87	1.20	4.84	0.87
	BMI0	30	2.98	3.04	2.03	4.50	0.67
	SMI0	30	4.42	3.85	1.03	10.37	2.26
	SMI1	30	4.10	3.89	1.34	7.76	1.68
	SMI2	30	3.95	3.52	1.42	9.86	1.94
	SMI3	30	4.21	3.72	1.34	10.57	2.01
	SMI4	30	3.95	3.74	1.33	10.49	1.74
	SMI5	29	3.95	3.92	1.36	7.65	1.65
	SSI0	29	4.71	3.46	0.66	15.09	3.91
	SSI1	29	3.89	3.35	1.03	9.77	2.25
	SSI2	30	3.81	3.13	0.54	11.96	2.47
	SSI3	30	4.02	3.29	1.59	11.52	2.33
	SSI4	30	3.92	3.27	1.16	10.89	2.17
	SSI5	29	3.66	3.47	0.87	8.62	1.74
Y	BFL1-2	30	2.79	2.69	1.55	5.24	0.80
	BMI0	30	2.95	3.05	1.62	4.96	0.74
	SMI0	29	3.87	3.25	1.35	11.55	2.23
	SMI1	29	3.79	3.11	1.19	9.88	2.22
	SMI2	30	3.69	3.13	0.61	9.95	2.23
	SMI3	29	3.74	3.16	1.59	8.57	1.87
	SMI4	29	3.75	3.41	1.52	10.18	1.98
	SMI5	29	3.83	3.41	1.47	12.78	2.19
	SSI0	29	3.81	3.16	0.61	12.20	2.44
	SSI1	29	3.81	2.83	1.05	11.73	2.60
	SSI2	29	3.91	2.89	0.18	11.44	2.86
	SSI3	28	3.92	2.81	1.33	10.39	2.67
	SSI4	28	3.72	2.89	1.37	14.39	2.77
	SSI5	28	3.63	3.11	1.30	10.38	2.07

5.7.5 Graphical presentation of the changes in CSAs

During tidal breathing (SMI0-5) the impact of the vertical diameter of the stepped mouthpiece on the CSAs was larger than the effect of the mandibular advancement for CSA1 and CSA3 for both groups (Figure 5.10). However, for CSA2 in the non-OSA group the change in the size of CSA2 was larger following mandibular advancement (SMI5-SMI0 = 0.91 cm^2) than following the introduction of the stepped mouthpiece (SMI0 - BMI0 = 0.74 cm^2). The change in size of CSA2 in the OSA group was also larger following mandibular advancement (0.33 cm^2) than following the introduction of the stepped mouthpiece (0.13 cm^2) although the magnitude was smaller.

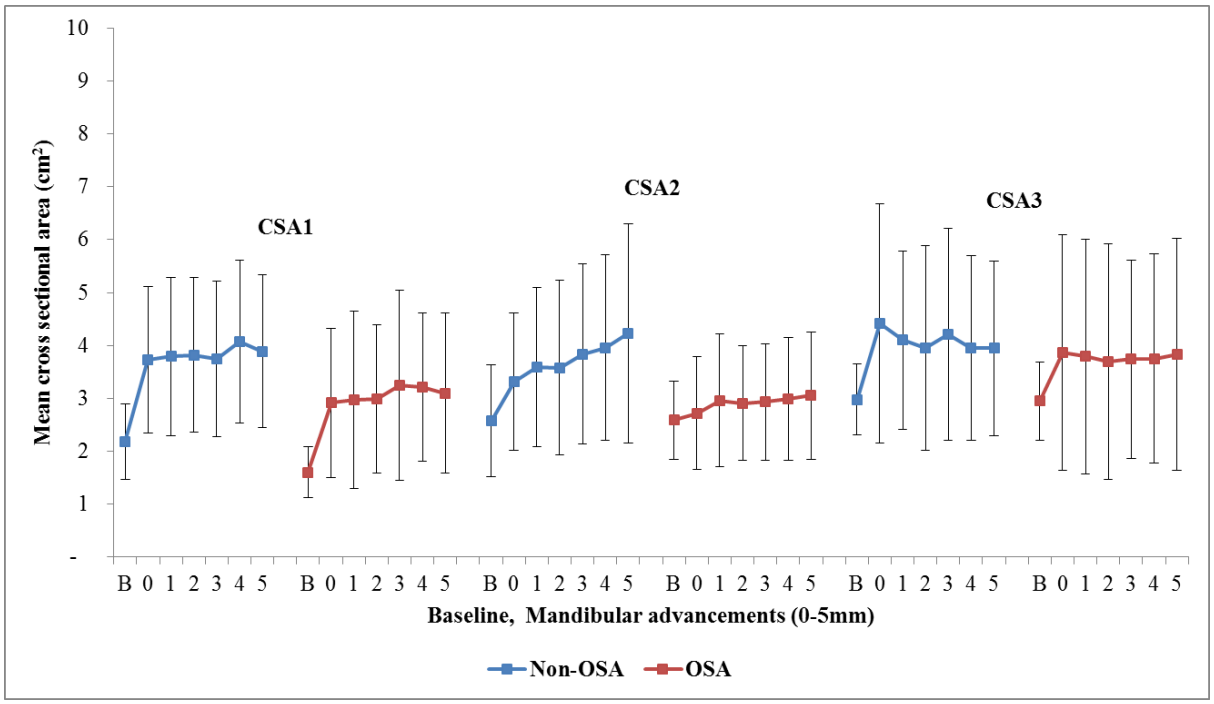


Figure 5.10: The CSAs are presented as mean values (± SD) for tidal breathing per group (non-OSA, OSA) following introduction of the stepped mouthpiece (B = baseline BMI0) and following the mandibular advancements (0-5 mm, SMI0 to SMI5).

During slow and deep breathing (SSI0-5) the impact of the vertical diameter of the stepped mouthpiece on the CSAs was larger in relation to the effect of the mandibular advancements for all of the CSAs (Figure 5.11). In the non-OSA group the change in the size of CSA2 was smaller

following full mandibular advancement (SSI5 - SSI0 = 0.34 cm^2) and larger (difference between SSI0 - BMI0 = 0.80 cm^2; Figure 5.11) following the introduction of the stepped mouthpiece (vertical change). The change in size of CSA2 in the OSA group followed the same pattern with smaller change following mandibular advancement (0.18 cm^2) and larger (0.25 cm^2) following the introduction of the stepped mouthpiece (vertical change).

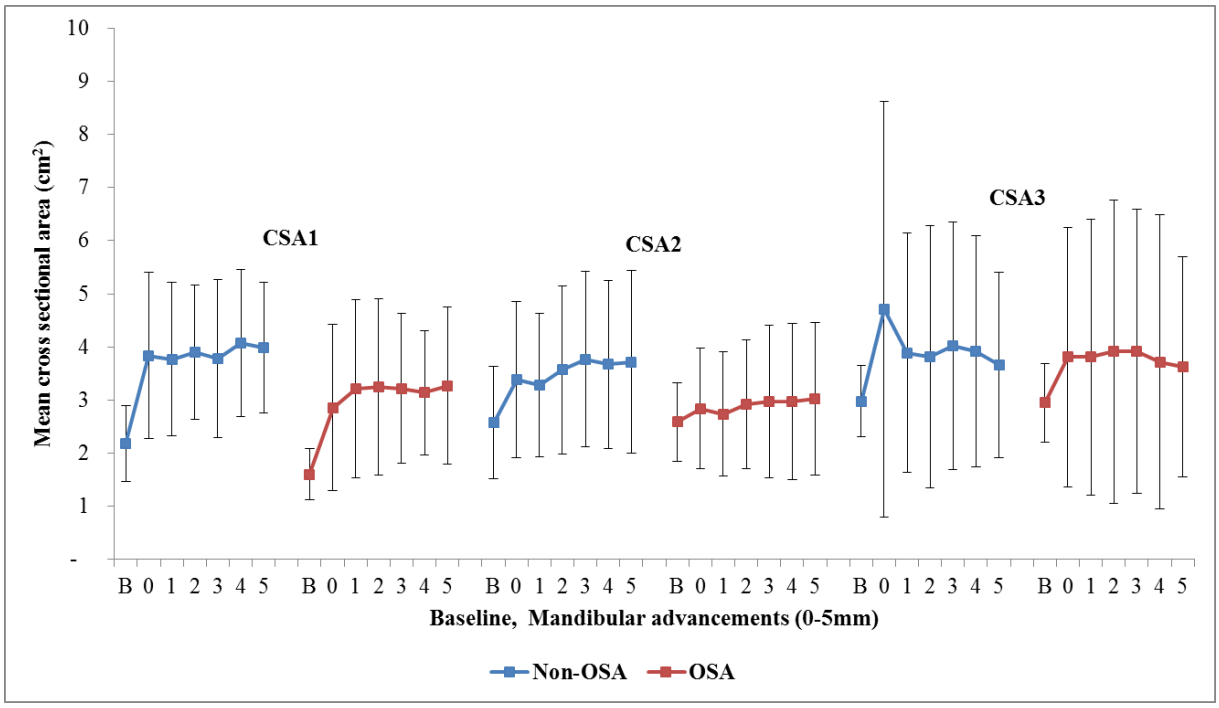


Figure 5.11: The CSAs are presented as mean values (± SD) for slow and deep breathing per group (non-OSA, OSA) following introduction of the stepped mouthpiece (B = baseline BMI0) and following the mandibular advancements (0-5 mm, SSI0 to SSI5).

5.7.6 Summary statistics for AUC1-3 for the non-OSA and OSA groups

Summary statistics for the AUCs are presented in subsections 5.7.6.1 to 5.7.6.3 and Tables 5.19-5.21 for the non-OSA and OSA groups.

5.7.6.1 Summary statistics for AUC1

Table 5.19: Summary statistics for AUC1 (cm^3) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	36.27	36.14	18.75	49.54	7.13
	BMI0	30	35.57	36.24	21.95	49.15	7.14
	SMI0	30	45.84	46.09	29.41	62.23	8.39
	SMI1	30	44.78	44.02	28.57	65.49	8.55
	SMI2	30	44.37	45.44	28.23	60.53	8.66
	SMI3	30	44.11	45.14	29.52	64.69	9.42
	SMI4	30	45.23	45.80	28.02	58.85	8.58
	SMI5	29	44.06	44.29	25.31	59.33	9.63
	SSI0	30	46.13	46.52	23.95	69.26	9.68
	SSI1	30	46.09	45.91	28.70	61.40	8.84
	SSI2	30	44.21	44.51	31.23	61.10	8.29
	SSI3	30	44.49	44.53	28.97	59.87	8.40
	SSI4	30	45.38	46.51	29.87	60.81	8.93
	SSI5	29	44.87	46.21	27.18	60.79	9.19
Y	BFL1-2	30	33.12	34.30	24.41	45.54	5.46
	BMI0	30	33.99	34.37	21.23	46.59	6.46
	SMI0	30	46.14	46.71	29.58	60.66	6.95
	SMI1	30	45.81	45.95	31.59	61.00	6.83
	SMI2	30	45.42	45.76	28.90	61.07	7.55
	SMI3	29	46.32	45.67	32.92	60.62	6.86
	SMI4	29	45.33	46.31	30.18	60.00	7.05
	SMI5	29	45.27	45.86	27.70	63.10	7.99
	SSI0	30	45.34	45.16	30.69	57.72	6.63
	SSI1	30	47.50	46.46	34.46	59.10	5.84
	SSI2	30	47.03	46.65	33.35	61.21	5.88
	SSI3	29	47.26	47.52	30.66	59.75	6.84
	SSI4	29	46.48	46.13	29.13	60.78	7.60
	SSI5	29	46.13	46.24	30.24	64.41	8.24

5.7.6.2 Summary statistics for AUC2

Table 5.20: Summary statistics for AUC2 (cm^3) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	9.37	8.91	4.60	19.02	3.46
	BMI0	30	9.20	8.67	4.57	18.67	3.28
	SMI0	30	10.92	10.41	4.58	19.91	3.51
	SMI1	30	11.72	11.12	5.08	21.99	3.79
	SMI2	30	11.75	11.84	4.86	19.66	3.81
	SMI3	30	12.16	11.49	4.48	22.67	4.27
	SMI4	30	13.12	12.07	4.73	22.84	4.49
	SMI5	29	13.40	13.77	4.61	28.00	4.76
	SSI0	30	10.98	10.72	4.47	19.11	3.62
	SSI1	30	11.06	10.05	4.30	20.91	4.03
	SSI2	30	11.68	12.25	4.60	18.03	3.31
	SSI3	30	11.86	11.30	5.24	20.48	3.50
	SSI4	30	12.37	12.08	4.87	22.92	4.19
	SSI5	29	12.33	11.86	4.63	22.13	4.01
Y	BFL1-2	30	7.96	7.90	4.44	11.03	1.79
	BMI0	30	7.90	7.49	4.40	14.12	2.33
	SMI0	30	8.75	8.20	4.67	15.26	2.61
	SMI1	30	9.07	8.34	4.30	17.63	2.96
	SMI2	30	9.30	9.18	4.31	15.53	2.51
	SMI3	29	9.67	9.18	4.48	17.37	2.80
	SMI4	29	9.64	9.32	4.53	13.91	2.45
	SMI5	29	9.69	9.45	4.23	15.31	2.90
	SSI0	30	8.40	8.11	3.89	17.94	2.88
	SSI1	30	8.94	8.34	4.16	16.47	3.08
	SSI2	30	9.26	8.61	4.68	16.23	3.07
	SSI3	29	9.37	8.51	4.08	15.72	3.38
	SSI4	29	9.41	8.76	4.63	15.34	3.24
	SSI5	29	9.73	9.35	5.07	16.03	3.28

5.7.6.3 Summary statistics for AUC3

Table 5.21: Summary statistics for AUC3 (cm^3) by OSA. The results are presented as mean, median, minimum, maximum, ± SD for baseline measurements (BFL1-2 during exhalation, BMI0 during inhalation), measurements during tidal breathing with the stepped mouthpiece (SMI0-5) and measurements during slow and deep breathing with the stepped mouthpiece (SSI0-5).

OSA	Measurement	N	Mean	Median	Min	Max	± SD
N	BFL1-2	30	27.49	27.23	14.62	44.81	8.14
	BMI0	30	27.00	27.37	11.99	45.33	8.46
	SMI0	30	29.83	29.41	12.30	48.46	9.35
	SMI1	30	30.78	30.47	14.39	55.31	9.63
	SMI2	30	30.12	29.95	15.52	49.70	9.08
	SMI3	30	30.61	31.90	13.88	51.23	8.98
	SMI4	30	30.19	30.72	13.89	55.78	9.56
	SMI5	29	31.31	32.04	11.05	53.66	10.88
	SSI0	30	28.63	27.13	14.28	55.52	9.70
	SSI1	30	28.20	27.76	15.01	45.90	8.43
	SSI2	30	28.27	28.40	14.32	43.59	8.02
	SSI3	30	29.03	29.25	15.63	53.19	8.88
	SSI4	30	28.28	29.45	15.65	41.94	7.78
	SSI5	29	29.00	29.91	14.84	43.37	7.61
Y	BFL1-2	30	26.62	25.71	14.22	48.94	7.90
	BMI0	30	26.51	24.42	14.23	49.32	7.29
	SMI0	30	27.80	25.07	16.09	49.47	8.74
	SMI1	30	27.92	25.84	15.70	52.55	8.61
	SMI2	30	27.65	25.93	16.50	44.77	7.88
	SMI3	29	27.54	23.78	15.89	46.43	8.49
	SMI4	29	28.00	26.46	16.09	46.69	8.17
	SMI5	29	28.05	25.31	17.05	49.32	8.90
	SSI0	30	27.34	25.37	13.15	50.39	8.36
	SSI1	30	27.43	25.56	15.29	51.50	8.77
	SSI2	30	27.24	26.62	15.30	51.86	8.82
	SSI3	29	28.14	28.06	15.91	53.77	9.91
	SSI4	29	26.84	24.90	16.30	52.89	8.99
	SSI5	29	27.90	25.56	16.65	52.83	9.64

5.7.7 Graphical presentation of the changes in AUCs

During tidal breathing (SMI0-5) the impact of the vertical diameter of the stepped mouthpiece on AUC1 was larger than the effect of the mandibular advancements for both groups (Figure 5.12). For AUC2 in the non-OSA group the change in the size of AUC2 was larger following mandibular advancement (SMI5 - SMI0 = 2.48 cm^3) than following the introduction of the stepped mouthpiece (SMI0 - BMI0 = 1.72 cm^3).

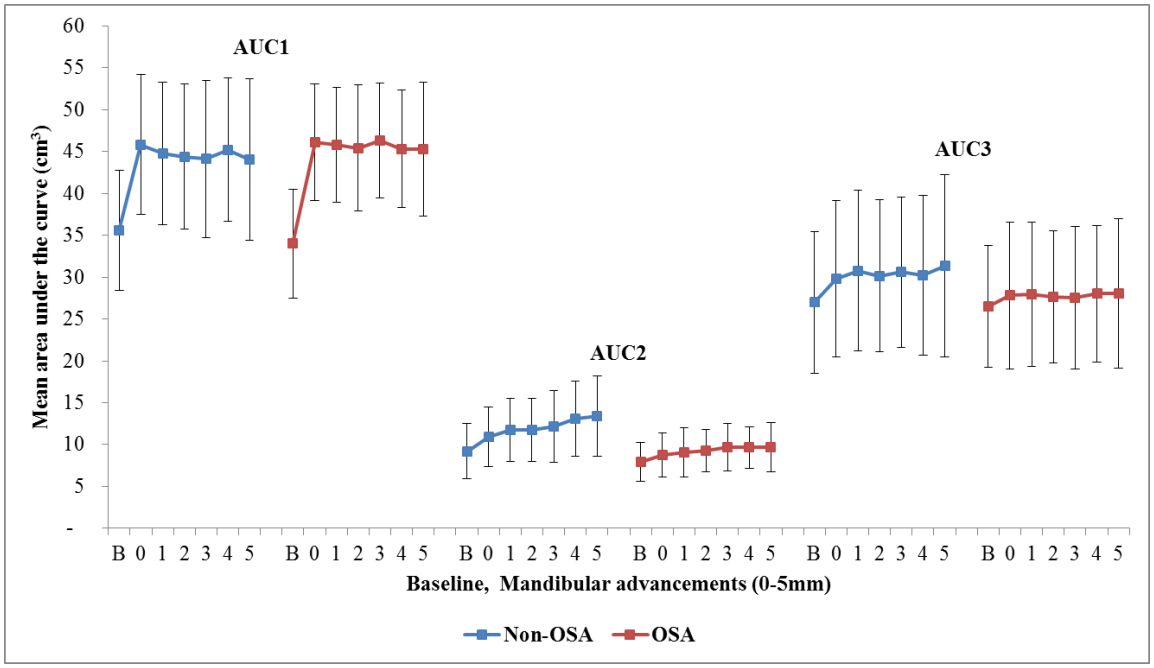


Figure 5.12: The AUCs are presented as mean values (± SD) for tidal breathing per group (non-OSA, OSA) following introduction of the stepped mouthpiece (B = baseline BMI0) and following the mandibular advancements (0-5 mm, SMI0 to SMI5).

The change in size of AUC2 in the OSA group followed the same pattern with larger change following mandibular advancement (0.94 cm^3) than following the introduction of the stepped mouthpiece (0.85 cm^3) although the magnitude was smaller. The changes in size of AUC3 were small in relation to the volume of the AUC3 and the impact of the vertical diameter of the stepped mouthpiece on the AUC3 was larger than the effect of the mandibular advancements (non-OSA

group, SSI0 - BMI0 = 2.83 cm^3 *versus* SSI5 - SSI0 = 1.48 cm^3; OSA group, SSI0 - BMI0 = 1.63 cm^3 *versus* SSI5 - SSI0 = 0.37 cm^3).

During slow and deep breathing (SSI0-5) the impact of the vertical diameter of the stepped mouthpiece on AUC1 was larger than the effect of the mandibular advancements for both groups (Figure 5.13). This was also the case for the changes in AUC2 in the non-OSA group (SSI0 - BMI0 = 1.78 cm^3 and *versus* SSI5 - SSI0 = 1.35 cm^3).

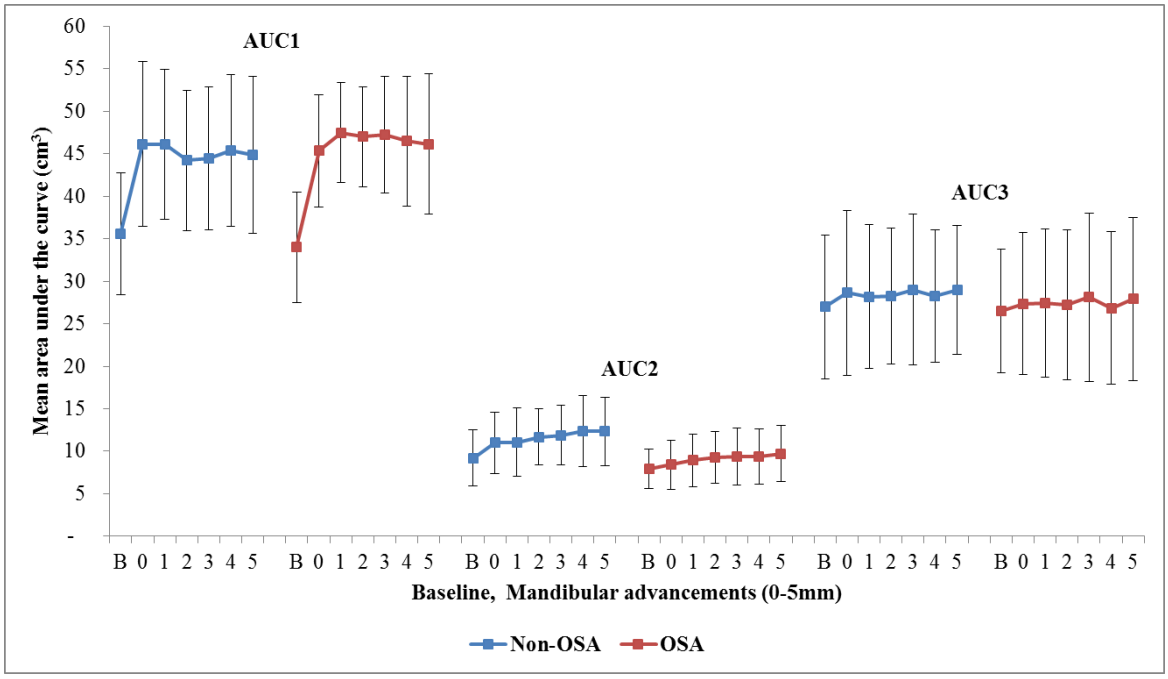


Figure 5.13: The AUCs are presented as mean values (± SD) for slow and deep breathing per group (non-OSA, OSA) following introduction of the stepped mouthpiece (B = baseline BMI0) and following the mandibular advancements (0-5 mm, SSI0 to SSI5).

However, in the OSA group the effects were the opposite (SSI0 - BMI0 = 0.50 cm^3 *versus* SSI5 - SSI0 = 1.33 cm^3). The changes in size of AUC3 were small in relation to the volume of the AUC3 and the impact of the vertical diameter of the stepped mouthpiece on the AUC3 was larger than the effect of the mandibular advancements (non-OSA group, SSI0 - BMI0 = 1.63 cm^3 *versus* SSI5 - SSI0 = 0.37 cm^3; OSA group, SSI0 - BMI0 = 0.83 cm^3 *versus* SSI5 - SSI0 = 0.56 cm^3).

5.7.8 Statistics - effects of the vertical diameter of the stepped mouthpiece

The effects of the introduction of the stepped mouthpiece (incisal opening) on CSA1-3 and AUC1-3 were investigated by ANOVA for data from BMI0, SMI0 and SSI0 (Tables 5.22-5.23). The data from tidal, and slow and deep breathing patterns were pooled for this analysis.

Table 5.22: The non-OSA group, summary statistics and results of ANOVA based on data from SMI0 and SSI0 for changes in CSA1-3 (cm^2) and AUC1-3 (cm^3) depending on the introduction of the stepped mouthpiece (incisal opening).

Endpoint	Means with mouthpiece	Means without mouthpiece	p-values
CSA1	3.78	2.18	<0.0001
CSA2	3.35	2.58	0.0004
CSA3	4.62	2.98	0.0033
AUC1	45.99	35.57	<0.0001
AUC2	10.95	9.20	0.0001
AUC3	29.23	27.00	0.0091

Table 5.23: The OSA group, summary statistics and results of ANOVA based on data from SMI0 and SSI0 for changes in CSA1-3 (cm^2) and AUC1-3 (cm^3) depending on the introduction of the stepped mouthpiece (incisal opening).

Endpoint	Means with mouthpiece	Means without mouthpiece	p-values
CSA1	2.89	1.60	<0.0001
CSA2	2.78	2.59	0.1602
CSA3	3.87	2.95	0.0257
AUC1	45.74	33.99	<0.0001
AUC2	8.57	7.90	0.0679
AUC3	27.57	26.51	0.1709

The results for the non-OSA group (Table 5.22) showed that the changes in CSA1-3 and AUC1-3 following the introduction of the stepped mouthpiece (incisal opening) were all statistically significant. This was in contrast with the results for the OSA group (Table 5.23) which only showed statistically significant results for CSA1, CSA3 and AUC1.

5.7.9 Statistics- effects by OSA, gender, breathing pattern, and mouthpiece position

An ANOVA with fixed factors OSA, gender, breathing pattern and mouthpiece position was performed for endpoints CSA1-3 and AUC1-3 for data from SMI0-5 and SSI0-5. The results of the analysis (p-values) is presented in Table 5.24.

Table 5.24: Results of ANOVA (p-values) for endpoints CSA1-3 and AUC1-3 based on data from SMI0-5 and SSI0-5 evaluating effects by factors OSA, gender, breathing pattern and mouthpiece position.

Endpoint	OSA	Gender	Breathing pattern	Mouthpiece position
CSA1	<0.0001	0.7915	0.5365	0.7378
CSA2	<0.0001	0.3492	0.3374	0.1461
CSA3	0.1383	0.0001	0.7748	0.7392
AUC1	0.0419	<0.0001	0.2264	0.9261
AUC2	<0.0001	0.8825	0.2235	0.0058
AUC3	0.0046	0.0968	0.0879	0.9829

The breathing pattern had no statistically significant effects on any of the endpoints whereas OSA was statistically significant for all endpoints but CSA3, and gender was statistically significant only for CSA3 and AUC1. Mouthpiece position was statistically significant for AUC2. To assess if effects were associated with relevant differences the overall means for factors OSA, gender and breathing pattern are presented in Table 5.25, and for mouthpiece position in Table 5.26.

Table 5.25: Main effects from ANOVA for endpoints CSA1-3 (cm^2) and AUC1-3 (cm^3) for factors OSA status, gender and breathing pattern.

Endpoint	**OSA**		**Gender**		**Breathing pattern**	
	No	Yes	Female	Male	SMI	SSI
CSA1	3.86	3.11	3.48	3.50	3.46	3.52
CSA2	3.66	2.92	3.24	3.34	3.34	3.24
CSA3	4.05	3.79	4.25	3.59	3.94	3.90
AUC1	44.97	46.17	42.86	48.24	45.22	45.90
AUC2	11.94	9.26	10.60	10.62	10.77	10.45
AUC3	29.52	27.65	28.05	29.13	29.15	28.03

A couple of examples from factors OSA and gender might be of interest. The statistically significant effect by OSA on CSA2 was 0.74 cm^2 or equal to a ~25% difference, whereas the statistically significant effect by gender on AUC1 was 5.38 cm^3 or equal to a ~13% difference.

Table 5.26: Main effects from ANOVA for endpoints CSA1-3 (cm^2) and AUC1-3 (cm^3) for factor mouthpiece position.

Endpoint	**Mouthpiece position**					
	0 mm	1 mm	2 mm	3 mm	4 mm	5 mm
CSA1	3.34	3.43	3.49	3.50	3.63	3.56
CSA2	3.06	3.14	3.25	3.38	3.41	3.51
CSA3	4.20	3.90	3.84	3.97	3.84	3.77
AUC1	45.86	46.05	45.26	45.52	45.60	45.08
AUC2	9.76	10.20	10.50	10.79	11.16	11.29
AUC3	28.40	28.58	28.32	28.85	28.34	29.06

The statistically significant effect by mouthpiece position on AUC2 (non-OSA and OSA data combined) corresponded to an increase in volume from 9.76 to 11.29 cm^3, a difference of 1.53 cm^3 or ~16% of the 0 mm value. In Table 5.27 the mean AUC2 is presented in a cross-tabulation for mouthpiece position v*ersus* non-OSA and OSA groups.

Table 5.27: AUC2 (cm3) for stepped mouthpiece position *versus* non-OSA and OSA groups.

Mouthpiece position (mm)	Non-OSA (cm^3)	OSA (cm^3)
0	10.95	8.57
1	11.39	9.00
2	11.71	9.28
3	12.01	9.52
4	12.75	9.52
5	12.87	9.71
Change (%)	17.5%	13.3%

The effect of shifting the stepped mouthpiece position from 0 to 5 mm was relatively comparable for the non-OSA and OSA groups, although the degree of change was different between the groups.

5.7.10 Statistics - effect of extreme endpoints

A number of relatively extreme CSA1-3 and AUC1-3 results were recorded (Tables 5.16-5.18 in 5.7.4.1 - 5.7.4.3; Tables 5.19-5.21 in 5.7.6.1 - 5.7.6.3) and an analysis of these was performed. For each measurement and each of the CSA1-3 and AUC1-3 endpoints the mean and SD was calculated based on the 60 subjects. Based on these calculations upper and lower limits for normal measurements were defined as mean ± 3 SDs.

Through the analysis 51 results were identified from 46 measurements, and of these 15 concerned CSA1, 6 CSA2, 17 CSA3, 9 AUC2 and 4 AUC3. The atypical results concerned 17 subjects and the subject with the highest number of extreme results was subject 46 with 12 outliers, all of which were related to CSA1 and of these all but one were related to measurements with the stepped mouthpiece. Out of the 17 CSA3 outliers, 4 were related to baseline measurements and 13 to measurements in 5 subjects (11, 12, 16, 25 and 34) with the stepped mouthpiece.

A main effects ANOVA with fixed factors inhalation mode, mouthpiece position, OSA status and gender was performed for each of the 6 endpoints (excluding data for BFL and BMI) using the data set without "outliers" (Table 5.28).

Table 5.28: p-values from ANOVA evaluating effects by OSA and gender, based on data without "outliers".

Endpoint	OSA	Gender	Inhalation mode	Mouthpiece position
CSA1	<0.0001	0.0098	0.3139	0.3308
CSA2	<0.0001	0.2423	0.3320	0.1103
CSA3	0.0656	0.0001	0.5024	0.7720
AUC1	0.0419	<0.0001	0.2264	0.9261
AUC2	<0.0001	0.7194	0.4571	0.0039
AUC3	0.0043	0.1459	0.0514	0.9521

A number of statistically significant effects are presented in Table 5.28. Most importantly, the same conclusions as found using the full data set can be draw. This shows that the "outliers" did not affect the analysis to a significant degree. To assess if the significant effects were associated with relevant differences the overall means for each level of the 4 factors are presented.

Table 5.29: Main effects from ANOVA: OSA, gender & inhalation mode – based on data without "outliers".

Endpoint	OSA		Gender		Breathing pattern	
	No	Yes	Female	Male	SMI	SSI
CSA1	3.87	2.90	3.27	3.50	3.34	3.43
CSA2	3.64	2.92	3.22	3.34	3.33	3.23
CSA3	3.91	3.63	4.06	3.47	3.82	3.72
AUC1	44.98	46.12	42.87	48.23	45.21	45.89
AUC2	11.82	9.27	10.59	10.50	10.64	10.45
AUC3	29.45	27.57	28.03	28.99	29.15	27.87

Table 5.30: Main effects from ANOVA: mouthpiece position – based on cleaned data without "outliers".

Endpoint	Mouthpiece position					
	0 mm	1 mm	2 mm	3 mm	4 mm	5 mm
CSA1	3.22	3.30	3.38	3.38	3.57	3.46
CSA2	3.06	3.14	3.17	3.38	3.40	3.51
CSA3	3.96	3.77	3.77	3.86	3.62	3.62
AUC1	45.86	46.05	45.26	45.49	45.56	45.08
AUC2	9.69	10.11	10.50	10.68	11.14	11.15
AUC3	28.18	28.58	28.32	28.82	28.10	29.06

The main effects presented in Tables 5.29 and 5.30 agree very closely to those presented in Tables 5.25 and 5.26 based on the full data set.

5.7.11 Graphical presentation of the endpoints

In order to visually highlight the range of the endpoints including the "outliers", these have been plotted in increasing order in Figure 5.14 (CSA1-3) and Figure 5.15 (AUC1-3). The endpoints are from measurements BMI0, SMI0-5 and SSI0-5 for all 60 subjects.

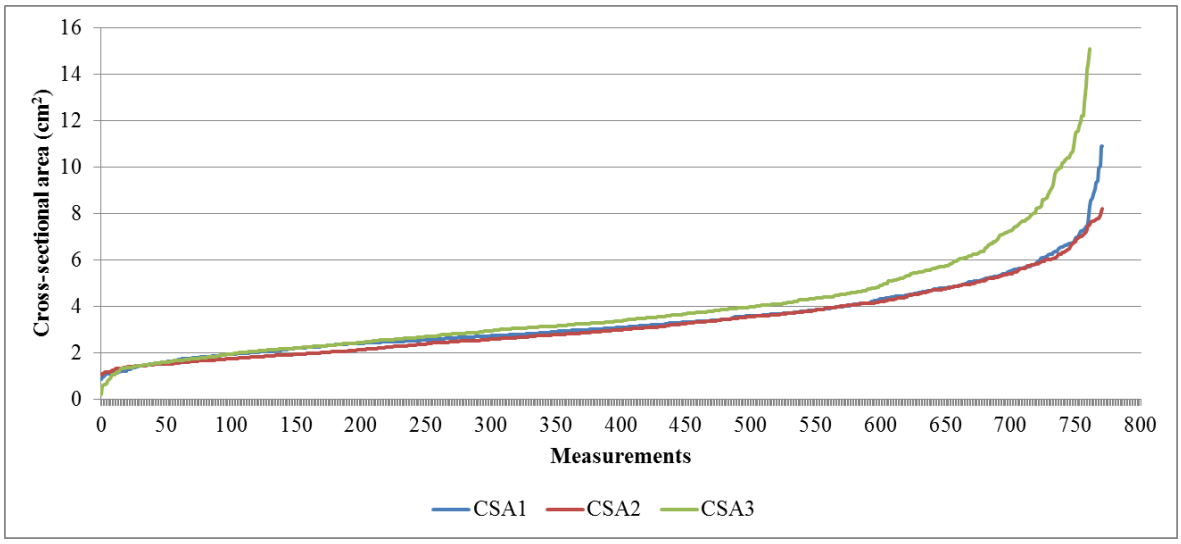


Figure 5.14: The mean CSA1-3 (mean of 4 pharyngograms) endpoints from measurements BMI0, SMI0-5 and SSI0-5 plotted in increasing order for the 60 subjects.

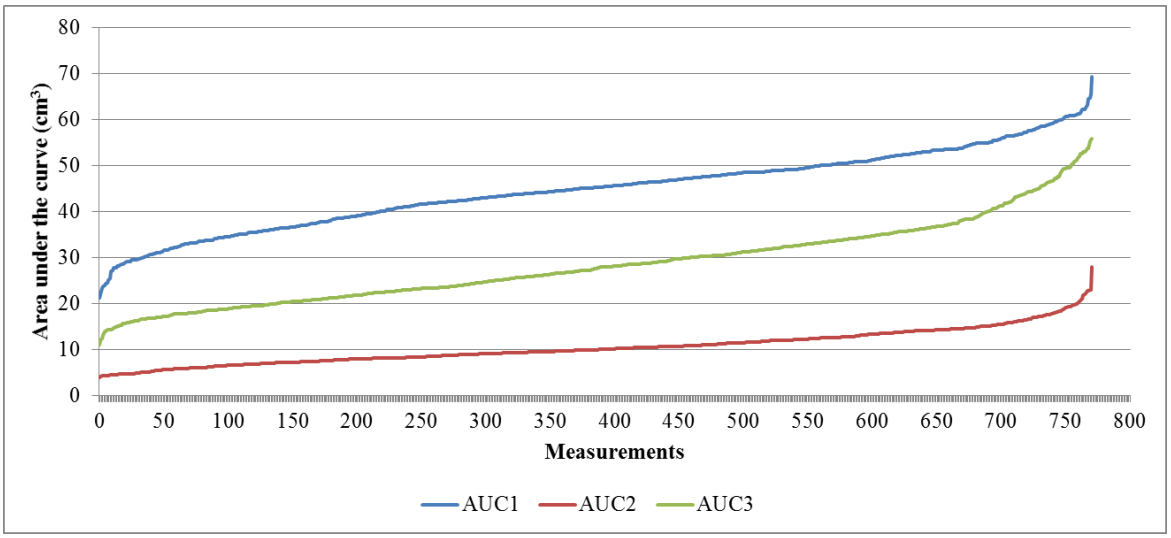


Figure 5.15: The mean AUC1-3 (mean of 4 pharyngograms) endpoints from measurements BMI0, SMI0-5 and SSI0-5 plotted in increasing order for the 60 subjects.

5.7.12 Measurements (SMI, SSI) in relation to the baseline value (BMI0)

As some of the SMI0-5 and/or the SSI0-5 measurements had endpoint (CSA1-3, AUC1-3) values which were lower than the corresponding baseline measurement (BMI0) endpoint values, it was of interest to present the number of such measurements per endpoint per the non-OSA and OSA groups (Table 5.31).

Table 5.31: The number of measurements (SMI0-5, SSI0-5) per endpoint (CSA1-3, AUC1-3) smaller than the baseline measurement (BMI0) per OSA group in percent of the total number of measurements.

	CSA1	**CSA2**	**CSA3**	**AUC1**	**AUC2**	**AUC3**
Total number of measurements	712	712	712	712	712	712
Non-OSA, SMI, SSI <BMI0	19	88	123	24	61	104
% of total	2.7	12.4	17.3	3.4	8.6	14.6
OSA, SMI, SSI <BMI0	15	140	154	4	120	165
% of total	2.1	19.7	21.6	0.6	16.9	23.2

5.7.13 Statistics - the subjects' comfort with stepped mouthpiece positions

The comfort with different mandibular advancement positions achieved with the stepped mouthpiece was assessed for each subject during both tidal and slow deep breathing. For each of the 6 stepped mouthpiece positions (0-5 mm) the subject scored the degree of comfort as follows:

- 1 = Very Uncomfortable.
- 2 = Uncomfortable.
- 3 = Acceptable.
- 4 = Comfortable.
- 5 = Very Comfortable.

In Table 5.32 the number of subjects recording each of the 5 scores for different breathing patterns and mandibular advancements are presented.

Table 5.32: Comfort scores presented by position (1-6) and breathing pattern. The comfort scores (1-5) were scored by the subjects when testing the different mandibular advancements (positions 1-6, 0-5 mm mandibular advancements) achieved with the stepped mouthpiece during both tidal (T) and slow and deep (S) breathing.

Score	**Positions 1-6 (mandibular advancement 0-5 mm)**											
	1		2		3		4		5		6	
	T	S	T	S	T	S	T	S	T	S	T	S
1	0	0	0	0	1	1	0	2	3	1	5	8
2	2	0	4	3	7	4	13	7	12	15	18	20
3	10	12	10	12	16	16	20	22	21	22	18	14
4	30	34	35	35	27	34	20	24	19	17	16	16
5	18	14	11	10	9	5	4	6	4	4	2	1

The most common score was 4 (comfortable) and the degree of comfort decreased with increasing mandibular advancement as highlighted in the decrease of scores 4 and 5 from position 1 to 6. The mean scores scored during tidal (3.48) and slow and deep (3.45) breathing were not statistically significantly different.

The plot of the scores shown in Figure 5.16 presents the mean score by position and breathing pattern. The plot highlights the minimal difference between the scores for the two breathing

patterns up to position 5 (4 mm mandibular advancement). However, for position 6 (5 mm mandibular advancement) there was a statistically significant (p = 0.0171) difference in the scores between the two breathing patterns although the difference was small (0.2 units).

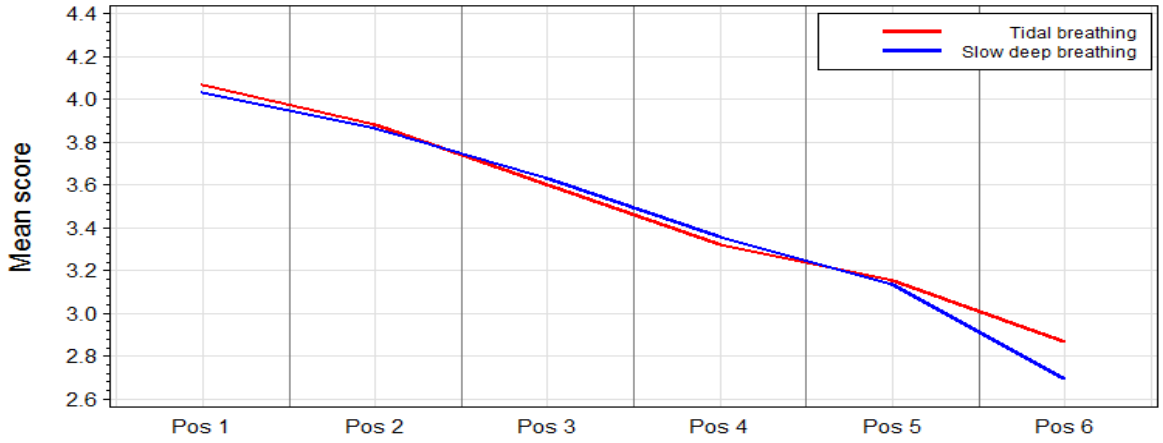


Figure 5.16: Mean comfort scores presented by stepped mouthpiece position for tidal, and slow and deep breathing patterns. The scores were scored using the stepped mouthpiece in six different positions (position 1 to position 6; 0-5 mm mandibular advancements).

When the most "comfortable position" with the stepped mouthpiece during both tidal (3.40) and slow and deep (3.70) breathing had been established, the subject was asked to hold both positions for 3 minutes during both tidal, and slow and deep breathing. After 3 minutes per breathing pattern, the subject's level of comfort with the stepped mouthpiece was re-evaluated using the same scoring system. The results are shown in Figure 5.17 in which the individual comfort scores after 3 minutes in the most comfortable mandibular advancement position for tidal, and slow and deep breathing are shown. Some variability can be seen between the two breathing patterns. For example, the subjects scoring 4 during tidal breathing scored 1-5 during slow and deep breathing. Neither the most comfortable positions nor the scores after 3 minutes of tidal (3.62) and slow and deep (3.40) breathing were statistically significantly different. The results have also been presented in a Bland-Altman plot (Figure 5.18).

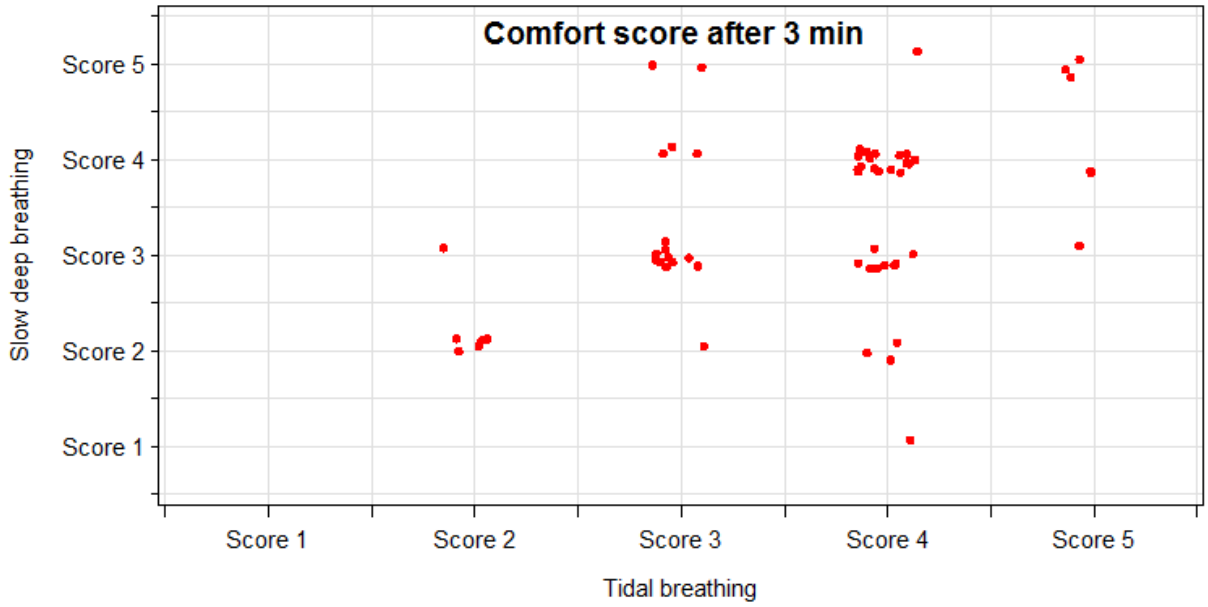


Figure 5.17: The individual comfort scores after holding the most comfortable stepped mouthpiece mandibular advancement position for 3 minutes during both tidal, and slow and deep breathing.

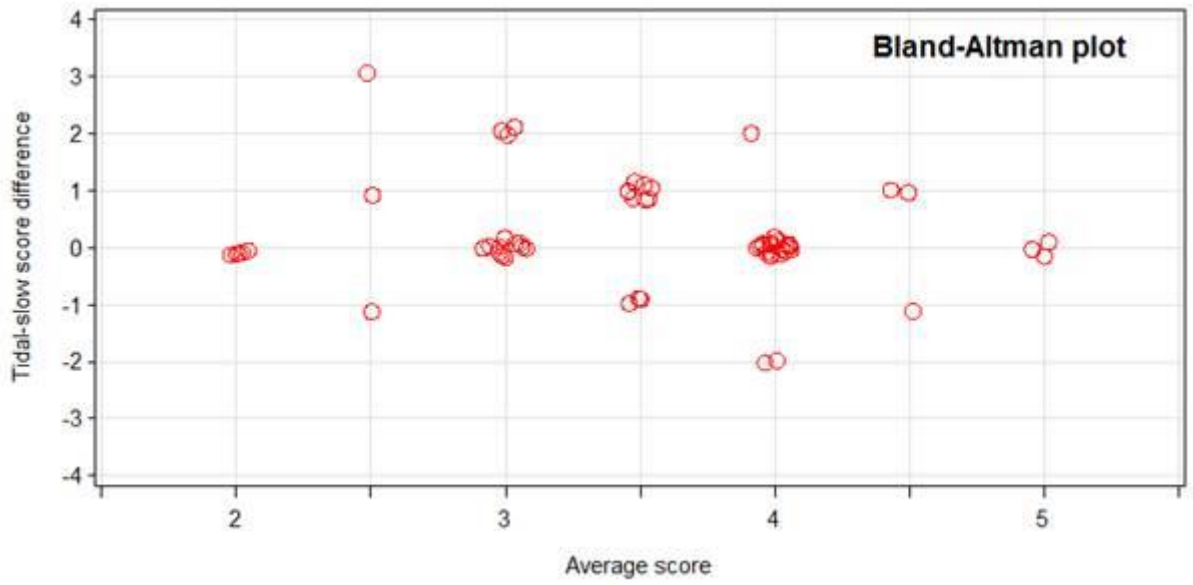


Figure 5.18: A Bland-Altman plot of the individual comfort scores after holding the most comfortable stepped mouthpiece mandibular advancement position for 3 minutes during both tidal, and slow and deep breathing.

An analysis of the impact of demographic factors on the comfort score showed that only gender (p=0.0232) and age (p=0.0277) had statistically significant influences on the most comfortable position, whereas for example OSA did not. For each subject the mean endpoint was determined from the two breathing patterns as there was no statistically significant effect by breathing pattern. The mean most comfortable position was 2.7 for females and 4.5 for males indicating that men preferred a somewhat larger mandibular advancement. The analysis of the impact of age indicated that for males the most comfortable position increased with increasing age, whereas for females the preference was independent of age.

5.8 Discussion

The primary objective of the study was to measure through acoustic pharyngometry the impact of different horizontal mandibular advancements on the size of the upper airways in subjects without (non-OSA group) and with OSA (OSA group). The mandibular advancement was maximised to 5 mm from an incisal edge-to edge position and was achieved through a stepped mouthpiece attached to the wavetube of the acoustic pharyngometer wavetube. The part of the upper airways that was investigated included the area from the oral cavity to the GL. The measurements were performed while the subjects were seated and inhaled room air during tidal breathing through the stepped mouthpiece. The secondary objectives included measurements of the impact of the stepped mouthpiece on the upper airways during slow and deep breathing, and assessment of the most comfortable mandibular advancement position during both tidal and slow and deep breathing. No adverse events were recorded.

The were some differences in demographic data between the non-OSA and the OSA groups including age, weight, BMI, neck circumference and mandibular movement (protrusive, retrusive). Subjects with OSA were approximately 10 years older than non-OSA subjects, whereas females

with OSA were heavier (~17 kg), had a larger BMI (~6) and a larger neck circumference (~5 cm) than the non-OSA females. There were also differences regarding the mandibular movement from an incisal edge-to-edge position as the males with OSA had a larger protrusive movement of the mandible (2.8 mm) and a smaller retrusive movement of the mandible (2.2 mm) than the non-OSA males.

The stepped mouthpiece design was based on the design of the stepped mouthpieces used in the proof-of-concept study presented in Chapter 4. The addition of a slider and a tongue depressor to the design created a stepped mouthpiece with which mandibular advancements from 1 to 6 mm could be achieved measured from an incisal edge-to-edge position. The incisal edge-to-edge position was selected as basis for the stepwise advancements due to the limited adjustability of the stepped mouthpiece. As a 6 mm protrusion from an incisal edge-to-edge position proved to be difficult to achieve in the proof-of-concept study, the maximal advancement in the present study was limited to 5 mm. The difficulty in achieving a larger than 5-6 mm protrusion from an incisal edge-to-edge position with the stepped mouthpieces was somewhat related to the vertical diameter of 18 mm. The larger the vertical diameter of the stepped mouthpieces and hence the vertical opening of the mouth was in the proof-of-concept study (10, 15 and 20 mm), the more difficult it was to advance the mandible. The baseline pharyngograms differed from the pharyngograms with the stepped mouthpiece as the addition of the stepped mouthpiece to the acoustic pharyngometry wavetube created a shift to the right on the x-axis of ~5 cm. In other aspects the pharyngograms performed with the stepped mouthpiece attached to the wavetube were similar to the baseline pharyngograms performed with the standard mouthpiece apart from differences in CSAs and AUCs due to the stepped mouthpiece settings.

As in the studies presented in Chapters 3 and 4, acoustic pharyngometry was chosen in favour of other techniques such as MRI and CT as the subjects could be seated instead of being supine during

the measurements. Acoustic pharyngometry is a non-invasive, fast and relatively cheap technique which is ideal for numerous measurements (Kamal, 2001; Kamal, 2002; Viviano, 2002a; Viviano, 2002b; Kamal, 2004a; Kamal, 2004b; Jung et al., 2004; Viviano, 2004; Monahan et al., 2005; Shiota et al., 2007; Gelardi et al., 2007; Patel et al., 2008; Busetto et al., 2009; Allen et al., 2014; Oliver et al., 2014; Kumar et al., 2015). The pharyngograms were analysed in terms of landmarks (CSAs) for the OPJ (CSA1), the EG (CSA2) and the GL (CSA3), and in terms of volume (AUCs) for the oral cavity (AUC1), the oropharynx (AUC2) and the hypopharynx (AUC3).

The mean baseline (BFL0) CSAs measured during expiration without the stepped mouthpiece were for the non-OSA group 2.13 cm^2 (CSA1), 2.64 cm^2 (CSA2) and 2.88 cm^2 (CSA3), and for the OSA group 1.58 cm^2 (CSA1), 2.59 cm^2 (CSA2) and 2.79 cm^2 (CSA3). The CSAs were relatively similar for the groups but for CSA1. A comparison of mean CSAs of the non-OSA group with the mean CSAs of the healthy subjects in the study presented in Chapter 3 shows that the mean CSA1 and CSA2 of the non-OSA group were somewhat smaller than those found in the previous study whereas the mean CSA3 of the non-OSA group was ~50% of the CSA3 in the previous study. As highlighted in Chapters 2 and 3, data on CSAs (OPJ, EG and GL) from pharyngograms in adult healthy subjects from measurements with the Eccovision ARP during expiration have been published by several authors (Table 5.33).

Table 5.33: The mean CSAs of the upper airways from studies in adult healthy subjects in which the Eccovision ARP has been used. The table includes data from Chapters 3, 4 and 5. The measurements were performed during expiration while the subjects were seated - if supine this has been highlighted specifically.

1st author, year published	**Healthy subjects (male)**	**CSA (cm^2, mean), presented with one decimal Range (minimum – maximum) included if available**
Allen et al., 2014	80 (*), 20/ethnic group	***Mouth to larynx*** Caucasian = 2.7 cm^2 ; Chinese = 2.9 cm^2 ; Japanese = 2.6 cm^2 ; Korean = 2.9 cm^2
Busetto et al., 2009	145 (no male)	Seated: ***Pharynx*** = 2.6 cm^2 (0.7-5.8); ***OPJ*** = 1.6 cm^2 (0.3-4.0); ***GL*** = 2.2 cm^2 (0.5-4.4) Supine: ***Pharynx*** = 2.2 cm^2 (0.7-4.4); ***OPJ*** = 1.2 cm^2 (0.5-2.0); ***GL*** = 2.0 cm^2 (0.5-3.9)
Jung et al., 2004	16 (14)	Seated: ***Pharynx*** = 2.5 cm^2; ***OPJ*** = 1.6 cm^2; ***GL*** = 1.8 cm^2 Supine: ***Pharynx*** = 1.9 cm^2; ***OPJ*** = 1.3 cm^2; ***GL*** = 1.4 cm^2
Kamal, 2001	350 (271)	***Pharynx***: Men = 2.7 - 3.8 cm^2; Women = 2.1 - 3.4 cm^2 ***GL***: Men = 0.9 - 1.2 cm^2; Women = 0.8 - 1.1 cm^2
Kamal, 2002	40 (29)	***Pharynx***: Men = 3.2 cm^2, Women = 2.8 cm^2
Kamal, 2004b	20 (16)	***Pharynx***: Test 1 = 3.2 cm^2, Test 2 = 3.2 cm^2, Test 3 = 3.2 cm^2
Monahan et al., 2005	75 (36) white 62 (23) black	***Oropharynx***: White = 2.7 cm^2, 1.9 - 3.8 cm^2; Black = 2.4 cm^2, 1.7 - 3.3 cm^2 ***OPJ***: White = 2.4 cm^2; Black = 2.0 cm^2 ***EG***: White = 2.2 cm^2; Black = 2.6 cm^2
Chapter 3	9 (2)	***CSA1*** = 3.2 cm^2; ***CSA2*** = 3.5 cm^2; ***CSA3*** = 5.0 cm^2
Chapter 4	4 (4)	***Upper airway***: CSA = 3.4 cm^2
Chapter 5	30 (15)	***Non-OSA group***: CSA1 = 2.1 cm^2; CSA2 = 2.6 cm^2; CSA3 = 2.9 cm^2

*No data.

Most of the mean CSAs are in the same range apart from the mean CSA3 value in the Chapter 3 study. Ranges for CSA3 were, however, included in the Busetto et al (2009) paper and ranged from 0.5 to 4.4 cm^2 (CSA3). As the Busetto et al (2009) CSA3 study only included female subjects it seems reasonable to assume that the CSA3 data in the Chapter 3 study is in the normal range as men tend to have larger upper airways (Martin et al., 1997).

Data on CSAs from pharyngograms in subjects with OSA from measurements with the Eccovision ARP have also been published by several authors (Table 5.33). The CSAs of the OSA group in the

present study were similar to the CSAs (OPJ, EG, GL) in the papers by Jung et al (2004), Monahan et al (2005) and Patel et al (2008) (Table 5.34).

Table 5.34: The CSAs of the upper airways from studies in which the Eccovision ARP has been used to measure the upper airways of subjects with OSA including Chapter 5 data on subjects with OSA. The measurements were performed during expiration while the subjects were seated - if supine this has been highlighted specifically.

1st author, year published	**Adult healthy subjects (male)**	**CSA (cm^2, mean) Range (minimum – maximum) included if available**
Jung et al., 2004	54 (13)	Seated: ***Pharynx*** = 2.4 cm^2; ***OPJ*** = 1.4 cm^2; ***GL*** = 1.9 cm^2 Supine: ***Pharynx*** = 1.6 cm^2; ***OPJ*** = 0.8 cm^2; ***GL*** = 1.4 cm^2
Monahan et al., 2005	32 (32) White 41 (27) Black	***Oropharynx***: White = 2.4 cm^2 (1.6-3.5 cm^2); Black = 2.1 cm^2 (1.5–2.8 cm^2) ***OPJ***: White = 1.9 cm^2; Black = 2.0 cm^2 ***EG***: White = 2.5 cm^2; Black = 2.0 cm^2
Patel et al., 2008	229 (102) White 339 (140) Black	***Oropharynx***: White = 2.7 cm^2 (1.9-3.2 cm^2); Black = 2.3 cm^2 (1.8–2.8 cm^2) ***OPJ***: White = 2.1 cm^2; Black = 1.9 cm^2 ***EG***: White = 2.6 cm^2; Black = 2.4 cm^2
Chapter 5	30 (15)	***OSA group***: CSA1 = 1.6 cm^2; CSA2 = 2.6 cm^2; CSA3 = 2.8 cm^2

The mean baseline (BFL0) AUCs were for the non-OSA group in the present study 36.27 cm^3 (AUC1), 9.37 cm^3 (AUC2) and 27.49 cm^3 (AUC3) and for the OSA group 33.12 cm^3 (AUC1), 7.96 cm^3 (AUC2) and 26.62 cm^3 (AUC3). The mean AUC1 and AUC2 of both groups were considerably smaller than those found in the study presented in Chapter 3, whereas the AUC3 values were similar. The mean volumes of the upper airways were 73.2 cm^3 (AUC1-3 non-OSA group) and 67.7 cm^3 (AUC1-3 OSA group) which were considerably smaller than the mean volume of 100 cm^3 of the previous study (Chapter 3) but similar to the mean volume of the proof-of-concept study 62.0 cm^3. A comparison of the AUC1-3 values from the studies in Chapters 3-5 with published data on upper airway volumes in healthy subjects shows a relatively large variability (Ehtezazi et al., 2004; Pritchard et al., 2004; Ehtezazi et al., 2005; McRobbie et al.,

2005). In these 4 studies the impact of inhalation from different inhalers on the upper airway volume was measured with MRI during inhalation through inhalers or during tidal breathing with the subjects in a supine position. As shown in Table 5.35 the volumes of the upper airways reported in the studies ranged from 38 cm^3 (McRobbie et al., 2005) to 101 cm^3 (Ehtezazi et al., 2005).

Table 5.35: The mean AUCs from the present study (Chapter 5), the studies presented in Chapters 3 and 4, from published studies.

1st author, year published	**Technique, position**	**Healthy subjects (male)**	**Upper airway volume (cm^3, mean)**
Ehtezazi et al., 2004	MRI, supine	10 (6)	pMDI = 56 cm^3, spacer = 59 cm^3, DPI = 70 cm^3
Ehtezazi et al., 2005	MRI, supine	7 (5)	Orifice 1 = 72 cm^3, orifice 6 = 101 m^3
McRobbie et al., 2005	MRI, supine	5 (3)	Tidal breathing = 38 cm^3
Pritchard et al., 2004	MRI, supine	20 (10)	Tidal breathing: males = 47 cm^3, females = 43 cm^3
Chapter 3	ARP, seated	9 (2)	AUC1-3 = 100 cm^3
Chapter 4	ARP, seated	4 (4)	AUC = 62.0 cm^3
Chapter 5	ARP, seated	30 (15)	***Non-OSA***: AUC1-3 = 73.2 cm^3

The descriptive analyses of CSAs and AUCs indicated that both the vertical diameter and the mandibular advancements of the stepped mouthpiece had an impact on the CSAs and AUCs although the impact was somewhat different for different parts of the upper airways. During tidal breathing the impact of the mandibular advancements of the stepped mouthpiece on the CSAs were in both the non-OSA and the OSA groups larger than the effect of the vertical diameter for CSA2 but smaller for CSA1 and CSA3. The same trend was true for the changes in the AUCs in which the impact of the mandibular advancements of the stepped mouthpiece was in both groups larger than the effect of the vertical diameter for AUC2 but smaller for AUC1 and AUC3. During slow and deep breathing the impact of the vertical diameter of the stepped mouthpiece on the CSAs

was larger in relation to the effect of the mandibular advancements for all of the CSAs. The same trend was true also for the AUCs but for AUC2 in the OSA group in which the effects of the mandibular advancements were larger.

A larger pharyngeal lumen should have a positive effect on inhaled oral drug delivery. Changes in the vertical diameter of inhaler mouthpieces together with changes in peak inspiratory flows and particle sizes have been shown *in vitro* to affect the deposition efficiency of inhaled aerosol (Lin et al., 2001). The results of the Kumazawa et al clinical pharyngeal, laryngeal and lung deposition study (1997) support the results of the Lin et al *in vitro* study (check Chapter 2, section 2.5.1). An open GL lead to a higher lung deposition than a closed GL. An expansion of both the OPJ, the EG and the GL should follow the same trend and lead to a higher lung deposition of inhaled droplets and particles especially in tandem with a slow and deep inhalation.

The statistical analysis of the impact of the incisal opening following the introduction of the stepped mouthpiece without any mandibular advancement showed that the changes in CSAs and AUCs were statistically significant for both CSA1-3 and AUC1-3 for the non-OSA group. For the OSA-group only the changes in CSA1, CSA3 and AUC1 were statistically significant. The results are in line with published data indicating that the upper airways of subjects diagnosed with OSA differ from those of healthy subjects during wakefulness (Schwab et al., 1995; Ciscar et al., 2001). The results of the study by Pritchard et al (2004) support the vertical effects of the stepped mouthpiece on AUC1 and CSA1 for both groups in the present study. In the Pritchard et al study the impact of 4 dummy inhalation devices on the size of the upper airways in 20 healthy subjects were measured with the subjects in a supine position through an inhalation-gated MRI technique. The results of 2 of the mouthpieces (Device A, diameter 25 mm; Device C, diameter 14 mm) are of interest as the diameters were both somewhat smaller and larger than the diameter of the stepped mouthpiece and had low resistances. The results showed that the size of the buccal volume was

statistically significantly larger with the large diameter (mean 33.2 cm^3) in comparison with the small diameter mouthpiece (mean 22.4 cm^3), and the significant dependence was mainly related to the buccal volume. The results were, however, a bit more complex and the authors summarize in the abstract as follows:

"Individual subjects showed varied device dependent changes: 45% having an increase in regional airway volumes, particularly in the nasopharynx (+46% volume increase) and laryngo-pharynx (+36% volume increase) for the high resistance devices compared with the low-resistance ones. However, 30% of subjects showed the opposite behaviour, a reduction in nasopharynx volume (-17%), laryngo-pharynx volume (-17%), and laryngeal cavity (-11%). 25% showed no significant difference in airway volume between high- and low-resistance devices."

These results are in agreement with the results of the study by Vroegop et al (2012) in which a vertical opening of up to 20 mm was tested in subjects with OSA through different oral appliances during sleep endoscopy. The results showed that 80% showed an adverse effect of the vertical opening, 2.5% had a positive effect and 17.5% an indifferent effect.

The statistical analysis of the mandibular advancement achieved with the stepped mouthpiece showed a statistically significant effect only for AUC2 with a larger change in the non-OSA (18%) group in comparison with the OSA group (13%). It is not surprising that the changes in CSA1 and AUC1 following mandibular advancements did not show a statistically significant effect as the introduction of the stepped mouthpiece had already expanded the oral cavity considerably.

5.9 Conclusions

The study hypothesis: "mandibular advancement together with incisal opening during tidal breathing achieved through a stepped mouthpiece design affects the size of the upper airways in subjects without and with OSA" was based on the results of the proof-of-concept study in Chapter 4. The results following the introduction of the stepped mouthpiece (incisal opening) showed that in the non-OSA group the changes in CSA1-3 and AUC1-3 were all statistically significant in

contrast with the results for the OSA group which only showed statistically significant results for CSA1, CSA3 and AUC1. The results following mandibular advancement showed that there was a statistically significant effect on the oropharynx (AUC2) in both the non-OSA and the OSA groups. Thus the study confirmed the study hypothesis, highlighted the impact of the combined effect of the incisal opening and the mandibular advancement of the stepped mouthpiece on the size of the upper airways, and the large inter-subject variability in respons to the stepped mouthpiece.

The results of the present study highlight a number of questions that might be addressed in future research of the stepped mouthpiece. These include research of stepped mouthpieces with variable vertical dimensions in addition to a number of mandibular advancement positions, tests of new stepped mouthpieces in both healthy subjects and subjects diagnosed with asthma, COPD and the "overlap syndrome" - that is patients diagnosed with both COPD and OSA (Weitzenblum et al., 2008) - and studies of lung deposition when adding the stepped mouthpiece to inhalers.

Chapter 6 An *in vitro* evaluation of acoustic pharyngometry when using a cast of a human upper airway

6.1 Introduction

6.1.1 Acoustic pharyngometry and the open velum effect

To ensure oral breathing during acoustic pharyngometer measurements, nose clips have been used although there is limited information on the possible impact of these on the pharyngogram. Rubinstein et al (1987) did not find any significant difference in mean pharyngeal, glottal or tracheal areas following use of nose clip or not. In contrast Molfino et al (1990) in a letter to an editor made comments regarding a published paper (Brooks, 1990), discussed possible artefacts during acoustic pharyngometer measurements, and proposed an open nasopharyngeal velum (soft palate) following use of nose clips as the possible reason for an overestimation of the distal pharynx, GL, and trachea. The reason for the overestimation would be the propagation of acoustic pulses from the mouth through the pharynx, GL, and trachea, and in addition propagation of acoustic pulses from the mouth to the nasopharynx and the paranasal sinuses. The acoustic pulses from the nasal airways would then propagate along the GL and trachea creating a falsely large measurement of GL and tracheal areas. The example included in the letter (Chapter 2, subsection 2.7.5.3) showed how the use of a nose clip led to an overestimation of the GL and tracheal area which was reversed by the removal of the nose clip. They also noted that the velum could be partially open even after removal of the nose clip (Molfino et al., 1990).

Marshall et al (1993) presented the effects of different soft palate (velum) positions on the echogram (0-30 cm) which were controlled by the mode of breathing. A mixed oral and nasal breathing placed the soft palate in an intermediate position which should create an open velum effect (Chapter 2, subsection 2.7.5.3). The CSA of the OPJ did not change whereas the CSAs of

the EG and the GL increased and the AUC increased. Based on the graphic presentations of the echograms in the Molfino et al (1990) and the Marshall et al (1993) articles the increase of the CSAs could be quite variable with increases from ~4 cm^2 to ~14 cm^2 (Molfino et al., 1990) and from ~2 cm^2 to ~5 cm^2 (Marshall et al., 1993).

6.1.2 Study background – Chapter 3 study

This study was designed to investigate the possible artefact found in Chapter 3 which was related to the use of nose clips during the acoustic pharyngometer measurements. Based on the Molfino et al (1990) and Marshall et al (1993) articles, the open velum might be the cause for the increase in the CSAs and AUCs. It would therefore be of interest to shortly review the effects on the CSAs and AUCs in the Chapter 3 study. Summary statistics for the CSAs (Table 6.1) and AUCs (Table 6.2) are presented below.

Table 6.1: Summary statistics (cm^2; mean ± SD) for CSA1, CSA2 and CSA3 for each of the 14 study measurements (without and with nose clip).

Measurement	**Without nose clip (CSAs in cm^2)**			**With nose clip (CSAs in cm^2)**		
	CSA1	CSA2	CSA3	CSA1	CSA2	CSA3
A	3.2 ± 2.0	3.5 ± 1.3	5.0 ± 1.4	3.3 ± 1.6	4.0 ± 1.1	4.3 ± 1.3
B	3.8 ± 1.6	3.0 ± 0.7	4.9 ± 1.2	3.7 ± 1.4	3.9 ± 1.5	5.4 ± 1.8
C	4.4 ± 2.8	2.9 ± 0.7	4.5 ± 1.2	3.9 ± 1.3	3.8 ± 1.6	5.0 ± 1.4
D 20	4.0 ± 2.0	3.0 ± 0.7	4.7 ± 1.3	3.9 ± 1.5	4.1 ± 1.5	5.1 ± 0.9
D 30	4.5 ± 1.5	3.3 ± 1.1	5.0 ± 1.6	3.9 ± 1.5	4.0 ± 1.5	4.6 ± 0.7
E 20	3.8 ± 1.6	2.9 ± 0.7	4.5 ± 1.2	3.5 ± 1.5	3.9 ± 1.7	4.7 ± 0.5
E 30	4.1 ± 1.7	3.0 ± 0.8	4.6 ± 1.2	3.9 ± 1.2	3.9 ± 1.5	5.3 ± 1.8

The possible effect by "nose clip" on CSAs was investigated using measurements B and C (without nose clip) and BNC and CNC (with nose clip) as outlined in 3.6.3. The data was assessed using a main effects ANOVA including subject as one factor. The use of a nose clip was statistically significant for CSA2 ($p = 0.0108$) and increased CSA2 by 26%. Although measurements without

or with nose clip did not have a statistically significant effect for CSA3 the effect was an increase by 11%.

Table 6.2: Summary statistics (cm^3; mean ± SD) for AUC1-3 for each of the 14 study measurements (without and with nose clip).

Measurement	**Without nose clip (AUCs in cm^3)**			**With nose clip (AUCs in cm^3)**		
	AUC1	AUC2	AUC3	AUC1	AUC2	AUC3
A	55 ± 13	31 ± 7	28 ± 10	52 ± 9	33 ± 10	29 ± 8
B	56 ± 12	23 ± 3	21 ± 8	54 ± 10	30 ± 6	29 ± 11
C	59 ± 15	24 ± 5	21 ± 8	55 ± 13	29 ± 7	28 ± 11
D 20	57 ± 12	24 ± 5	22 ± 8	53 ± 10	31 ± 6	30 ± 11
D 30	56 ± 12	27 ± 4	24 ± 10	54 ± 12	30 ± 7	27 ± 10
E 20	56 ± 12	23 ± 5	21 ± 7	52 ± 10	30 ± 7	28 ± 11
E 30	57 ± 13	25 ± 5	20 ± 7	54 ± 11	31 ± 7	28 ± 11

The possible effect by "Nose clip" on AUCs was investigated using measurements B and C (without nose clip) and BNC and CNC (with nose clip). The data was assessed using a main effects ANOVA including subject as one factor. The use of a nose clip had a statistically significant effect for AUC2 ($p = 0.0006$) and AUC3 ($p = 0.0002$) and increased the airway volume with 23% (AUC2) and 31% (AUC3). For AUC1 the effect was the opposite and smaller (6.8%).

6.1.3 The nasopharynx and the open velum

Why would an open velum create an artefact during acoustic pharyngometer measurements? According to Molfino et al (1990) an open nasopharyngeal velum during acoustic pharyngometry leads to an over-estimation of the lower upper airway (distal pharynx, GL and trachea) as the acoustic pulses will propagate from the mouth to the nasopharynx and the paranasal sinuses where they are reflected in order to propagate along the rest of the upper airway. Kamal (2004a) described the velum as the port to the nasopharynx and an open velum would pass acoustic impulses from the pharyngometer wavetube further up through the nasopharynx into the sinuses creating a form of acoustic leak. The consequence would be an overestimation of the assumed oropharyngeal CSA

(Kamal, 2004a). As with problems related to tongue position during pharyngometer measurements, asking the subject to think or utter "oooh" during the measurement would close the velum (Kamal 2004a; Kamal, 2004b). Thus the volume of the nasopharynx and the sinuses is of interest as the addition of that volume to the pharyngogram should based on the assumptions by Kamal be equal to the artefact (Figure 6.1).

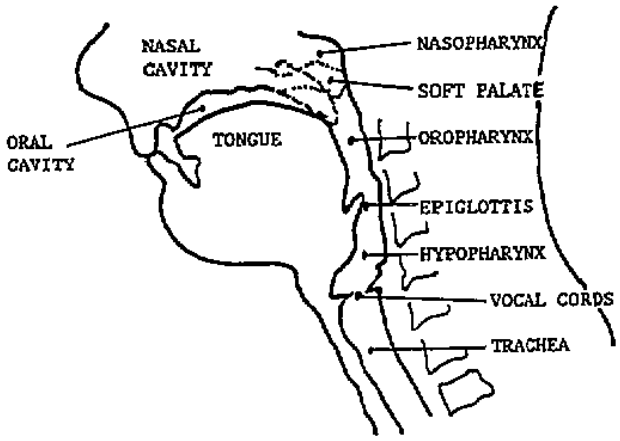


Figure 6.1: The figure highlights the soft palate (velum) position when breathing through the mouth (dotted curve, the passage between nasopharynx and the pharyngeal area closed) and when breathing through the nose (broken curve, the passage between nasopharynx and the pharyngeal area open. (Marshall et al., 1993).

Based on the location and size of the nasopharynx and the function as a connection between the sinuses and the oropharynx, it seems plausible that an open velum might create an artefact during acoustic pharyngometer measurements. The volume of the nasal cavity seems to be quite variable as shown in the study by Guilmette et al (1997) in which 21 male and 24 female subjects were included for MRI scans of the nasal cavity. The mean left side volume was 9.10 ±2.77 cm^3 and the mean right side volume 8.69 ±2.11 cm^3, with a total volume of 17.79 cm^3 with a large range (Guilmette et al., 1997; Figure 6.2).

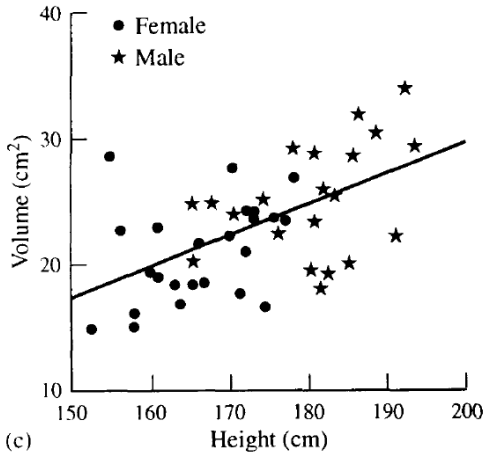


Figure 6.2: Nasal airway volumes plotted against the height of the subjects. The volume in the figure is for some reason given as "cm^2" although the volumes are given in "cm^3" in the text by the authors (Guilmette et al., 1997).

The Guilmette et al (1997) nasal cavity volumes are quite close to the nasal cavity volumes measured by Garcia et al (2009) in 4 healthy adult subjects using MRI. They reported volumes of 18.0, 15.4, 26.5 and 23.8 millilitres (mL). Acoustic rhinometry has also been used to measure the volume of the nasal cavity volume and for example de Paula Santos et al (2006) report a mean baseline volume of 38.91 cm^3 in 21 male and 19 female subjects. This was somewhat larger than the results of the Guilmette et al (1997) study.

6.1.4 *In vitro* tests of the "open velum" hypothesis

It would be difficult to reproducibly evaluate the "open velum" hypothesis in human subjects without control of the velum during a series of pharyngometer measurements. An *in vitro* study would be preferable as it would allow controlled acoustic pharyngometer measurements to be made with a surrogate for a closed or an open velum. A cast of the human upper airways similar to the polyester resin cast presented by Cheng et al (1990) could be used in order to mimic both a closed and an open velum (Figure 6.3).

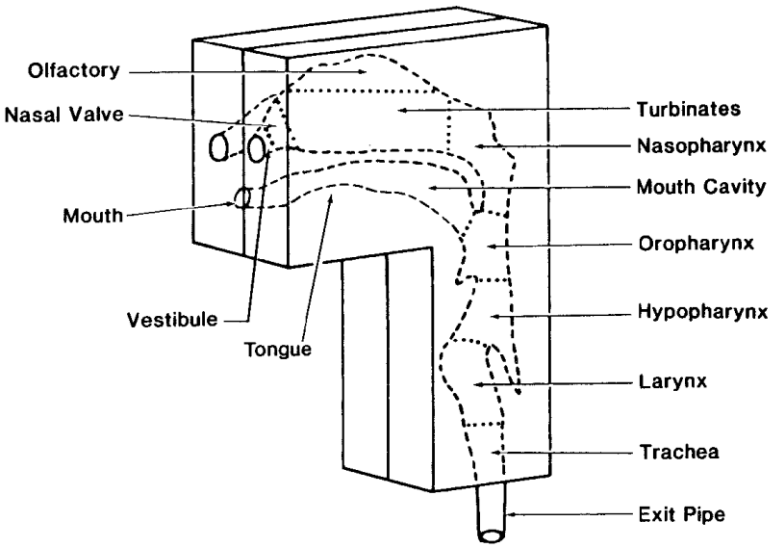


Figure 6.3: Line drawing of a cast of an adult human upper airway which highlights the different compartments from the nasal valve to the trachea (Cheng et al., 1990).

6.1.5 Study hypothesis

During acoustic pharyngometer measurements an open velum would pass acoustic impulses from the wavetube further up through the nasopharynx into the nasal airways creating a form of acoustic leak. The consequence would be an overestimation of the volume of the upper airways from the pharynx to the GL as displayed on the pharyngogram.

6.2 Methods

6.2.1 Overall study design

The *in vitro* test setup consisted of an acoustic pharyngometer (Eccovision ARP, Hood Laboratories, Pembroke, MA, USA; presently www.sleepgroupsolutions.com), a cast of a human upper airway (oral cavity to the GL), a PP tube as a surrogate for the upper airways, and green elastomeric-lipped ISO connectors (Intersurgical Ltd, Wokingham, UK) to connect the cast and the PP tube to the pharyngometer wavetube. Acoustic pharyngometer measurements were

performed without and with surrogate “open velums” in the form of holes of different sizes at the back of the cast and the PP tube, and with small or large “nasal cavities” (denoted “T-piece” below) attached to the hole. Measurements without any T-piece were also made and these measurements had an infinite size of the nasal cavity. In addition baseline measurements were performed with an open end of the cast (GL), with a closed end of the cast and with a 2 m hose attached to the end of the cast.

6.2.2 Cast of the human upper airway

The cast of the human upper airway used in the study was a copy of one of the original Swift casts made in resin (Figure 6.4). The original Swift casts of the human upper airways were constructed from *post mortem* anatomical casting and reconstruction of the *in vivo* airways from MRI measurements.

Figure 6.4: Photographs of the original Swift cast of the human upper airway from which a copy was made for the *in vitro* study (Swift, 1991; Swift et al., 1994).

The technique used in the creation of the Swift casts has been described in detail by Swift and co-workers (Swift, 1991; Swift et al., 1994). The cast available for the study was labeled “Cast M3”, did not include the nasal airways and was on loan from AstraZeneca, Lund, Sweden (Figure 6.4). As the cast was unique and could not be used in an *in vitro* study, a copy of the original cast was

made. The original cast could not be used for 3D scanning so a 3-dimensional internal geometry was generated by measuring the internal dimensions of the cast. This was done in sections and the CSA per section from section 6 to section 163 is presented in Figure 6.5.

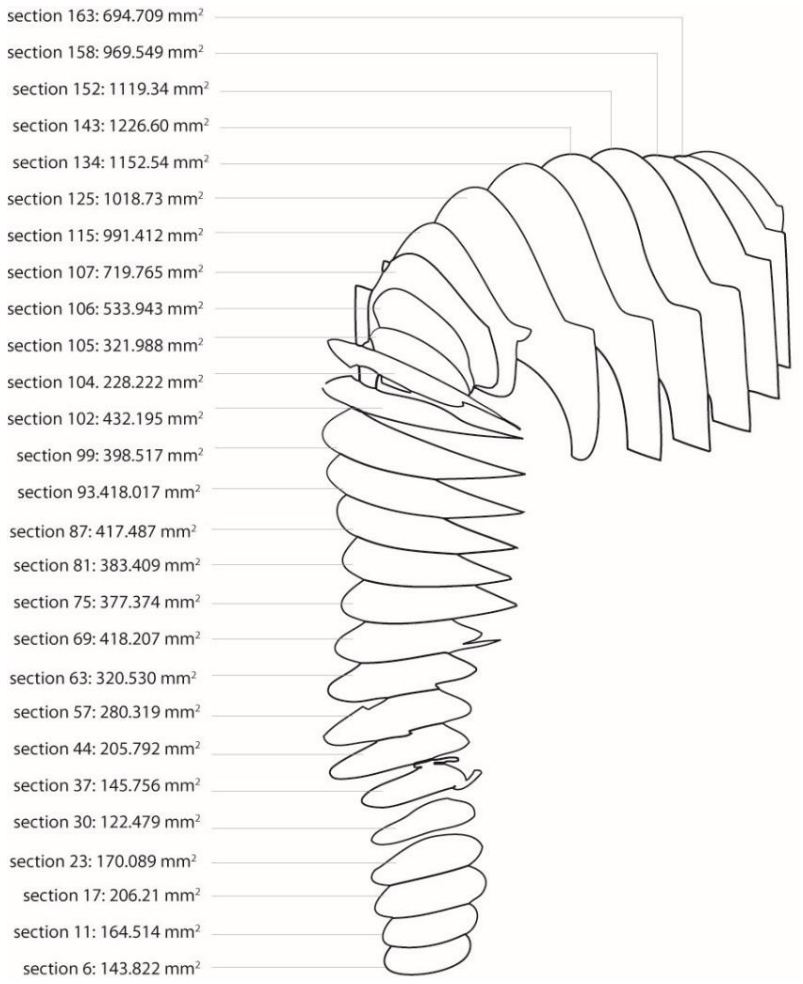


Figure 6.5: The CSAs of the cast shown for section 6 to section 163.

The length of the new cast "airway" through the middle of the lumen was ~20 cm. A copy of the original cast was made through stereolithography based on the internal geometry; Figure 6.6). The external dimensions of the new cast were: maximal height ~14 cm, maximal width of oral cavity ~7 cm, maximal height of oral cavity ~4.5 cm, length of throat ~12 cm. The volume of the cast was ~110 mL when filled with water. The cast was made in two parts so that it could be taken

apart and cleaned. For the purposes of the *in vitro* study with acoustic pharyngometry, the two halves were carefully glued together to prevent the acoustic pulses to leak through the splits.

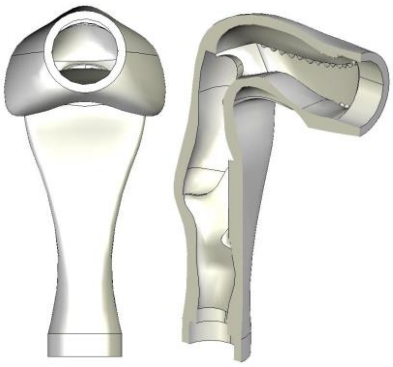


Figure 6.6: The new cast based on the original Swift "M3" cast and shown with the external geometry (left) and the internal geometry (right).

The internal geometry of the cast is shown in Figure 6.7 from 4 different angles. Both "ends" of the cast were circular, the mouth with an inner diameter of 29 mm and the bottom with an inner diameter of 24 mm.

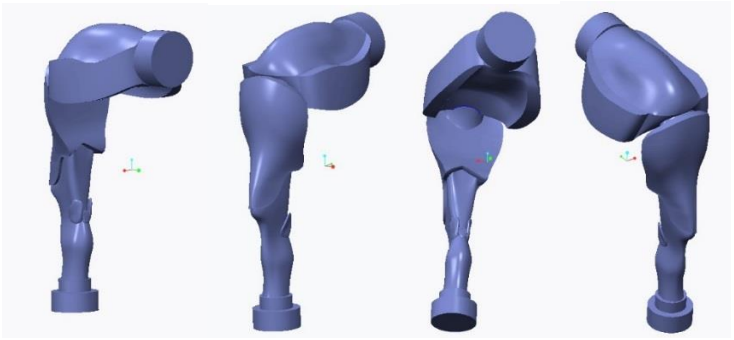


Figure 6.7: The throat of the cast shown from 4 different angles.

6.2.3 A PP tube as surrogate of the human upper airway

A PP tube (Dearborn Brass, Cleveland, Ohio, USA) with a slip joint washer connecting the long piece with the short piece after the 90° bend (Figure 6.8) was purchased to function as a second surrogate upper airway. The washer created a leak free seal between the 2 pieces of the tube. The

inner diameter of the tube was ~35 mm (area 962.1 mm^2 or 9.62 cm^2) and the inner length of the "airway" through the middle of the lumen as used in the *in vitro* tests was ~24 cm.

6.2.4 Creation of an open velum in the surrogates of the upper airways

In order to mimic an open velum in the cast and the tube the approximate position of the velum was identified based on the line drawing in Figure 6.3 at ~9 cm from the mouth (Figure 6.8). A round attachment made of PVC with threads was glued to the cast at that position. The attachment was taken from a PVC T-piece (D2466; IPEX, Pineville, North Carolina, USA) so that T-pieces mimicking nasal cavity volumes could be fitted to the attachment.

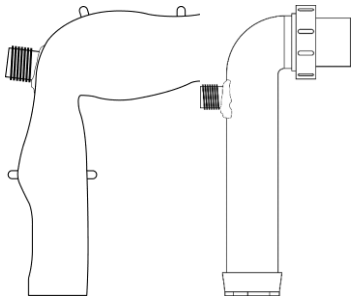


Figure 6.8: The cast (left) and the tube (right) with a round attachment made of PVC with threads glued to the "velum" position.

The same procedure was repeated with the tube with the attachment glued ~9 cm from the "mouth" (Figure 6.8).

A total of 12 different holes were drilled in both the cast and the tube in order to mimic different velum sizes. The first hole in the cast was drilled with a dentist drill 3 mm in diameter (area 7.1 mm^2, Figure 6.9) and the hole was subsequently enlarged with the same drill creating 11 incrementally larger holes (Figure 6.9). The same procedure was followed with the tube.

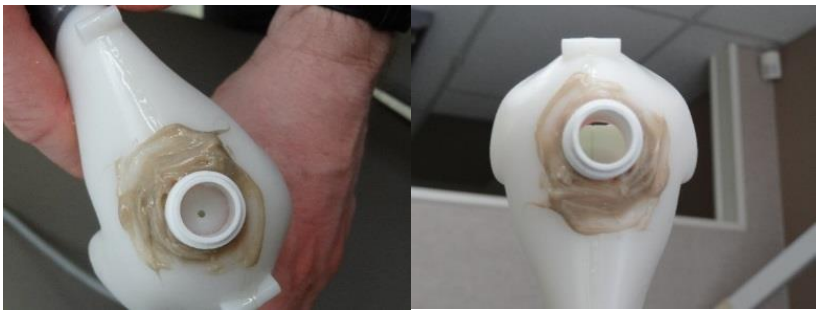


Figure 6.9: The cast shown with the round attachment made of PVC with threads glued (glue light brown) to the back of the cast. Holes of different sizes were drilled in the round attachment. The initial hole is shown to the left and the final hole to the right.

The largest hole was perfectly circular with a 14 mm diameter equal to the inner diameter of the attachment (area 153.9 mm^2 or 1.54 cm^2) and was prepared with a 7 mm diameter dentist drill (Figure 6.9). This was close to the sizes of the velopharynx measured with videoendoscopy in healthy subjects and subjects with OSA (Ferguson et al., 1997a). Four pharyngometer measurements were performed for each hole size for the cast and the same procedure was followed for the tube.

6.2.5 Addition of 'nasal cavities' to the cast

Two PVC T-pieces (IPEX, Pineville, North Carolina, USA) of different sizes were used in order to create nasal cavities that could be fitted on the attachment at the back of the cast and the tube at the velum position (Figure 6.10). The large T-piece (D2466) had an ~96 mm long T with an outer diameter of ~27 mm and a stem ~20 mm long. The small T-piece (D2464) had an ~85 mm long T with an outer diameter of ~18 mm and a stem ~18 mm long. The volumes of the T-pieces measured through water displacement were ~20 mL (small T-piece) and ~30 mL (large T-piece).

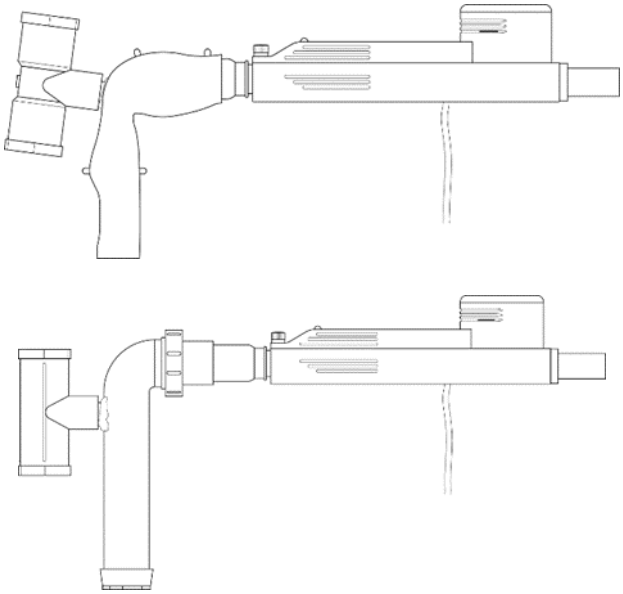


Figure 6.10: Cast (top) and tube (bottom) with large T-pieces connected via green elastomeric-lipped ISO connectors to the acoustic pharyngometer wavetube.

The "mouths" of the cast and the tube were connected to the acoustic pharyngometer wavetube through green elastomeric-lipped ISO connectors (Intersurgical Ltd, Wokingham, UK) as shown in Figure 6.10.

6.2.6 Summary of cast *in vitro* acoustic pharyngometer measurements

1. Baseline pharyngometer measurements were performed with the cast before any hole mimicking an open velum had been drilled into it. In these pharyngometer measurements the bottom end of the cast was initially open, then closed and after that opened again and connected to a ~2 m long vinyl hose with 26 mm inner diameter;
2. In the pharyngometer measurements with a hole mimicking an open velum, the first measurement was performed with a 3 mm diameter hole with the bottom end of the cast connected to the long vinyl hose. Four pharyngometer measurements were performed with

the velum hole open, 4 with the small T-piece connected to the hole and an additional 4 with the large T-piece connected to the hole, in that order.

3. A total of 12 different holes were created in order to mimic different velum sizes. The hole size was subsequently enlarged from the initial hole creating 11 larger holes, in total 12 different hole sizes to be tested with the cast connected to the pharyngometer wavetube. The same set of pharyngometer measurements were performed as with the 3 mm hole creating a total of 12 hole sizes × 12 measurements (hole open, small T-piece, large T-piece) = 144 pharyngometer measurements.

6.2.7 Summary of tube *in vitro* acoustic pharyngometer measurements

1. Baseline pharyngometer measurements were performed with the tube before any hole had been drilled into it and with the bottom end closed;
2. The first 3 mm diameter hole was drilled at the back of the tube in the same position as in the cast. The bottom end of the tube was closed with green putty. Four pharyngometer measurements were performed with the hole open, 4 with the small T-piece connected to the hole and an additional 4 with the large T-piece connected to the hole, in that order.
3. The hole size was subsequently enlarged creating 11 incrementally larger holes, in total 12 different hole sizes to be tested when connecting the tube to the pharyngometer wavetube. The same set of pharyngometer measurements were performed as with the 3 mm hole creating a total of 12 hole sizes × 12 measurements (hole open, small T-piece, large T-piece) = 144 pharyngometer measurements.

6.3 Statistical analysis

6.3.1 Data analysis

The data has been analysed descriptively. The main analysis of the pharyngograms was focused on the impact of the hole sizes on the CSAs and AUCs. The effect by hole size and T-piece on the endpoints was investigated using ANOVA including an interaction term. The significance level was established at 0.05.

6.4 Results

6.4.1 Cast – baseline pharyngograms

The baseline pharyngometer measurements were performed with the cast in pristine condition without a hole to mimic the open velum. Four pharyngograms per measurements were recorded with the bottom end of the cast either open, closed or connected to a long vinyl hose (Figure 6.11). The variability between the mean baseline pharyngograms was relatively small with two minima (CSA1 and CSA2).

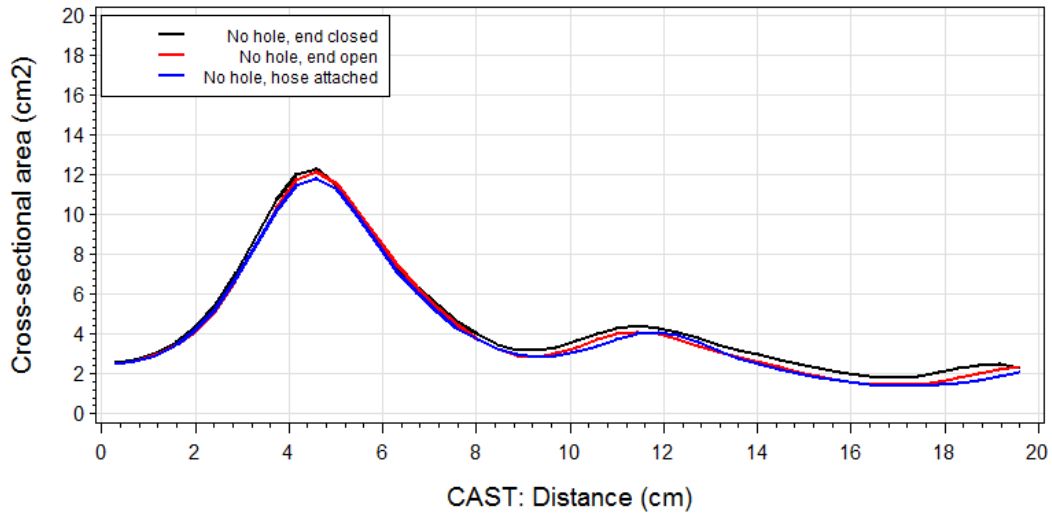


Figure 6.11: Cast - baseline mean pharyngograms from measurements with the cast in pristine condition. The bottom end was either open, closed or connected to a long vinyl hose.

The pharyngograms were relatively reproducible and as examples the mean SD for the cast CSA1 was 0.01 ranging from 0 to 0.04 (median 0.01) and for CSA2 0.01 ranging from 0.01 to 0.07 (median 0.02).

6.4.2 Cast - impact of hole size on pharyngograms

The mean pharyngograms from the measurements with open holes (no T-piece attached) of different sizes at the velum position of the cast (vinyl hose attached to the end of the cast) are shown in Figure 6.12.

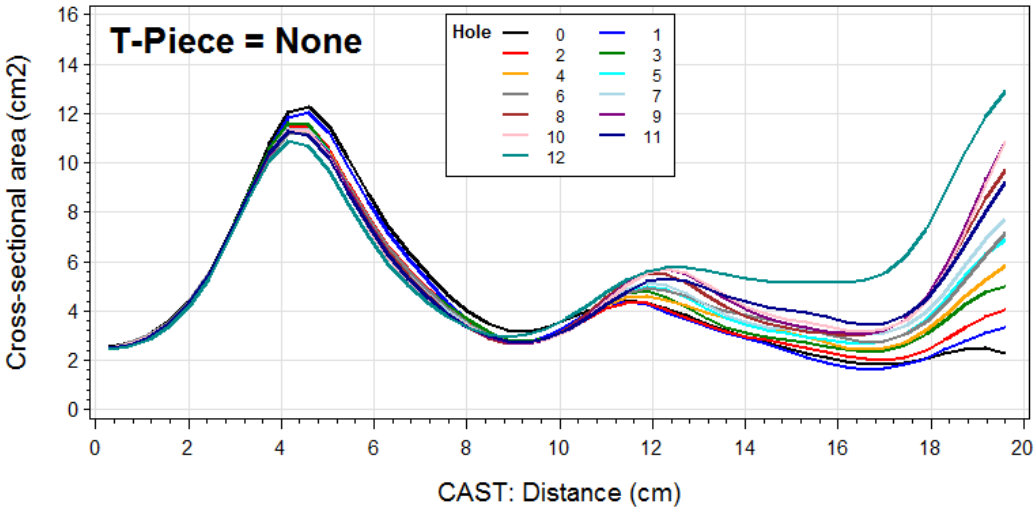


Figure 6.12: Cast - mean pharyngograms from measurements with the cast with holes of different sizes and without a T-piece attached.

The size of the hole had an impact on the mean pharyngograms. The hole was drilled ~9 cm from the mouth of the cast and there was some separation of the mean pharyngograms in the area covering the oral cavity at 4 to 9 cm (x-axis). The CSA of the mean pharyngograms decreased at ~4.5 cm (x-axis) depending on hole size from ~12 cm^2 (baseline) to ~11 cm^2 with the largest the hole size. Between ~11 cm and ~17 cm (x-axis) a second separation could be seen between the

mean pharyngograms. After ~17 cm (x-axis) the separation between the mean pharyngograms became more prominent and at 20 cm the mean pharyngograms followed an almost perfect order in relation to hole size.

6.4.3 Cast - impact of hole size and small T-piece on pharyngograms

The mean pharyngograms from the measurements with the small T-piece attached to the cast and with holes of different sizes at the velum position of the cast (vinyl hose attached to the end of the cast) are shown in Figure 6.13.

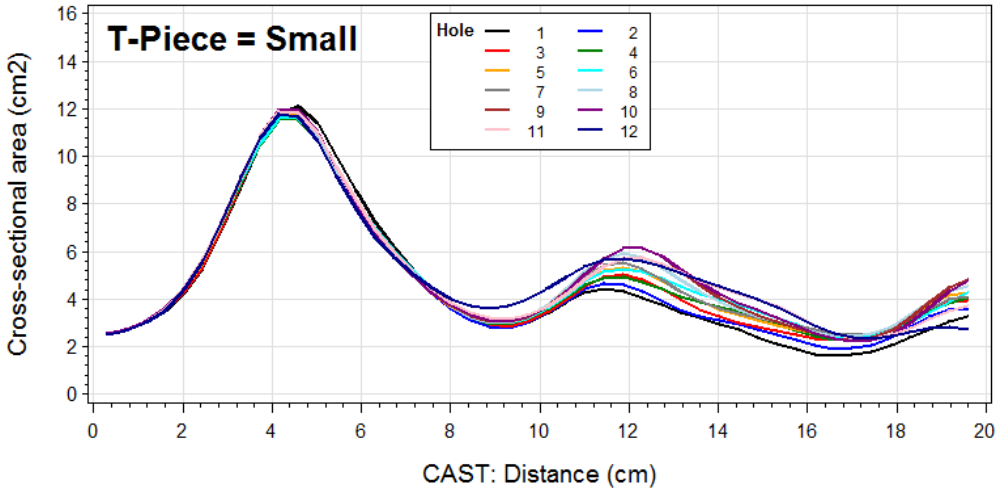


Figure 6.13: Cast - mean pharyngograms from measurements with the cast with holes of different sizes and the small T-piece attached.

With the small T-piece attached to the cast, the size of the hole had in comparison with the mean pharyngograms measured with an open hole less impact on the mean pharyngograms. The addition of the small T-piece eliminated the separation of the mean pharyngograms in the area covering the oral cavity at 4 to 9 cm (x-axis). The mean pharyngogram from the measurement with the largest hole size was somewhat separated from the other mean pharyngograms at ~8 cm but joined the large bundle of mean pharyngograms at ~11 cm. There was some further separation of the mean

pharyngograms between ~11 cm and 20 cm but without the order of the mean pharyngograms seen in the measurements without a T-piece attached to the hole.

6.4.4 Cast - impact of hole size and large T-piece on pharyngograms

The mean pharyngograms from the measurements with the large T-piece attached and with holes of different sizes at the velum position of the cast (vinyl hose attached to the end of the cast) are shown in Figure 6.14.

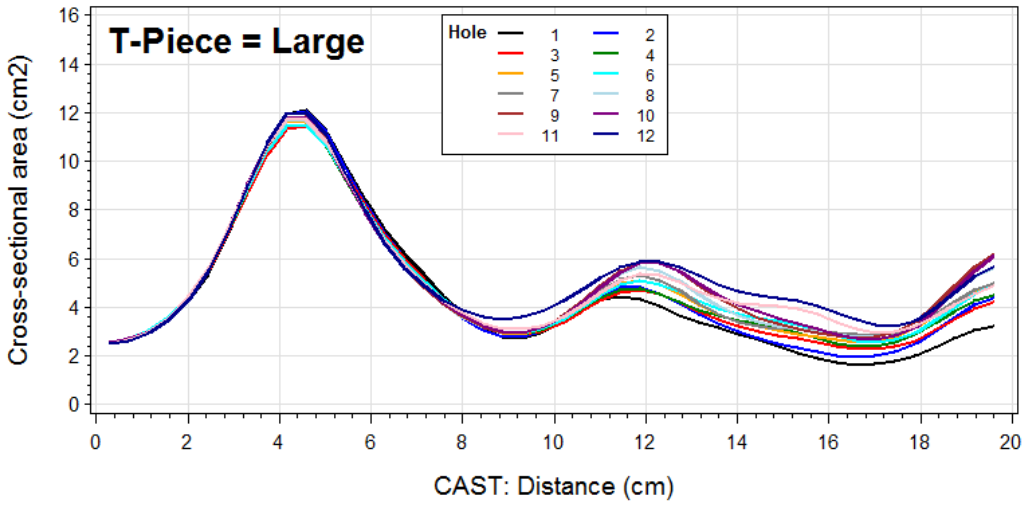


Figure 6.14: Cast - mean pharyngograms from measurements with the cast with holes of different sizes and a large T-piece attached.

The pharyngograms measured with the large T-piece attached to the cast resembled the pharyngograms measured with the small T-piece attached to the cast. The bundle of mean pharyngograms from ~11 cm was somewhat more separated in comparison with mean pharyngograms from the measurements with the small T-piece but still lacked the order of the mean pharyngograms seen in the measurements without a T-piece attached to the hole.

6.4.5 Cast – statistical analysis of CSAs and AUCs

As with the analyses of the pharyngograms reported in Chapters 3-5, the same endpoints (CSAs, AUCs) were studied. Based on the average baseline recording for the cast (closed end, no hole, black curve in Figure 6.11) the position of the two minima (CSA1, CSA2) following the initial peak (oral cavity) were determined with CSA1 positioned at 9.73 cm and CSA2 at 17.02 cm (x-axis). In addition to the CSAs, the AUCs were calculated for each hole size and T-piece; AUC1 between the mouth (x = -0.13) and CSA1, AUC2 between CSA1 and CSA2, and AUC3 between CSA2 and end of cast (x=20.02). The effect by hole size and T-piece on the endpoints was investigated using ANOVA including an interaction term (Table 6.3).

Table 6.3: Cast - results (p-values) from ANOVA exploring effects by hole size and T-piece.

Endpoint	Hole	T-piece	Interaction
CSA1	<0.0001	0.92	<0.0001
CSA2	<0.0001	<0.0001	<0.0001
AUC1	<0.0001	0.60	<0.0001
AUC2	<0.0001	<0.0001	<0.0001
AUC3	<0.0001	<0.0001	<0.0001

The statistically significant interactions indicated that the effect by hole size differed depending on T-piece used. The numerical effects are presented in the summary statistics (means) for the endpoints (CSA1-2, AUC1-3) per hole size and T-piece in Table 6.4. The volume of the cast including the green connector when connected to the wavetube was 169.42 cm^3 (Table 6.4).

Table 6.4: Cast – summary statistics for CSA1-2 and AUC1-3 (presented as means) per hole size and T-piece.

	T-piece	**H 0**	**H 1**	**H 2**	**H 3**	**H 4**	**H 5**	**H 6**	**H 7**	**H 8**	**H 9**	**H 10**	**H 11**	**H 12**
CSA1	None	2.91	2.97	2.89	2.88	2.89	2.90	2.86	2.88	2.86	2.82	2.83	2.90	3.30
	Small		2.99	3.00	3.03	3.13	3.20	3.18	3.26	3.23	3.24	3.26	3.32	4.00
	Large		2.94	2.98	3.01	3.06	3.11	3.09	3.13	3.14	3.15	3.13	3.22	3.82
CSA2	None	1.37	1.67	2.01	2.40	2.48	2.75	2.79	3.12	3.22	3.21	3.26	3.49	5.55
	Small		1.64	1.92	2.29	2.25	2.39	2.35	2.50	2.42	2.28	2.20	2.36	2.38
	Large		1.66	1.99	2.27	2.38	2.60	2.56	2.84	2.80	2.78	2.67	2.96	3.25
AUC1	None	118.64	119.0	115.2	115.4	114.2	114.1	114.4	113.3	113.1	112.8	112.5	112.6	109.2
	Small		119.8	117.1	118.1	117.9	118.4	119.5	118.9	119.2	119.8	120.0	120.9	120.3
	Large		120.0	119.3	117.1	116.6	117.0	117.9	117.3	117.4	118.0	118.2	118.7	120.0
AUC2	None	40.49	44.61	46.32	50.02	51.47	53.36	55.30	55.82	58.18	59.87	60.89	61.61	74.80
	Small		45.11	48.24	51.76	53.78	55.46	57.57	57.35	59.72	61.16	61.96	62.62	64.77
	Large		44.89	48.35	50.20	52.17	54.06	55.88	55.97	58.26	60.16	60.95	61.57	68.74
AUC3	None	10.29	15.56	18.50	23.25	25.54	29.70	29.92	32.76	39.28	42.11	41.48	38.12	57.59
	Small		15.23	17.57	19.47	19.52	20.72	19.82	20.15	21.38	21.51	20.64	18.05	15.66
	Large		15.15	19.68	19.77	21.30	23.23	22.60	23.73	25.83	27.14	26.26	23.49	26.35

The results presented in Table 6.4 indicated that:

- the effect by hole size was stronger without a T-piece.
- the effect of hole size was similar for the small and large T-piece.
- the effects were weakest for CSA1, AUC1, and strongest for CSA2, AUC2 and AUC3.

6.4.6 Tube - baseline pharyngograms

The baseline pharyngometer measurements were performed with the tube in pristine condition without a hole to mimic the open velum (Figure 6.15).

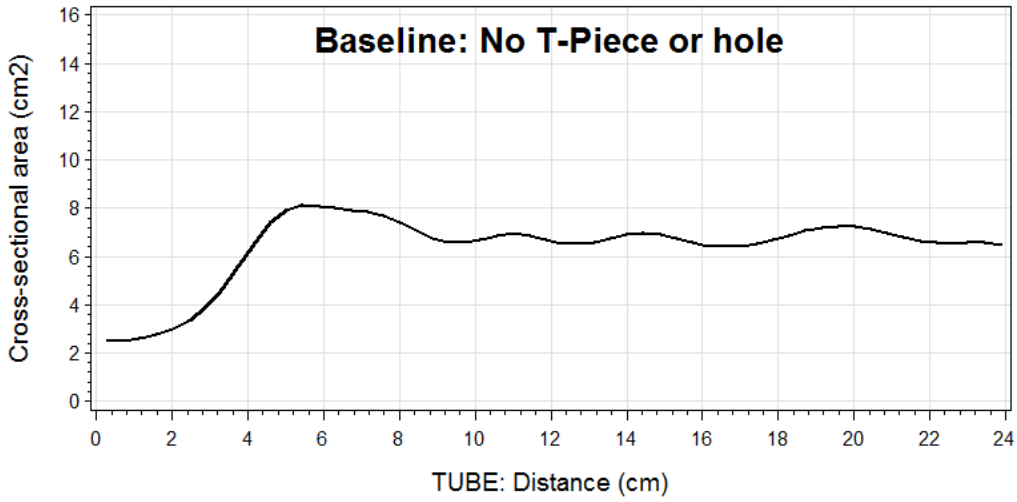


Figure 6.15: Tube - baseline mean pharyngogram from measurements with the tube in pristine condition. The bottom end was closed.

The baseline mean pharyngogram resembled a relatively flat waveform from ~9 cm (x-axis) onward. As the inner diameter of the tube was ~35 mm and the CSA of the tube therefore 9.62 cm^2, the tube baseline mean pharyngogram underestimated the true CSA. It was closest (~8 cm^2) to the true CSA at ~5 cm (x-axis) and then fluctuated about ~7.0 cm^2 between ~5 to 24 cm.

6.4.7 Tube – impact of hole size on pharyngograms

The mean pharyngograms from the measurements with open holes (no T-piece attached) of different sizes at the velum position of the tube are shown in Figure 6.16.

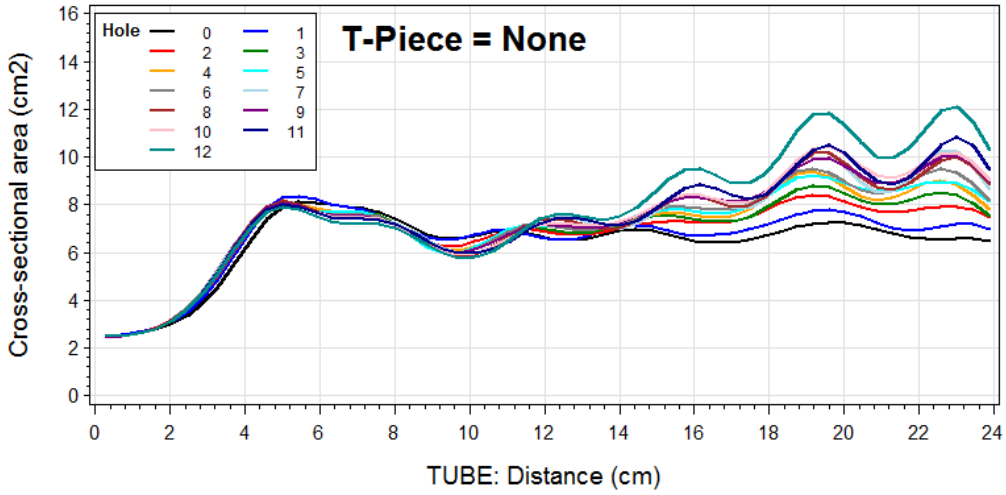


Figure 6.16: Tube - mean pharyngograms from measurements with holes of different sizes and without a T-piece attached.

The size of the hole had an impact on the mean pharyngograms which followed the same pattern as the baseline mean pharyngogram up to about ~14 cm (x-axis) after which some separation occurred. At ~15 cm the mean pharyngograms started to gradually separate more and more. As with the cast, the separation between the mean pharyngograms became more prominent and at ~19 cm followed an almost perfect order in relation to hole size. The mean pharyngograms underestimated the true tube CSA for the small and medium hole sizes and exceeded the true CSA only for the largest hole sizes.

In Figure 6.17 and Figure 6.18 the corresponding mean pharyngograms are shown with the addition of the small and large T-pieces to the tube.

6.4.8 Tube – impact of hole size and small T-piece on pharyngograms

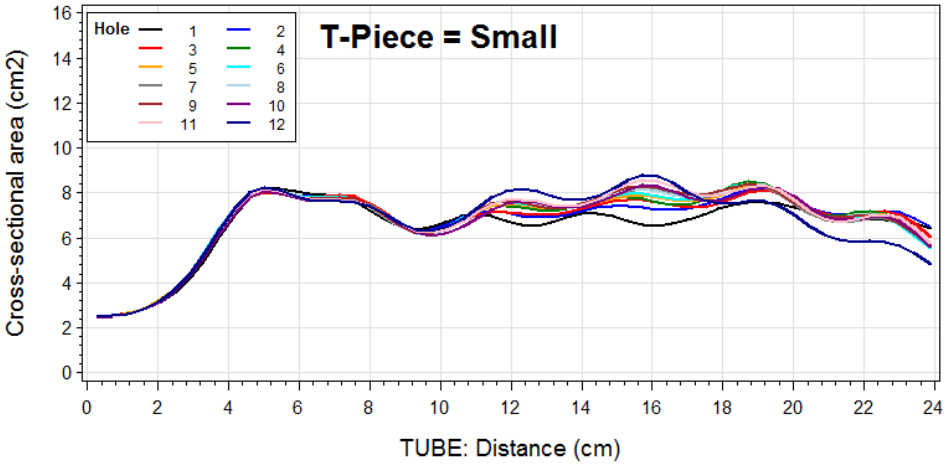


Figure 6.17: Tube - mean pharyngograms from measurements with the cast with holes of different sizes and the small T-piece attached.

6.4.9 Tube – impact of hole and large T-piece on pharyngograms

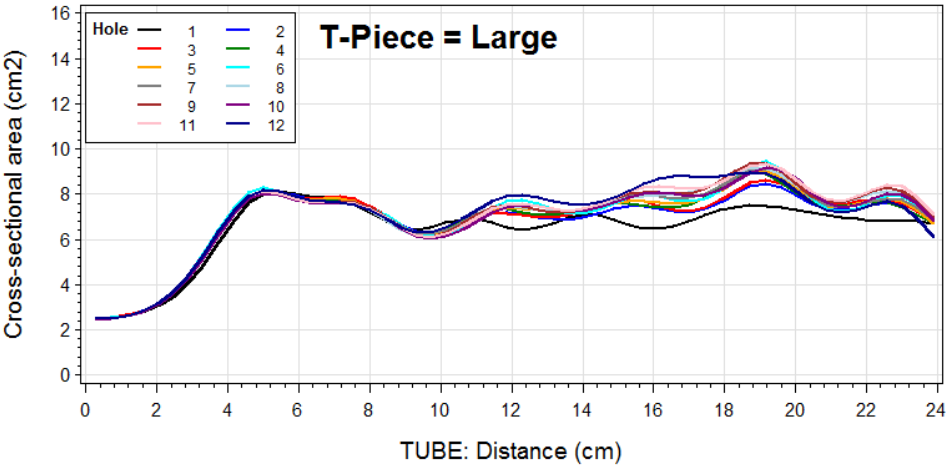


Figure 6.18: Tube - mean pharyngograms from measurements with the cast with holes of different sizes and the large T-piece attached.

As with the cast, the addition of the small T-piece almost eliminated the impact of the hole sizes on the mean pharyngograms. Some separation still occurred between the mean pharyngograms but this was minimal in comparison with the measurements without the T-piece. The changes in the

mean pharyngograms with the addition of the large T-piece were similar to those seen with the small T-piece, although an increase in CSA could be seen at ~19 cm (x-axis) which came close to the true tube CSA of 9.62 cm^2.

6.4.10 Tube – statistical analysis of CSAs and AUCs

Based on the average baseline pharyngogram for the tube (closed end, no hole, black curve in Figure 6.15) the position of three minima (CSAs) following the initial peak were determined; CSA1 at 9.73 cm, CSA2 at 17.02 cm and CSA3 at 22.6 cm (x-axis). There were more than three minima with growing hole size but the minima closest to the cast minima were used. The AUCs were calculated for each hole size and T-piece; AUC1 between the mouth (x = -0.13) and CSA1, AUC2 between CSA1 and CSA2, and AUC3 between CSA2 and end of tube (x=24.0). The effect by hole size and T-piece on the endpoints was investigated using ANOVA including an interaction term (Table 6.5).

Table 6.5: Results (p-values) from ANOVA exploring effects by hole size and T-piece for tube.

Endpoint	Hole	T-piece	Interaction
CSA1	<0.0001	0.95	<0.0001
CSA2	<0.0001	0.0104	<0.0001
CSA3	<0.0001	0.74	<0.0001
AUC1	<0.0001	0.0566	<0.0001
AUC2	<0.0001	0.0368	0.0417
AUC3	<0.0001	0.49	<0.0001

The statistically significant interactions indicated that the effect by hole size differed depending on T-piece used. The numerical effects are presented in the summary statistics (means) for the endpoints (CSA1-3, AUC1-3) per hole size and T-piece in Table 6.6.

Table 6.6: Tube – summary statistics for CSA1-3 and AUC1-3 (presented as means) per hole size and T-piece.

	T-piece	**H 0**	**H 1**	**H 2**	**H 3**	**H 4**	**H 5**	**H 6**	**H 7**	**H 8**	**H 9**	**H 10**	**H 11**	**H 12**
CSA1	None	6.59	6.60	6.26	6.09	6.07	5.98	5.96	5.98	5.88	5.98	5.90	5.97	5.77
	Small		6.46	6.35	6.26	6.26	6.28	6.19	6.25	6.14	6.19	6.09	6.24	6.29
	Large		6.53	6.33	6.24	6.21	6.22	6.14	6.23	6.08	6.11	6.02	6.11	6.30
CSA2	None	6.40	6.73	7.24	7.28	7.47	7.63	7.79	8.17	8.12	8.22	8.22	8.67	9.22
	Small		6.63	7.24	7.45	7.46	7.70	7.74	7.99	7.94	8.06	8.07	8.32	8.44
	Large		6.58	7.24	7.32	7.40	7.59	7.76	7.82	7.96	8.01	8.00	8.29	8.77
CSA3	None	6.55	7.05	7.93	8.52	9.00	8.98	9.50	10.24	9.84	10.00	10.13	10.53	11.92
	Small		6.81	7.18	7.17	7.09	7.03	6.86	6.86	6.99	6.93	6.94	7.05	5.83
	Large		6.80	7.61	7.75	7.61	7.82	7.93	7.82	8.12	8.31	8.00	8.42	7.67
AUC1	None	111.9	113.9	112.3	112.5	112.5	111.7	112.1	112.2	111.7	111.3	111.1	111.4	109.4
	Small		113.4	113.5	114.0	114.1	114.1	114.3	114.1	113.6	114.0	112.6	114.0	113.9
	Large		111.9	113.5	114.0	113.4	113.6	114.2	113.5	112.9	113.3	112.1	113.1	113.6
AUC2	None	92.54	93.88	95.72	96.63	98.05	98.61	98.70	99.58	99.79	100.1	100.1	100.8	105.0
	Small		93.53	97.58	98.81	100.9	101.4	102.4	102.8	102.7	103.6	102.3	104.6	108.0
	Large		92.46	97.02	98.53	99.22	99.95	100.9	101.1	101.0	101.6	100.4	102.3	105.9
AUC3	None	82.51	87.47	94.85	98.23	102.8	103.9	105.8	109.0	108.9	110.0	112.4	112.2	125.5
	Small		86.24	90.61	90.08	91.75	90.72	91.14	90.52	92.12	92.04	90.98	91.54	84.11
	Large		85.96	92.76	94.88	96.58	96.93	98.15	98.31	100.2	100.9	98.88	101.8	98.95

The results presented in Table 6.6 indicated that:

- the effect by hole size was stronger without a T-piece.
- the effect of hole size was similar for the small and large T-piece.
- the effects were weakest for CSA1, AUC1, and AUC2, and strongest for CSA2.

6.5 Graphical comparison of cast and tube data

6.5.1 Mean CSA1-3 by T-piece (none, small, large)

In Figure 6.19 the mean CSA1 results are shown by T-piece (none, small and large) for the cast and the tube per the different hole sizes. Apart from the size of the CSA1 which was obviously different, the trend following the addition of the T-pieces was positive for the cast and negative for the tube.

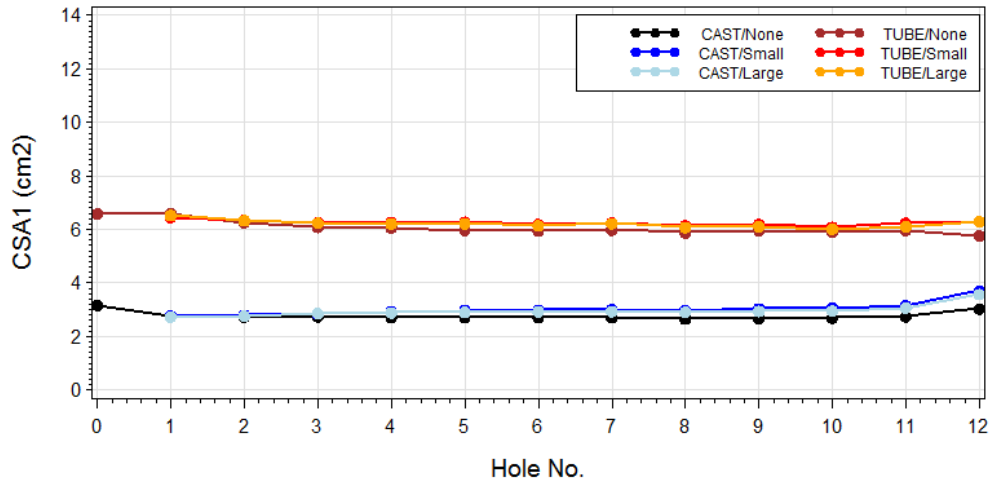


Figure 6.19: Mean CSA1 by T-piece (none, small, large) used with cast and tube per hole size.

In Figure 6.20 the mean CSA2 results and in Figure 6.21 the mean CSA3 results (no data for cast as a CSA3 was not identified) are shown by T-piece (none, small and large) for the cast and the tube per the different hole sizes.

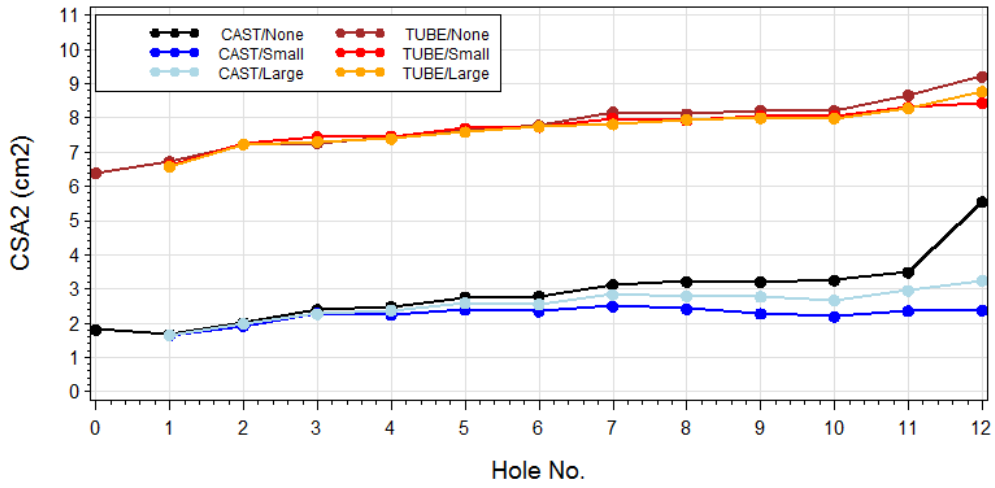


Figure 6.20: Mean CSA2 by T-piece (none, small, large) used with cast and tube per hole size.

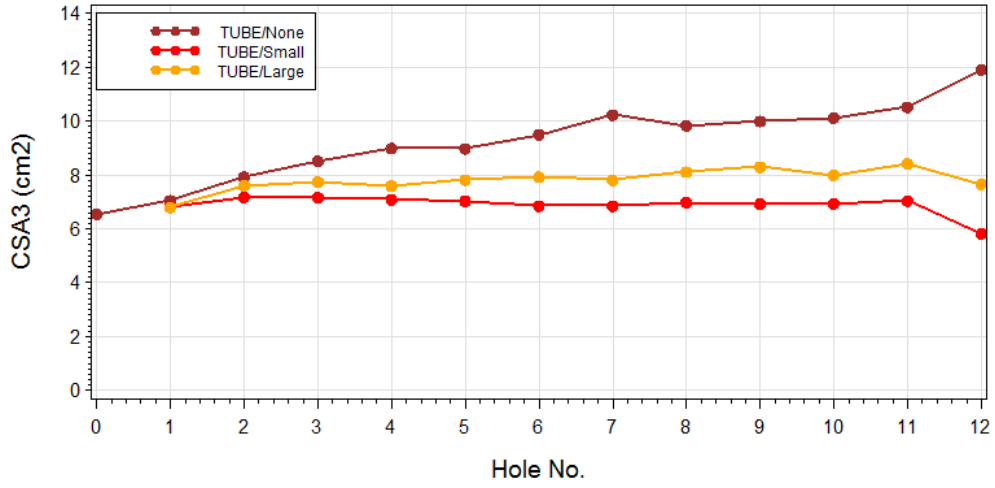


Figure 6.21: Mean CSA3 by T-piece (none, small, large) used with tube per hole size.

There was an increase in mean CSA2 both without and with T-pieces for both the cast and the tube following the increases in hole size. For the tube there was a relatively large increase and no separation between the 3 curves, whereas for the cast the curve without a T-piece showed the largest increase. The tube CSA3 curve without a T-piece increased with increasing hole size, whereas the addition of the T-pieces had no effect on the curves.

6.5.2 Mean AUC1-3 by T-piece (none, small, large)

In Figure 6.22 the mean AUC1 results are shown by T-piece (none, small and large) for the cast and the tube per the different hole sizes.

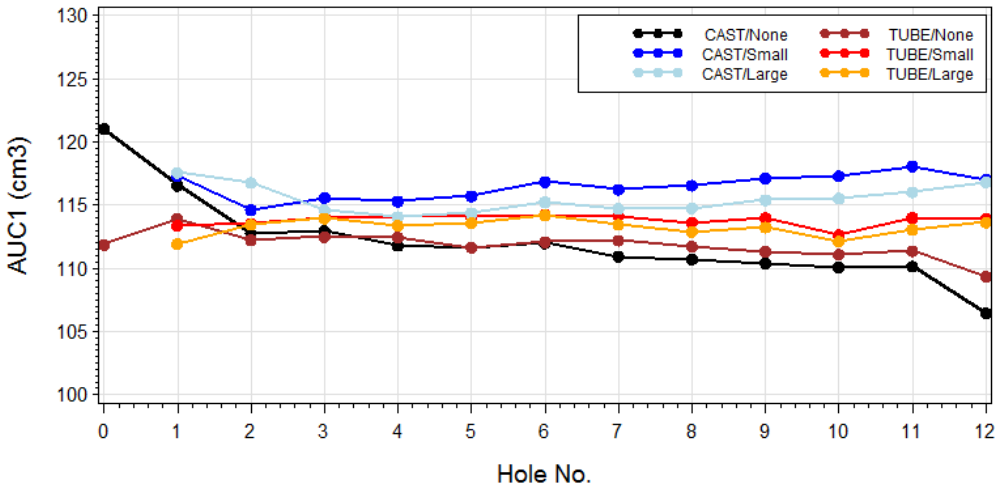


Figure 6.22: Mean AUC1 by T-piece (none, small, large) used with cast and tube per hole size.

The difference in AUC1 (without a T-piece) between the cast and the tube was ~9 cm^3 (~8%). The introduction of a hole led to a decrease in AUC1 (without a T-piece) for the cast but not for the tube, and the cast AUC1 decreased further with increasing hole size. The addition of the T-pieces had no major impact on the tube AUC1 values, whereas the cast AUC1 values increased.

In Figure 6.23 the mean AUC2 results and in Figure 6.24 the mean AUC3 results are shown by T-piece (none, small and large) for the cast and the tube per the different hole sizes. The cast and tube AUC2 values increased with increasing hole size and resembled the results for CSA2. The addition of a T-piece had no major effect on the AUC2 for either the cast or the tube.

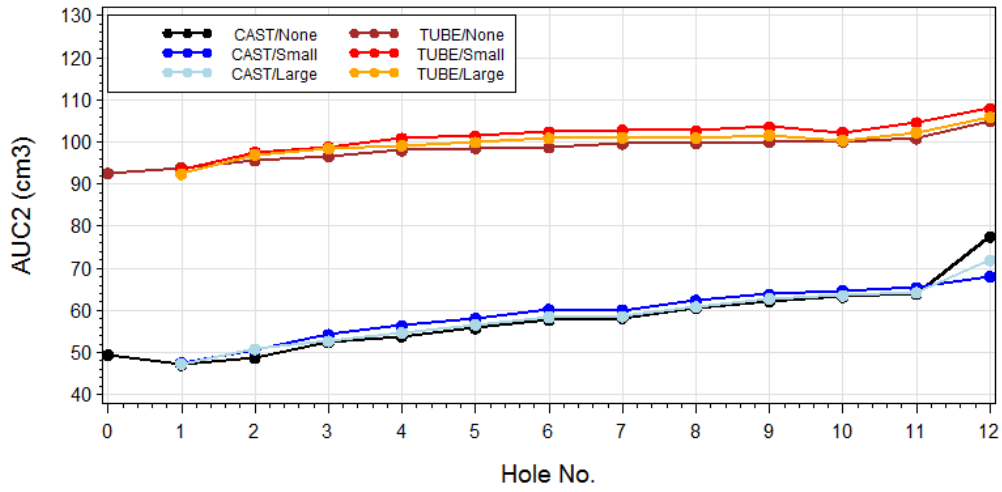


Figure 6.23: Mean AUC2 by T-piece (none, small, large) used with cast and tube per hole size.

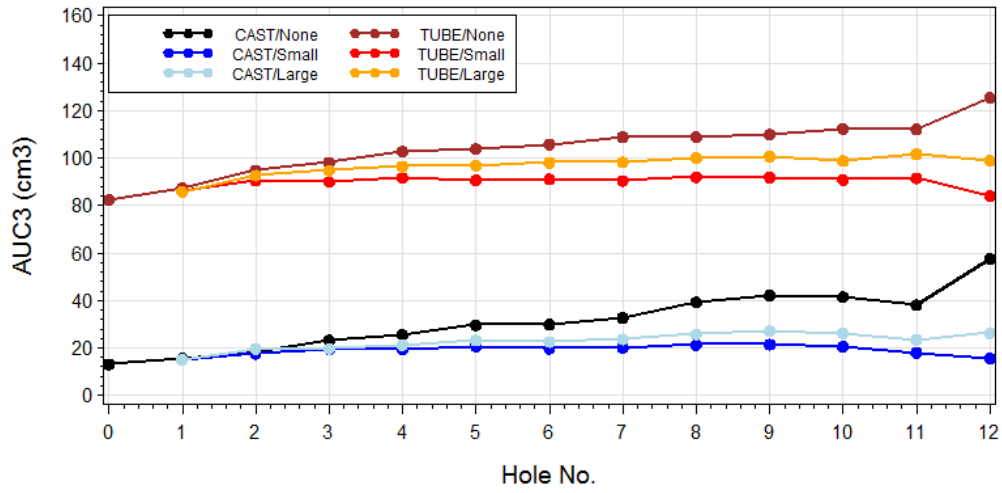


Figure 6.24: Mean AUC3 by T-piece (none, small, large) used with cast and tube per hole size.

The introduction of a hole led to an increase in AUC3 (without a T-piece) for both the cast and the tube, whereas the addition of the T-pieces had no or a small negative effect.

6.6 Discussion

The primary objective of the present study was to use an acoustic pharyngometer to study the effect of an open velum on acoustic pharyngograms. An open velum has been shown to affect the pharyngogram, when using prototype acoustic pharyngometers, resulting in overestimations of GL and tracheal areas (Molfino et al., 1990; Marshall et al., 1993). An evaluation of the impact of an open velum on acoustic pharyngometer measurements in human subjects would be of limited value without control of the velum and the amount of leak in terms of the CSA and the volume of the nasal cavity.

An *in vitro* study design was preferable as controlled acoustic pharyngometer measurements could be made with surrogates for a closed or an open velum and a nasal cavity. Two *in vitro* models were used, a cast of a human upper airway and a tube with a bend. The cast was based on a cast of the human upper airways without the nasal airways and was similar to the polyester resin cast presented by Cheng et al (1990). The shape of the different internal parts of the cast resembled the shape of the human upper airway when compared with the shape of a human upper airway presented by Strohl et al (2012) based on work by Proctor (1983). The similarity was of importance considering the origin of the cast from a post mortem anatomical casting and reconstruction from MRI measurements (Swift, 1991; Swift et al., 1994).

The open velum was mimicked through round holes of 12 different sizes (3 – 14 mm diameters) drilled in the posterior pharyngeal wall of the cast and the tube. The smallest hole size had a CSA of 0.07 cm^2 and largest hole size a CSA of 1.54 cm^2, which was close to the sizes of the velopharynx measured with videoendoscopy (Ryan et al., 1996; Ferguson et al., 1997a) and CT (Schwab et al., 1993b) in healthy subjects. The shape of the velopharynx in both healthy subjects and subjects with OSA presented in the study by Ryan et al (1996; Figure 6.25) indicate that the round shape selected for the surrogate velum was relevant.

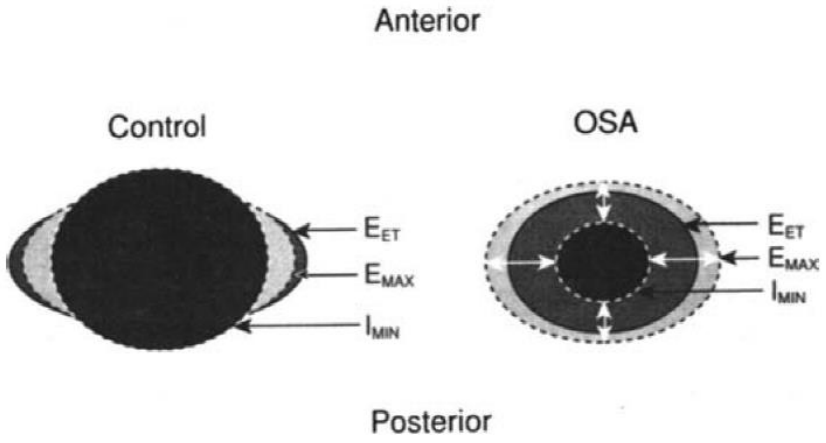


Figure 6.25: Schematic presentation of changes in velopharynx during a maximal VC manoeuvre against an unoccluded nasal airway in healthy subjects and subjects with OSA. EET = end-tidal expiration; EMAX = maximal airway CSA during expiration; IMIN = minimal airway CSA during inspiration (Ryan et al., 1996).

The *in vitro* nasal cavity in the shape of T-pieces was attached to the holes in the posterior pharyngeal walls of the cast and the tube. The T-piece volumes (~20 mL and ~30 mL) were based on published data of both male and female subjects (Guilmette et al., 1997). Measurements without any T-piece were also made in order to have a reference for larger nasal cavities.

There was a large difference between the shape of the internal parts of the cast and the "smooth" mean baseline pharyngogram of the cast. Marshall et al (1993) had observed the same kind of difference between an MRI of a subject's upper airway and a pharyngogram of the same subject and pointed out the apparent "smoothing" effect of the AR method. The cast mean baseline pharyngogram resembled, however, pharyngograms recorded in healthy subjects and subjects with OSA (Chapters 3-5) in terms of the waveform, but presented only 2 minima (CSA1 and CSA2) in contrast to the 3 minima (OPJ, EG and GL) found in pharyngograms from subjects in the studies presented in Chapters 3-5. This was probably a consequence of the origin of the cast. The mean positions of the minima were for example in the study presented in Chapter 5: OPJ (CSA1) at 8.48 cm, EG (CSA2) at 12.22 cm and GL (CSA3) at 20.72 cm. The position of the CSA1 of the cast

was ~9 cm which is close to the results found in Chapter 5, whereas the position of CSA2 was ~17 cm which is between the CSA2 and CSA3 positions found in Chapter 5. Three AUCs were, however, calculated although AUC3 was small due to the position of CSA2. The sizes of the cast CSA1 and CSA2 were similar to the mean CSAs found in the studies presented in Chapters 3-5, whereas the total volume of the cast (AUC1 + AUC2 + AUC3) was considerably larger. The main reason for the large total volume was probably the connection of the cast to the wavetube which increased AUC1. In clinical use the end of the wavetube would be within the mouthpiece inserted into the mouth of the subject whereas with the cast the end of the wavetube was connected to the green connector that was partically within the cast oral orifice. This is most probably the reason for the discrepancy between the volume of the cast when filled with water (~110 mL) and the volume of the AUC1-3 (~169 cm^3).

The tube mean baseline pharyngogram deviated from the waveform of the human pharyngogram, and the minima were therefore identified based on the positions of the minima in the cast mean baseline pharyngogram. The sinusoidal waveform of the tube mean baseline pharyngogram did, however, resemble the pharyngograms from measurements of endotracheal tubes performed with a prototype pharyngometer (Van Surell et al., 1994; Straus et al., 1998), and performed with the Eccovision ARP (Raphael et al., 2002). In the study by Straus et al the CSA of the endotracheal tube pharyngogram was somewhat smaller than the actual CSA calculated based on the diameter of 7.5 mm and showed a waveform pattern. This was in accordance with the results in the present study. In the studies by Van Surell et al (1994) and Raphael et al (2002) comparisons could not be made. The reasons for the sinusoidal waveform pattern in the tube and the underestimation of the true CSA are unclear.

The pharyngometer measurements with the largest hole size in the cast without T-pieces showed the largest increase in the pharyngograms. The pharyngograms were somewhat separated at the

peak CSA of the oral cavity with the pharyngogram presenting the largest hole sizes showing the smallest CSA. The increase in the pharyngograms after the oral cavity occurred at ~11 cm, reached a plateau at ~12 cm, and increased sharply at ~17 cm. The changes in the CSA were initially small with an early increase after the oral cavity from ~4 to ~6 cm^2 (~10 to ~12 cm) and the late increase from ~6 to ~13 cm^2 (~17 to ~20 cm). The increase in the pharyngograms at ~20 cm followed the order of the size of the holes with the pharyngogram based on the smallest hole size showing the least increase and vice versa. The addition of the T-pieces to the posterior pharyngeal wall of the cast reduced the increases seen without the addition of the T-pieces. The total increase in upper airway volume (difference between "no hole, total AUC1-3" and "total AUC1-3 for the largest hole size") was with the small T-piece ~33 mL and with the large T-piece ~46 mL and thus somewhat larger than the volumes of the T-pieces.

The pharyngometer measurements with the largest hole size in the tube without T-pieces showed as with the cast the largest increase in the pharyngograms. The pharyngograms did not show a separation at the peak CSA of the oral cavity as with the cast probably due to the simple geometry of the tube. As with the cast the increase in the pharyngograms at ~24 cm followed the order of the size of the holes with the pharyngogram based on the smallest hole size showing the least increase and vice versa. The addition of the T-pieces to the posterior pharyngeal wall of the tube did also reduce the increases seen without the addition of the T-pieces. Thus the changes in the pharyngograms did largely mimic those of the cast. The total increase in tube upper airway volume (difference between "no hole, total AUC1-3" and "total AUC1-3 for the largest hole size") was with the small T-piece ~19 mL and with the large T-piece ~32 mL and thus similar to the volumes of the T-pieces. The differences between the volumes of the cast and the tube were surprising. The differences between the surrogate upper airway models can be found in the different geometries,

the different materials and the different sizes of the surrogates. Could any of these or the combination be the reason for the differences?

The shape of the *in vitro* cast pharyngogram did not match the pharyngograms published by Molfino et al (1990) and Marshall et al (1993). A comparison of the *in vitro* pharyngograms with the artefact pharyngograms published by Molfino et al (1990) and Marshall et al (1993) is difficult due to the differences in x-axis scales as both measured the airways from the oral cavity to the trachea. Molfino et al used a 0-64 cm scale and Marshall et al a 0-30 cm scale (Chapter 2, section 2.5.5.4). Molfino et al showed an increase of the peak CSA from ~6 cm^2 to ~7.5 cm^2 of the oral cavity and a second large increase at ~19 cm which then increased up to ~52 cm reaching a CSA of ~13 cm^2. Thus the magnitude of the increases were similar to those of the *in vitro* pharyngogram but the locations on the x-axis did not match. Marshall et al, on the other hand, showed an increase of the peak CSA of the oral cavity from ~6 cm^2 to ~12 cm^2, whereas the next increase from ~4 cm^2 to ~5 cm^2 occurred at ~15 cm and then decreased to ~3 cm^2 at ~28 cm.

6.7 Conclusions

The study hypothesis: "during acoustic pharyngometer measurements an open velum would pass acoustic impulses from the wavetube through the nasopharynx into the nasal airways creating a form of acoustic leak, which could create an overestimation of the volume of the upper airways from the pharynx to the GL as displayed on the pharyngogram", was based on the assumption of an open velum based artefact as published by Molfino et al (1990) and Marshall et al (1993).

The pharyngometer measurements with the largest hole size in the cast with the T-pieces attached showed a total increase in the upper airway volume (difference between "no hole, total AUC1-3" and "total AUC1-3 for the largest hole size") which was with the small T-piece ~33 mL and with the large T-piece ~46 mL and thus somewhat larger than the volumes of the T-pieces. The same

analysis with the tube showed a total increase in upper airway volume (difference between “no hole, total AUC1-3” and “total AUC1-3 for the largest hole size”) which with the small T-piece was ~19 mL and with the large T-piece was ~32 mL and thus similar to the volumes of the T-pieces. The differences in the impact of the T-piece volumes on the pharyngograms between the volumes of the cast and the tube were surprising. The differences between the surrogate upper airway models can be found in the different geometries, materials and sizes of the surrogates. Could any of these or the combination be the reason for the differences?

The study did, however, confirm the study hypothesis that: “during acoustic pharyngometer measurements an open velum would pass acoustic impulses from the wavetube through the nasopharynx into the nasal airways creating a form of acoustic leak, which could create an overestimation of the volume of the upper airways from the pharynx to the GL as displayed on the pharyngogram”.

Chapter 7 General conclusions and future work

7.1 Summary

There are several advantages of the delivery of locally acting drugs as aerosols through the upper airways for treatment of diseases of the lungs in comparison with for example the oral route through the gastrointestinal tract. These include targeting the inhaled drugs directly to the airway surfaces, avoiding inactivation through hepatic first pass metabolism, minimising the amount of drug required and decreasing time to onset of action in comparison with swallowed drug (Newman et al., 2009). The mouth, the pharynx and the larynx are, however, potential sites of aerosol deposition during oral inhalation minimising the amount available for deposition in the lungs.

When using nebulisers for aerosol delivery parameters such as the aerosol characteristics (droplet size, FPF, solution *versus* suspension) and the subject's breathing pattern (and vocalization) will have an impact on the passage of aerosol through the upper airways to the lungs as shown in a number of studies (Newman et al., 1988; Zainudin et al., 1988; Newman et al., 1994; Svartengren et al., 1994; Svartengren et al., 1996; Kumazawa et al., 1997; Anderson et al., 1999; Häkkinen et al., 1999; Erzinger et al., 2007; Nikander et al., 2010c; van Velzen et al., 2015).

The clinical studies in this Thesis were focused on measurements of the size of the upper airways and the correlation of this to upper airway and lung deposition (Chapter 3), and on the possible impact of mandibular advancement and incisal opening achieved with novel stepped mouthpieces on the size of the upper airways (Chapters 4-5). The *in vitro* study was designed to investigate the impact of a possible artefact – an open velum - on the pharyngogram when using acoustic pharyngometry (Eccovision ARP) (Chapter 6).

In the first study (Chapter 3) 9 of 12 healthy subjects who had participated in a randomised, open-label, crossover lung deposition study (Nikander et al., 2010c) were included in a follow-up study

to measure the size of their upper airways from the oral cavity to the GL. The I-neb nebuliser had been used in both TBM and TIM breathing modes to deliver an aerosol with saline mixed with ^{99m}Tc-DTPA. The lung deposition of ^{99m}Tc-DTPA (with central and peripheral lung deposition shown separately), the upper airway deposition, and the exhaled fraction caught on the filter attached to the mouthpiece are shown in Chapter 3 and Figure 3.1. The variability in the upper airway deposition is difficult to explain as the aerosol MMD was relatively small (4.6 μm) considering the slow and deep breathing pattern (TIM) used as part of the study. It was therefore of interest to measure the subjects' upper airways through acoustic pharyngometry and to correlate this information with the upper airway and lung depositions from the previous study.

The 9 subjects' lung function data measured during the summer of 2008 were similar to the data during the past lung deposition study during the summer of 2006 (Nikander et al., 2010c). The summary statistics of the acoustic pharyngometer measurements during inhalation - with subjects using nose clips - showed that there was a relatively large inter-subject variability in the CSAs and the AUCs. As published information on acoustic pharyngometry data acquired during inhalation are lacking, the present data were compared to acoustic pharyngometry data acquired during exhalation. The comparison showed that the mean CSA values were similar to those found in previous studies with the Eccovision ARP (Kamal, 2001; Kamal, 2002; Monahan et al., 2005; Busetto et al., 2009) and the volume of the upper airway similar (Ehtezazi et al., 2004) or somewhat larger when compared to upper airway volumes measured with MRI during inhalation in the supine position (Pritchard et al., 2004; Ehtezazi et al., 2005; McRobbie et al., 2005). The correlation analysis between the CSAs and AUCs and the upper airway and lung deposition data showed that the correlations between the CSA3 and the AUC3 and the total oropharyngeal and lung depositions were statistically significant. This meant that the volume between the EG and the GL correlated with the previous lung deposition results, whereas the oral cavity volume, the OPJ CSA, the

volume between the OPJ and the EG, and the EG CSA did not. Thus the anatomy of the lower part of the upper airways seemed to have had an impact on oropharyngeal and lung depositions. This raised questions related to the expansion and contraction of the upper airways during inhalation and whether it would be possible to increase especially the size of the CSAs of the upper airways. Oral appliances have been used to facilitate the movement of the mandible and/or the tongue in order to increase the size of the CSAs and AUCs of the upper airways in subjects diagnosed with OSA (Chapter 2, Table 2.6). The question was whether the upper airways could be expanded with a mouthpiece that advanced the mandible during inhalation. This led to the invention of a new "stepped mouthpiece". The assumption was that as mandibular advancement expanded the size of the upper airways in subjects with OSA both during wakefulness and sleep, the same might be achieved during wakefulness in subjects not diagnosed with OSA.

The second study (Chapter 4) was a proof-of-concept study of the impact of mandibular advancement and incisal opening achieved with the new stepped mouthpiece (without tongue depressor) on the size of the upper airways in 4 healthy subjects. A set of 12 stepped mouthpieces were designed with a round back orifice to be connected to the pharyngometer wavetube and an oval front orifice to be kept between the front teeth as shown in Chapter 4 and Figure 4.2.

During inhalation through the stepped mouthpieces the mean CSAs were in 3 of 4 subjects affected by both the horizontal advancement of the mandible and the incisal opening (10-20 mm). The changes in the CSAs showed a large variability between the 4 subjects. The change in the CSA following mandibular advancement ranged for subject A from ~9% to ~34%, for subject B from ~-5% to ~18%, for subject C from ~56% to ~78% and for subject D from ~34% to ~45%. The impact of the incisal opening on these changes was in subjects A, B and D considerable especially when testing the large mouthpiece whereas this effect was almost the opposite when testing the small mouthpiece. The 10 mm incisal opening created by the small mouthpiece (-3 mm) had a

surprisingly negative effect in subjects A and B. The changes in the mean AUCs during inhalation followed the changes in the mean CSAs.

The analysis of the results of the proof-of-concept study showed that the response to the combination of mandibular advancement and incisal opening was far from linear and quite complex. The occurrence of negative results following mandibular advancement and incisal opening might have been due to the lack of a tongue depressor. This led to the development of a stepped mouthpiece with a tongue depressor which was tested in the next study.

The study presented in Chapter 5 covers the results of an open investigation including 60 subjects - 30 healthy subjects and 30 subjects diagnosed with OSA with equal numbers of male and female subjects – using the new stepped mouthpiece with a tongue depressor (Chapter 5 and Figure 5.1). The new stepped mouthpiece was 81 mm long fully extended and the 18 mm vertical external diameter of the mouthpiece was chosen partly based on the results of the previous proof-of-concept study in which the largest mouthpiece had an external vertical mouthpiece diameter of 20 mm and partly as this is a common vertical size of jet nebuliser and inhaler mouthpieces. Five of the 6 possible advancements of the stepped mouthpiece were used (1, 2, 3, 4, and 5 mm). As in the proof-of-concept study the back end of the stepped mouthpiece was connected to the acoustic pharyngometer wavetube. The primary objective of the study was to measure through acoustic pharyngometry the impact of horizontal mandibular advancement on the size of the upper airways in subjects without and with OSA while in a seated position.

The CSA related results during tidal breathing showed that the impact of the introduction (incisal opening) of the stepped mouthpiece was for CSA1 and CSA3 larger than the effect of the mandibular advancement in both groups of subjects. For CSA2 in the non-OSA group the change in size was larger following mandibular advancement than following the introduction of the

stepped mouthpiece (incisal opening). The change in size of CSA2 in the OSA group was also larger following mandibular advancement although the magnitude was smaller.

The AUC related results during tidal breathing showed that the impact of the vertical diameter of the stepped mouthpiece on AUC1 was larger than the effect of the mandibular advancements for both groups. For AUC2 in the non-OSA group the change in the size of AUC2 was larger following mandibular advancement than following the incisal opening. The change in size of AUC2 in the OSA group followed the same pattern with larger change following mandibular advancement although the magnitude was smaller. The changes in size of AUC3 were small in relation to the volume of the AUC3 and the impact of the vertical diameter of the stepped mouthpiece on the AUC3 was larger than the effect of the mandibular advancements for both groups.

The statistical analysis of the results for the non-OSA group showed that the changes in CSA1-3 and AUC1-3 following the introduction of the stepped mouthpiece were all statistically significant. This was in contrast with the results for the OSA group which only showed statistically significant results for CSA1, CSA3 and AUC1 and not for the important oropharyngeal area (CSA2, AUC2). This might reflect the mixed results of incisal openings in subject with OSA published in the past (Meurice et al., 1996; Vroegop et al., 2012). The mouthpiece position was, however, statistically significant for AUC2 and the significant effect (non-OSA and OSA data combined) corresponded to an increase in volume from 9.76 to 11.29 cm^3, a difference of 1.53 cm^3 or ~16% of the 0 mm value. The increases were ~18% in the non-OSA group and ~13% in the OSA group.

The *in vitro* study in Chapter 6 was designed to investigate the possible artefact found in Chapter 3 which was related to the use of nose clips during the acoustic pharyngometer measurements. Based on the Molfino et al (1990) and Marshall et al (1993) articles, an open velum might be the cause for the increase in the CSAs and AUCs. According to Molfino et al (1990) an open nasopharyngeal velum during acoustic pharyngometry leads to an over-estimation of the lower

upper airway (distal pharynx, GL and trachea) as the acoustic pulses will propagate from the mouth to the nasopharynx and the paranasal sinuses where they are reflected in order to propagate along the rest of the upper airway. Kamal (2004a) described the velum as the port to the nasopharynx and an open velum would pass acoustic impulses from the pharyngometer wavetube further up through the nasopharynx into the sinuses creating a form of acoustic leak. The consequence would be an overestimation of the assumed oropharyngeal CSA (Kamal, 2004a). Thus the volume of the nasopharynx and the sinuses would be of interest as the addition of that volume to the pharyngogram should, based on the assumptions by Kamal (2004a), be equal to the artefact.

The *in vitro* test setup consisted of an acoustic pharyngometer, a cast of a human upper airway (oral cavity to the GL) and an L-shaped tube. Acoustic pharyngometer measurements were performed without and with surrogate "open velums" in the form of holes of different sizes at the back of the cast and the tube at the probable location of the velum. Small and large "nasal cavities" of ~20 ml (small T-piece) and ~30 mL (large T-piece) volumes were attached to the hole. Measurements without any T-piece were also made and these measurements had an infinite size of the nasal cavity.

The pharyngometer measurements with the largest hole size in the cast without T-pieces showed the largest increase in the pharyngograms. The pharyngograms were somewhat separated at the peak CSA of the oral cavity with the pharyngogram presenting the largest hole sizes showing the smallest CSA. The increase in the pharyngograms after the oral cavity occurred at ~11 cm, reached a plateau at ~12 cm, and increased sharply at ~17 cm. The changes in the CSA were initially small with an early increase after the oral cavity from ~4 to ~6 cm^2 (~10 to ~12 cm) and the late increase from ~6 to ~13 cm^2 (~17 to ~20 cm). The increase in the pharyngograms at ~20 cm followed the order of the size of the holes with the pharyngogram based on the smallest hole size showing the least increase and vice versa. The addition of the T-pieces to the posterior pharyngeal wall of the

cast reduced the increases seen without the addition of the T-pieces. However, the total increase in upper airway volume (no hole, total AUC1-3 minus total AUC1-3 for the largest hole size) was with the small T-piece ~33 mL and with the large T-piece ~46 mL and thus somewhat larger than the volumes of the T-piece. The increase in the pharyngograms based on the largest hole size and the volumes of the T-pieces attached indicates that the *in vitro* model was a reasonable tool for evaluation of the open velum effect, but indicates that the open velum effect might be related to factors other than the volume of the nasal airways.

The pharyngometer measurements with the largest hole size in the tube without T-pieces showed as with the cast the largest increase in the pharyngograms. The addition of the T-pieces to the posterior pharyngeal wall of the tube did also reduce the increases seen without the addition of the T-pieces. Thus the changes in the pharyngograms did largely mimic those of the cast. The total increase in tube upper airway volume (difference between "no hole, total AUC1-3" and "total AUC1-3 for the largest hole size") was, however, with the small T-piece ~19 mL and with the large T-piece ~32 mL and thus similar to the volumes of the T-pieces. The differences between the volumes of the cast and the tube were surprising. The differences between the surrogate upper airway models can be found in the different geometries, materials and sizes of the surrogates.

In conclusion, the results of the studies of this Thesis have shown that:

- oropharyngeal and lung depositions of nebulised saline was related to the anatomy of the upper airways.
- the size of the upper airway can be increased with a stepped mouthpiece and that this effect is based on a combination of incisal opening and mandibular advancement.
- an open velum has an impact on the pharyngogram and that the volume of this effect seems to be larger than the size of the nasal cavity.

7.2 Future work

The results of the studies presented in Chapters 4 and 5 indicate that mandibular advancement and incisal opening would be a simple method for the enlargement of the area of the pharynx between the OPJ and the EG during inhalation. This should enhance the passage of aerosol through the lumen of the pharynx to the lungs. A number of questions do, however, remain to be answered before the optimal combination of mandibular advancement and incisal opening is identified. These questions are related to:

1. The impact of the incisal opening on the size of the pharynx in subjects with respiratory disorders treated with inhaled drugs.
2. The impact of the mandibular advancement on the size of the pharynx in subjects with respiratory disorders treated with inhaled drugs.
3. The impact of different combinations of incisal openings and mandibular advancements on the size of the pharynx in subjects with respiratory disorders treated with inhaled drugs.
4. The identification of the optimal individual combination of an incisal opening and mandibular advancement.
5. The impact of incisal openings and mandibular advancements on upper airway and lung deposition of nebulised saline.
6. How long could a subject comfortably inhale nebulised drug with a stepped mouthpiece with an optimal individual combination of an incisal opening and a mandibular advancement.
7. The impact of high peak inspiratory flows required for inhalation through DPIs on the size of pharynx during mandibular advancement with a stepped mouthpiece.

The results of the study presented in Chapter 3 indicated that the use of nose clips during recordings with an acoustic pharyngometer might lead to an open velum effect which would create an artefact in terms of too large CSAs. The results of the study presented in Chapter 5 showed some large CSA values that might have been a consequence of the movement of the subject's head or an open velum. Finally, the study presented in Chapter 6 showed how a well-defined open velum could affect the pharyngogram, but the results did not match published pharyngograms recorded with an open velum (Molfino et al., 1990; Marshall et al., 1993). A number of questions therefore remain to be answered regarding the possible open velum effect and the impact of the movement of the head during acoustic pharyngometry measurements.

These questions are related to:

1. The identification of a possible leakage through the velum during oral breathing *versus* nasal breathing with different combinations of mandibular advancement and incisal opening.
2. The quantification of the leakage through the open velum during oral breathing *versus* nasal breathing with different combinations of mandibular advancement and incisal opening.
3. The impact of a verified open velum on the pharyngogram with different combinations of mandibular advancements and incisal openings.
4. The collection of acoustic pharyngometry reference values of the size of the upper airways - including the CSAs of the OPJ, the EG and the GL and the related AUCs – during inspiration and expiration in healthy subjects of both genders and in different age groups.
5. Development of an algorithm for the acoustic pharyngometer equipment for identification of deviating pharyngograms following an open velum. This could be based on the GOF algorithm used in Chapters 3 and 5.

The stepped mouthpieces used in the studies presented in Chapters 4 and 5 could be further developed based on the results from the above outlined studies. The size and shape of the stepped mouthpiece including the size and shape of the tongue depressor could be further developed in order to fit the multitude of different teeth settings. This would include an increase in the mandibular advancement settings and most probably a range of vertical dimensions.

The variable results in acoustic pharyngometry recordings in some subjects following mandibular advancement and incisal opening (Chapter 5) indicate that the SOPs presented by Kamal (2004c) might require an update. If similar trends are observed during evaluations of oral applainces in subjects diagnosed with OSA, additional tools for standardizing the recordings would probably be required in the acoustic pharyngometers. These tools could include:

1. Measurements of the head position and upper body position.
2. Measurements of tongue movements.
3. Measurements of swallowing.
4. Timing the acoustic pharyngometer recording to either inhalation or exhalation as presented in Chapter 3.

Appendices

Appendix A 1

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

Appendix A 2

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

Appendix B 1

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

Appendix C 1

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

Appendix C 2

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

Appendix C 3

Presented as a softcopy (Microsoft Word 2013 format) on a DVD attached to the side back cover of the thesis.

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Canolfan Gwasanaethau Busnes
Business Services Centre

South East Wales Research Ethics Committee - Panel D

Telephone: 02920 376823
Facsimile: 02920 376835
Email: Carl.phillips@bsc.wales.nhs.uk

Dr Peter Dewland
Medical Director
Simbec Research Limited
Merthyr Tydfil Industrial Estate
Merthyr Tydfil
CF48 4DR

20 December 2007

Dear Dr Dewland

Full title of study: **Assessment of the upper airways in healthy subjects using acoustic pharyngometry**

REC reference number: **07/WSE04/131**

Thank you for your letter of 17 December 2007, responding to the Committee's request for further information on the above research, and for submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised].

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document.

You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	5.5	15 November 2007
Investigator CV	Dr P Dewland	25 September 2007
Protocol	Final	14 November 2007
Compensation Arrangements	Marsh Certificate of Insurance	19 July 2007
Letter of invitation to participant	1	15 November 2007
GP/Consultant Information Sheets	1	15 November 2007
Participant Information Sheet	2	17 December 2007
Participant Consent Form: Volunteer	2	17 December 2007
Response to Request for Further Information	C Edwards	17 December 2007
Insurance	Miller	07 August 2007
Volunteer Participation Card	1	15 November 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

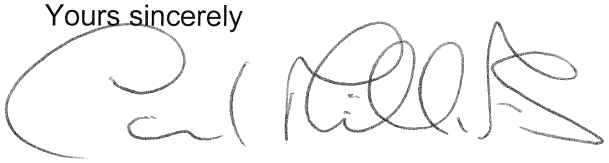
e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

07/WSE04/131	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely



Carl Phillips
Executive Officer
South East Wales Research Ethics Committees

Enclosures: Standard approval conditions SL-AC1

Site approval form

Copy to: Mr Steven Coughlin, Respironics International, Chichester Business Park, Tangmere, Chichester

South East Wales Research Ethics Committee - Panel D

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	07/WSE04/131	**Issue number:**	0	**Date of issue:**	20 December 2007
Chief Investigator:	Dr Peter Dewland				
Full title of study:	Assessment of the upper airways in healthy subjects using acoustic pharyngometry				

This study was given a favourable ethical opinion by South East Wales Research Ethics Committee - Panel D on 20 December 2007. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* (1)
Dr Peter Dewland	Medical Director	Simbec Research Ltd	South East Wales REC - Panel D	20/12/2007	

Approved by the Chair on behalf of the REC:

..... (Signature of Chair/Co-ordinator)

(delete as applicable)

..... (Name)

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

Appendix A-2

Chapter 3, Individual pharyngograms

The individual mean pharyngograms have been plotted for each of the 9 subjects (Figures 3.1 to 3.9). Each plot covers 7 mean pharyngograms with legend "Measurement": A, B, C, D20, D30, E20, E30 for the pharyngometry measurements without a nose clip, and 7 mean pharyngograms with legend "Measurement": ANC, BNC, CNC, D20NC, D30NC, E20NC, E30NC for the pharyngometry measurements with a nose clip. In the plots the y-axis presents the cross-sectional area (CSA; in cm^2) of the upper airway and the x-axis presents the length of the upper airway from the end of the pharyngometer wavetube (0) to the glottis (in cm). The position of the glottis is obviously different for different subjects and therefore not fixed at a certain point, for example 20 cm.

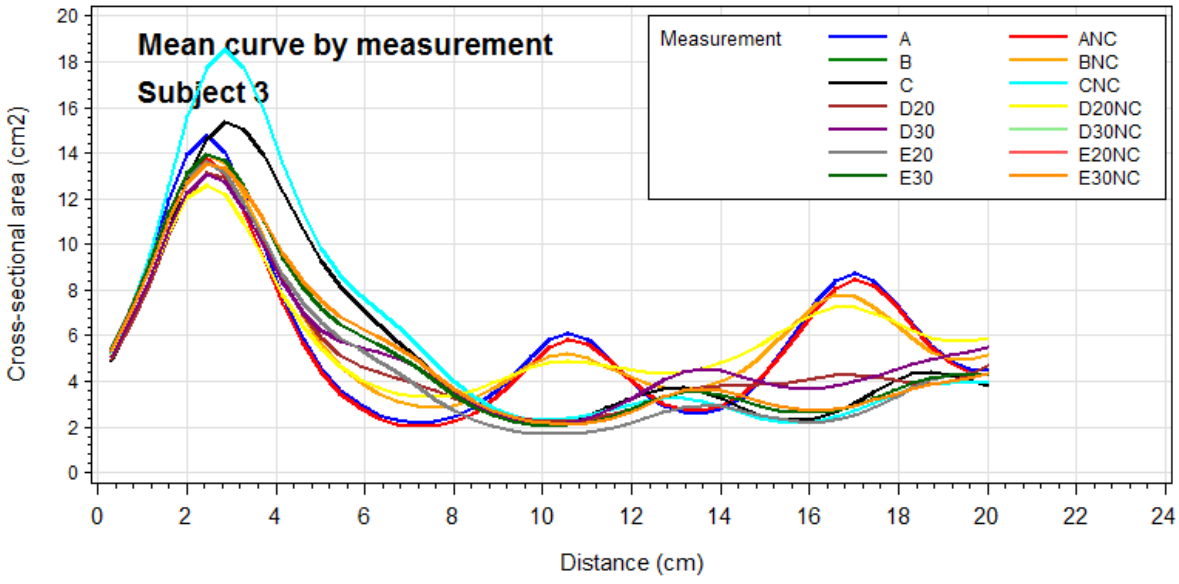


Figure 3.1: Subject 3, mean pharyngograms by measurement (measurements A to E without and with nose clip).

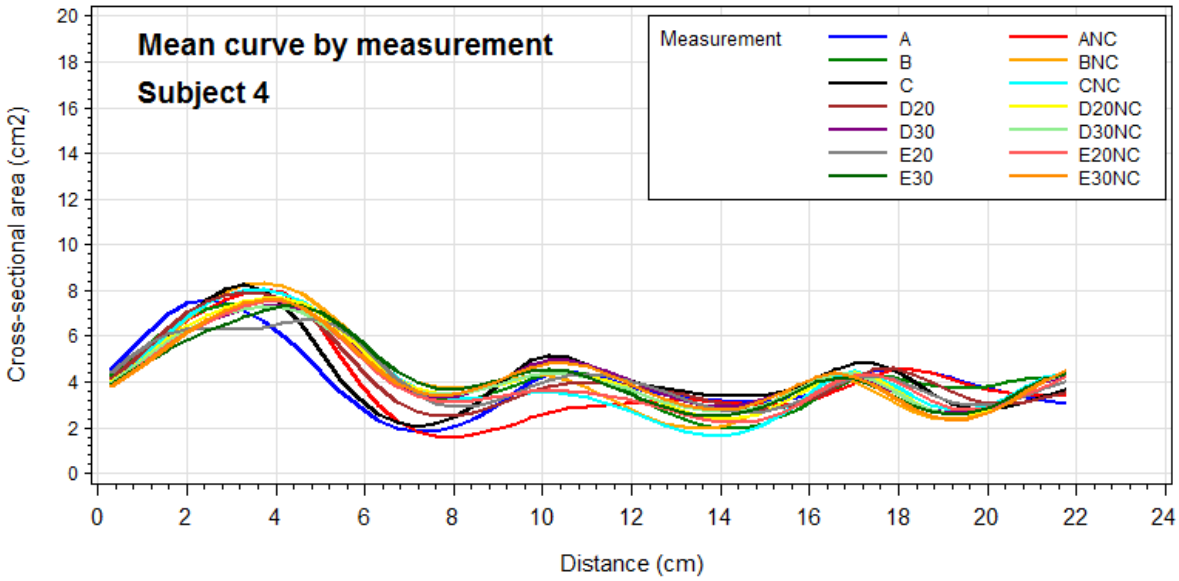


Figure 3.2: Subject 4, mean pharyngograms by measurement (measurements A to E without and with nose clip).

.

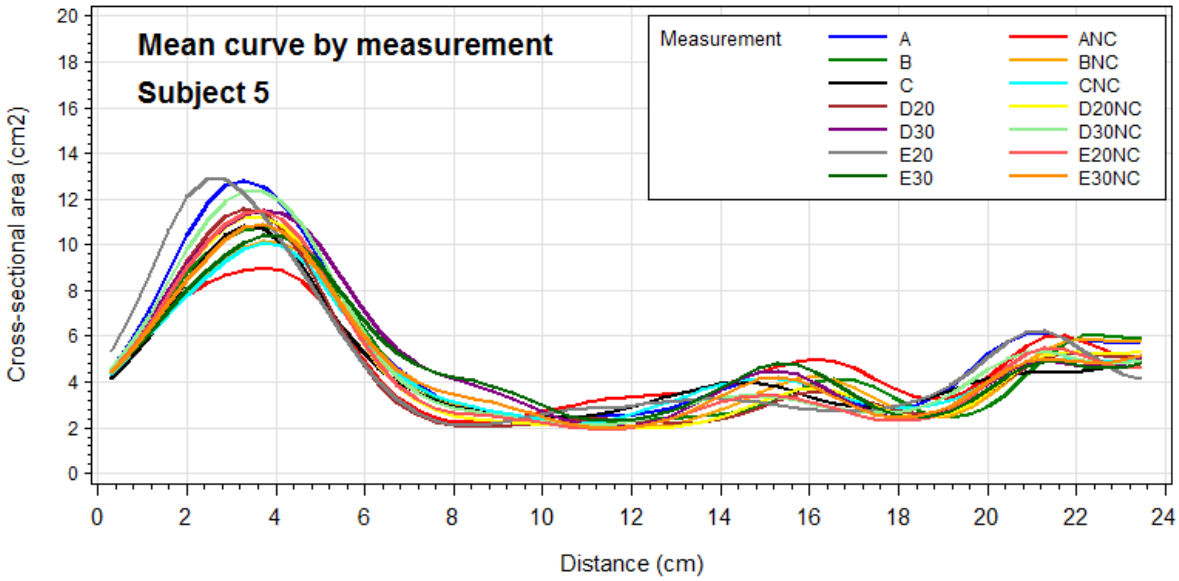


Figure 3.3: Subject 5, mean pharyngograms by measurement (measurements A to E without and with nose clip).

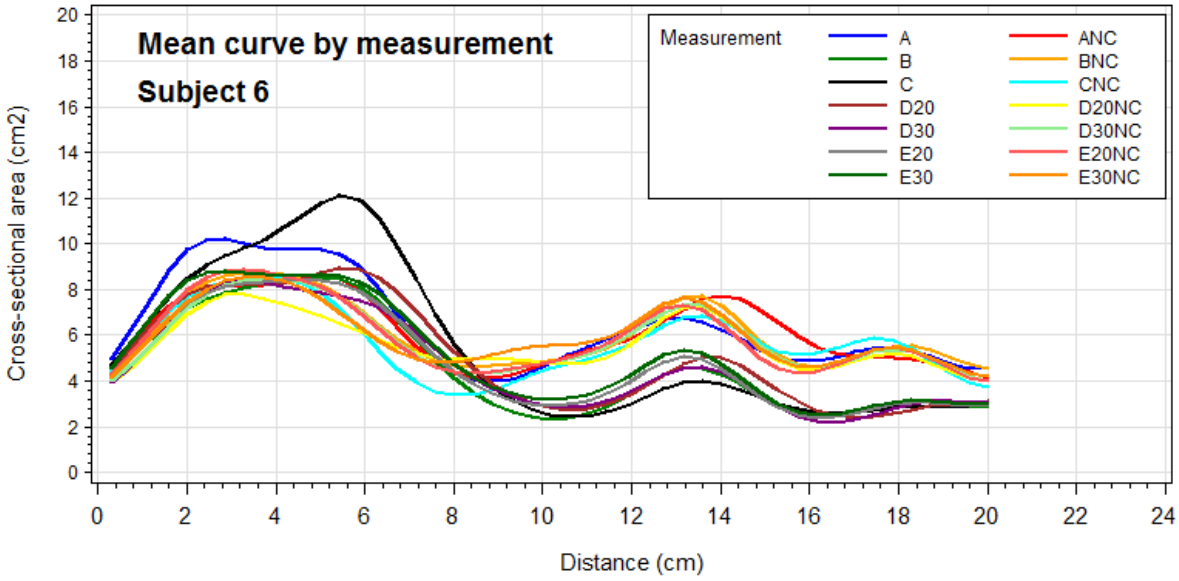


Figure 3.4: Subject 6, mean pharyngograms by measurement (measurements A to E without and with nose clip).

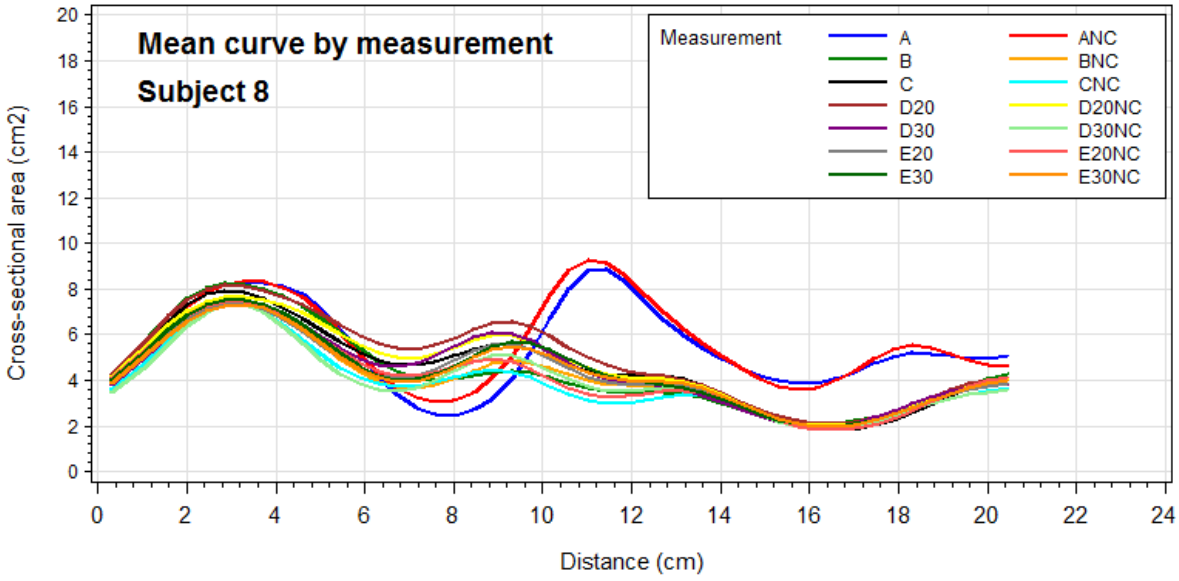


Figure 3.5: Subject 8, mean pharyngograms by measurement (measurements A to E without and with nose clip).

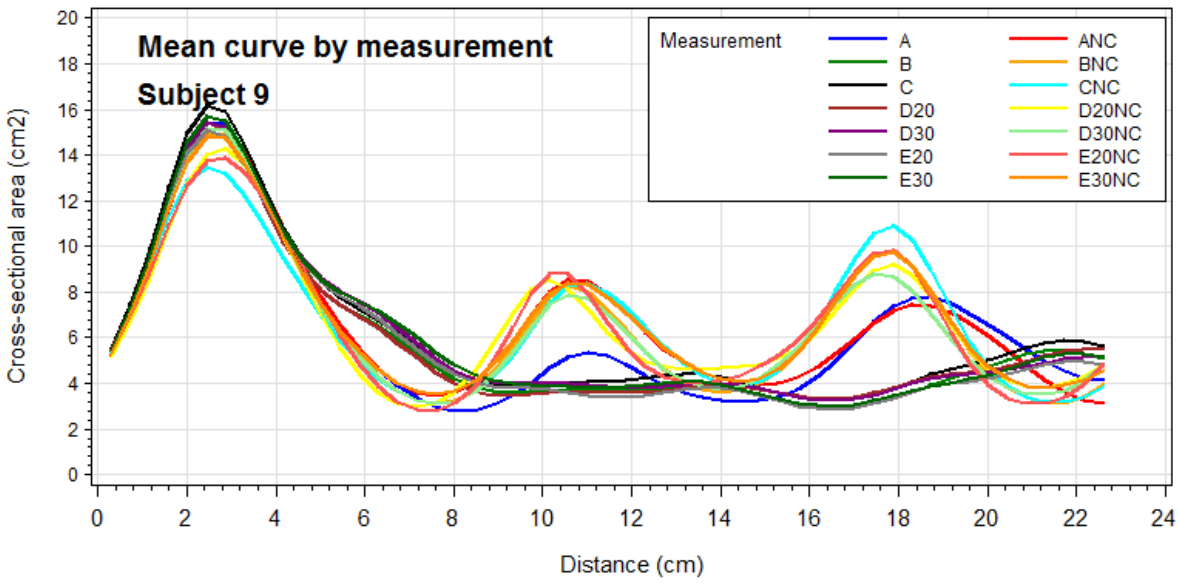


Figure 3.6: Subject 9, mean pharyngograms by measurement (measurements A to E without and with nose clip).

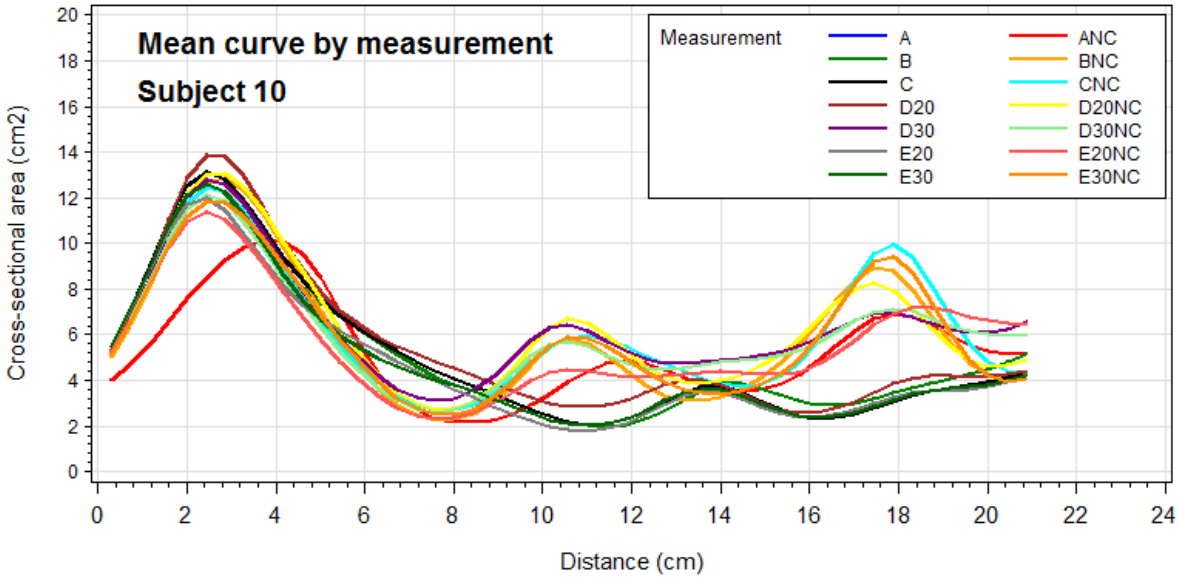


Figure 3.7: Subject 10, mean pharyngograms by measurement (measurements A to E without and with nose clip).

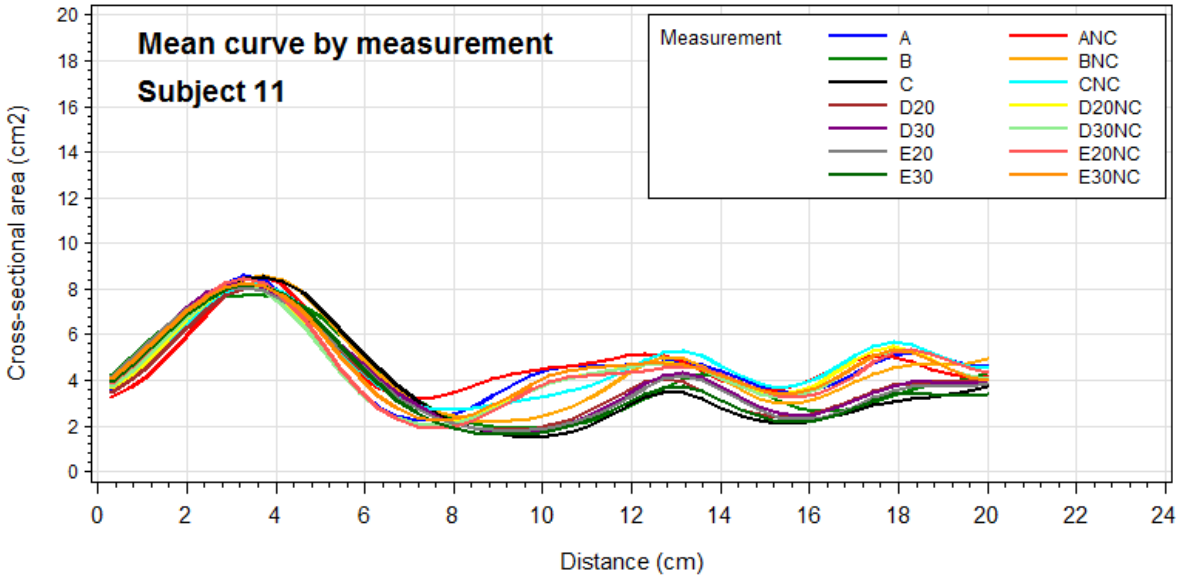


Figure 3.8: Subject 11, mean pharyngograms by measurement (measurements A to E without and with nose clip).

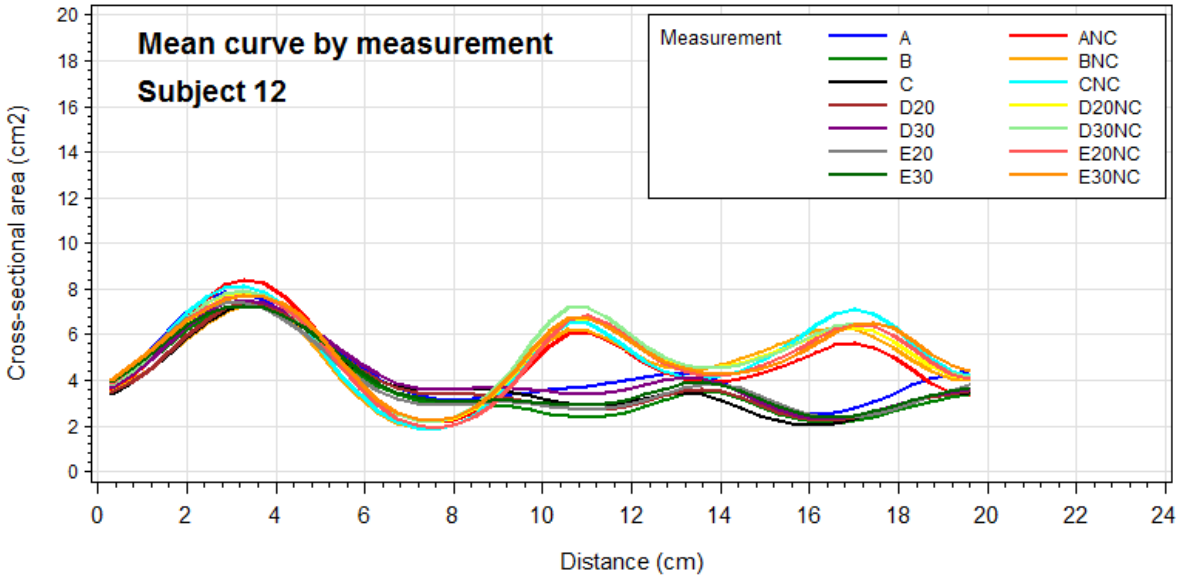


Figure 3.9: Subject 12, mean pharyngograms by measurement (measurements A to E without and with nose clip).

APPENDIX B-1

Chapter 4, Individual pharyngograms

The individual mean pharyngograms have been plotted for each of the 4 subjects (Figures 4.1 to 4.8). The plots in each figure cover 12 pharyngograms with separate legends for small (S = 10 mm orifice; -3 mm S, 0 mm S, 3 mm S, 6 mm S), medium (M = 15 mm orifice; -3 mm M, 0 mm M, 3 mm M, 6 mm M) and large (L = 20 mm orifice; -3 mm L, 0 mm L, 3 mm L, 6 mm L) stepped mouthpieces. In the plots the y-axis presents the cross-sectional area (CSA; in cm^2) of the upper airway and the x-axis presents the length of the upper airway from the end of the pharyngometer wavetube (0) to the glottis (in cm).

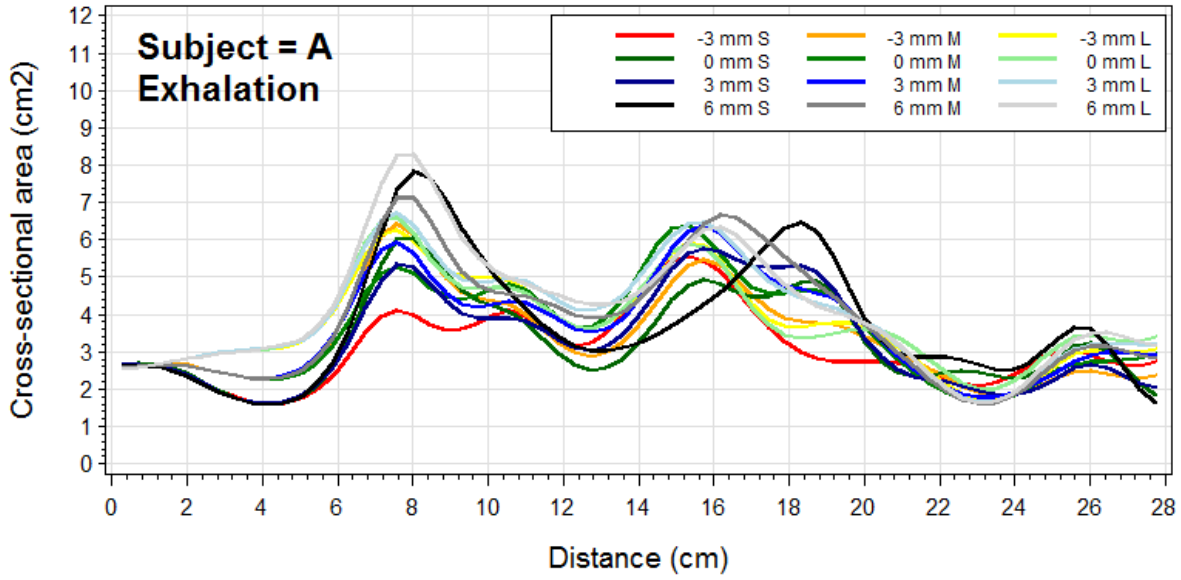


Figure 4.1: The pharyngograms measured during exhalation have been plotted for subject A for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

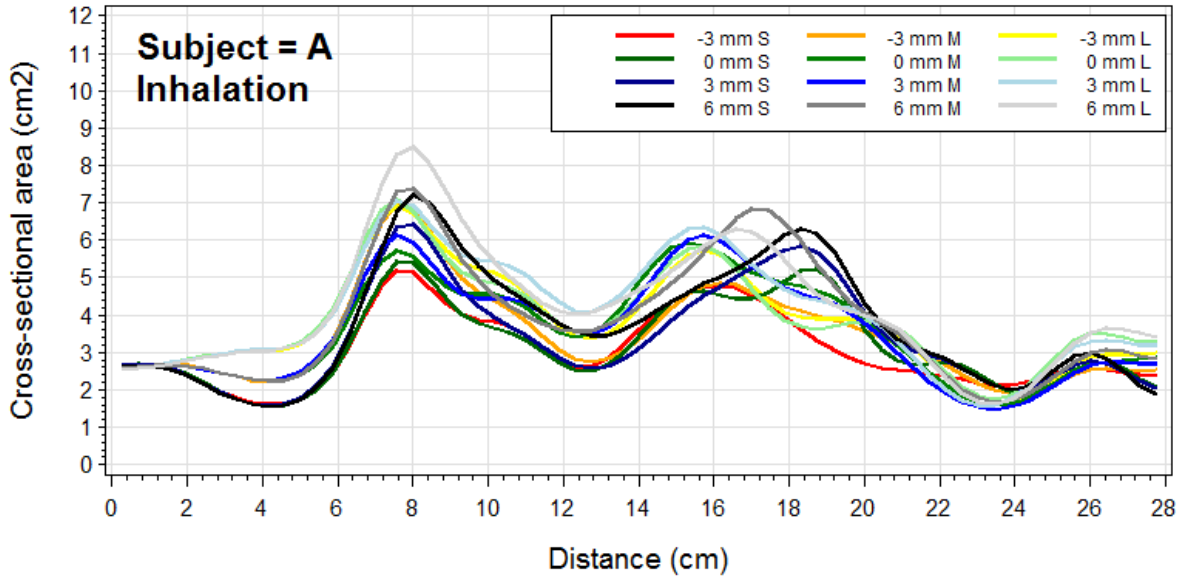


Figure 4.2: The pharyngograms measured during inhalation have been plotted for subject A for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

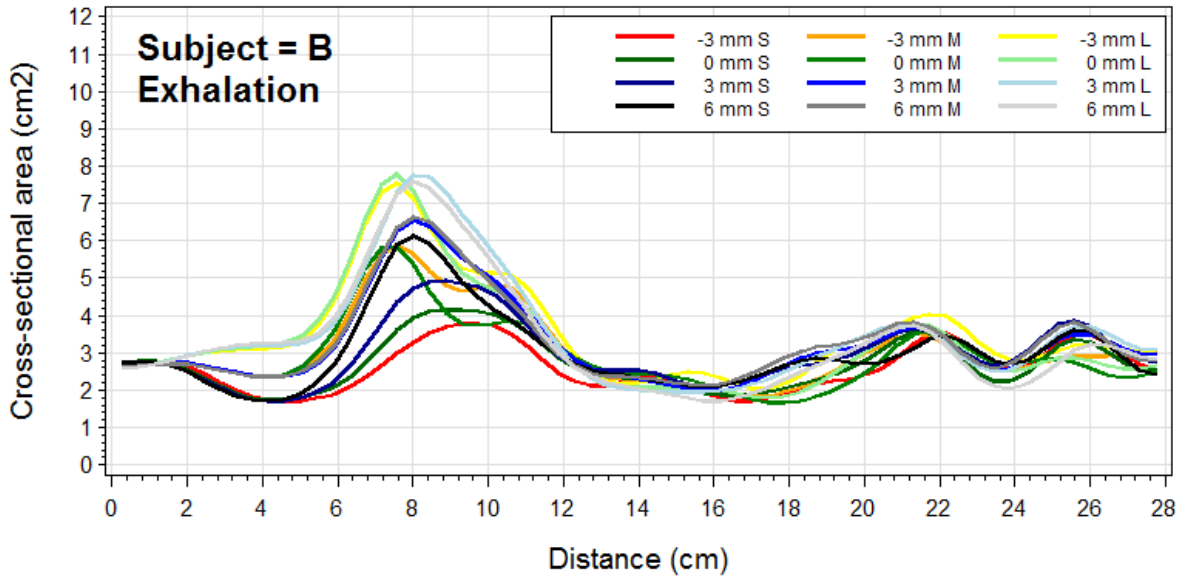


Figure 4.3: The pharyngograms measured during exhalation have been plotted for subject B for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

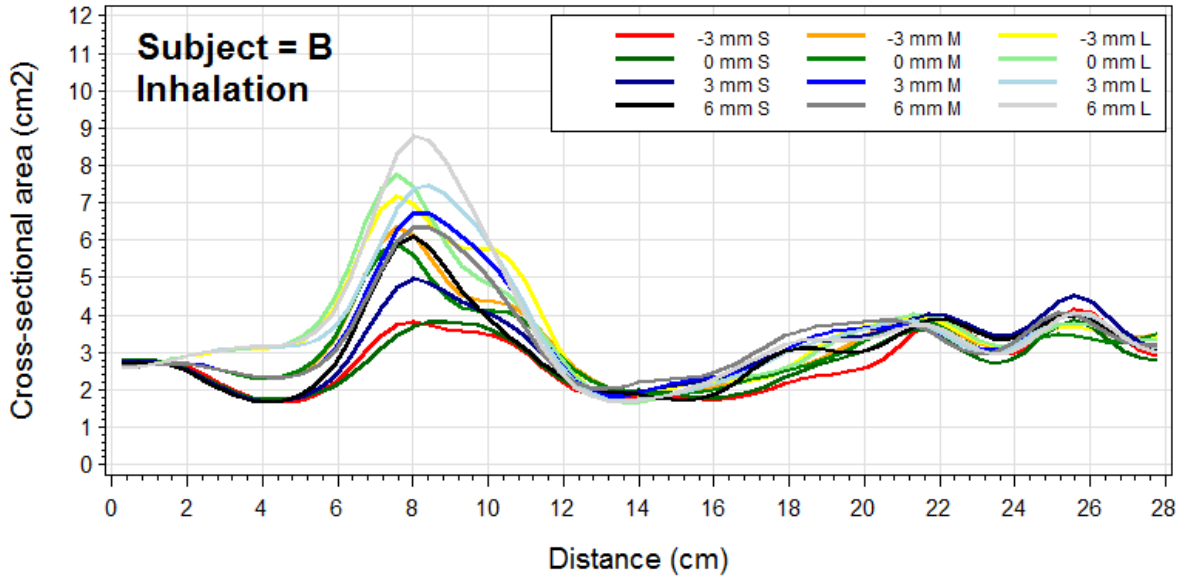


Figure 4.4: The pharyngograms measured during inhalation have been plotted for subject B for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

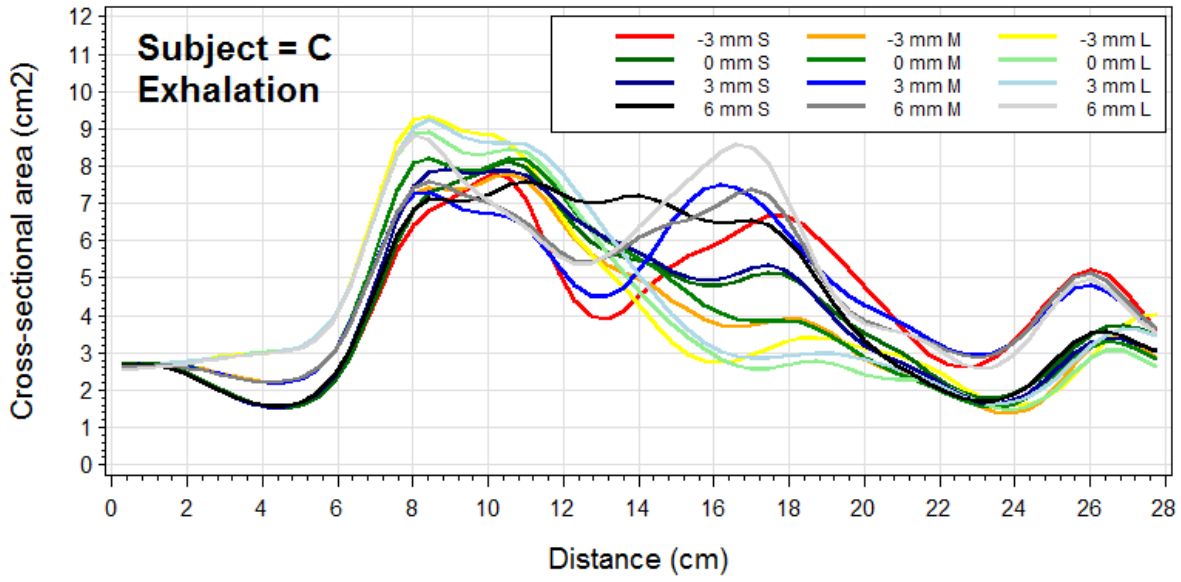


Figure 4.5: The pharyngograms measured during exhalation have been plotted for subject C for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

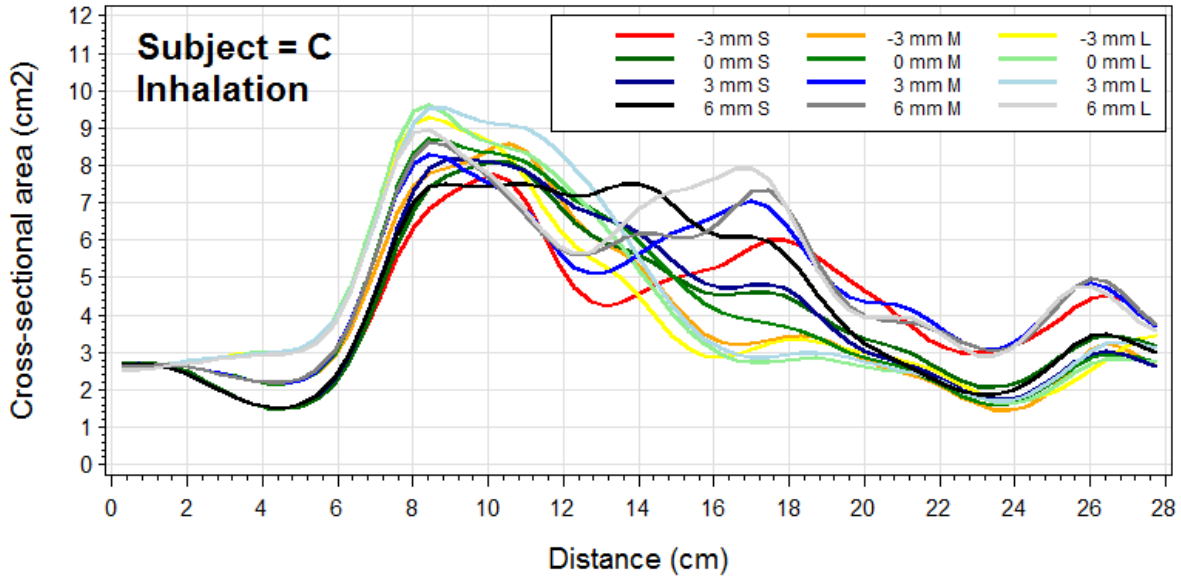


Figure 4.6: The pharyngograms measured during inhalation have been plotted for subject C for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

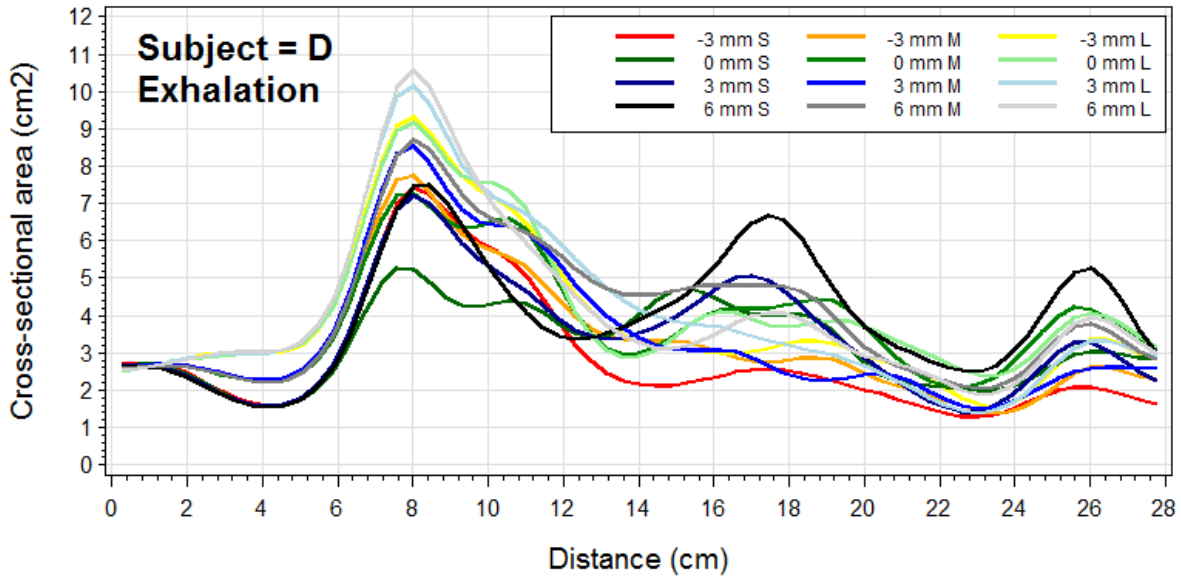


Figure 4.7: The pharyngograms measured during exhalation have been plotted for subject D for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

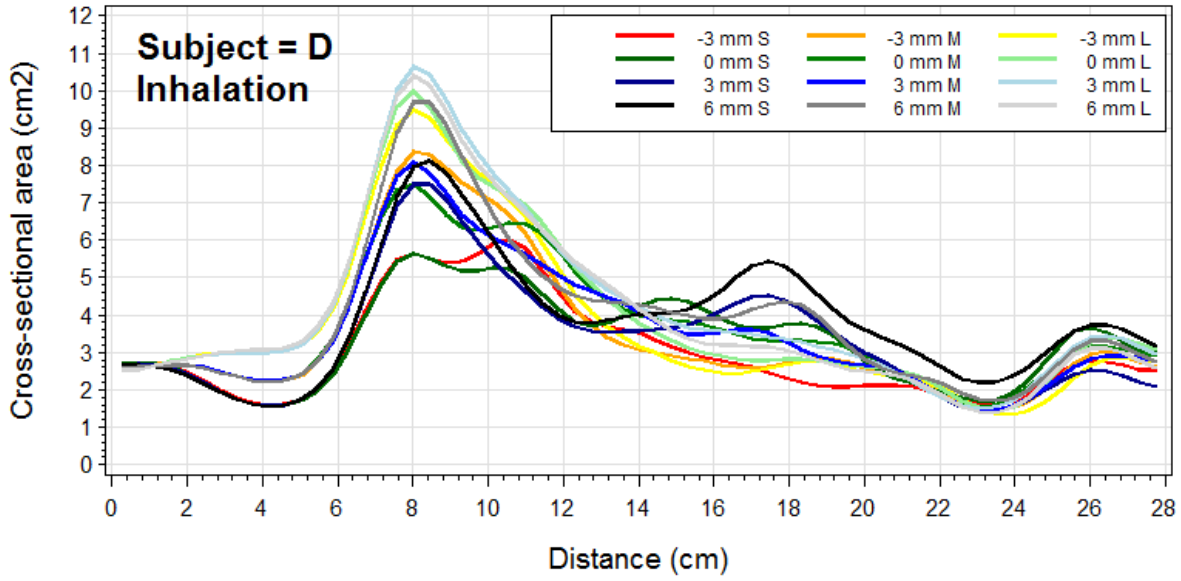


Figure 4.8: The pharyngograms measured during inhalation have been plotted for subject D for each of the stepped mouthpieces. Each pharyngogram represents the means of two recordings.

December 11, 2009

Dr. John S. Viviano
Dr. John Viviano and Associates
4099 Erin Mills Parkway, Suite 1
Mississauga, ON L5L 3P9

Re: Philips Respironics Protocol No: RDD-2009-001
INVESTIGATOR NOTICE OF IRB APPROVAL PACKAGE

You have been approved as the Investigator at your site for the above study. Enclosed is your IRB Approval Package, as described below:

- IRB Approval Form/REB Attestation Form: Expedited Review
- IRB Approved Patient Information and Consent Form dated December 4, 2009
- IRB Investigator Progress Report
- Change of Site Information Form

Study Visit Compensation/Reimbursement: The IRB has set an amount of $200 as maximum compensation to study subjects as reimbursement for study related expenses for each visit (such as travel, parking and babysitting) unless there are unusual case-by-case circumstances. If you wish to exceed this amount, please contact me.

Reporting Requirements:
Please note that you are required to file Progress Reports, Amendments, new Safety Information, e.g., Unexpected Serious Adverse Event Reports, changes increasing the risk to subject and/or affecting significantly the conduct of the trial, etc., on a timely basis. The Sponsor or CRO may file these reports on your behalf, but, under ICH GCP Guideline E6, the investigator is responsible for such reporting. However, receipt of these documents by the IRB will not routinely be acknowledged – you are to maintain evidence that you complied with this GCP requirement.

Please note that annual ethics approval is required therefore you must request an extension of IRB Approval prior to the expiry date of December 8, 2010.

Should you have any questions or concerns regarding the above, please do not hesitate to contact me at 905-727-7989 ext. 248 or via email at svillani@irbservices.com.

Sincerely,
Institutional Review Board Services

Sharon Villani
Team Leader
Protocol Review
(Phase I & Special Projects Team)

Enclosures (4)

IRB APPROVAL / REB ATTESTATION FORM: EXPEDITED REVIEW

STUDY APPROVAL DATE: DECEMBER 9, 2009

THE APPROVAL IS VALID FOR ONE YEAR AND EXPIRES ON DECEMBER 8, 2010.

ORIGINAL APPLICANT: Dr. John S. Viviano, Dr. John Viviano and Associates

INITIAL REVIEW:

The following protocol; Revised Patient Invitation Brochure (undated) and informed consent document dated 2009-DEC-04, qualify for and were reviewed by Dr. Allan Knight, Chairman of the ON Institutional Review Board Services under the Expedited Review provisions of the Institutional Review Board Services procedures on December 9, 2009.

Final Protocol Number and Date: RDD-2009-001 dated September 29, 2009

Final Protocol Title: Assessment of the impact of a stepped mouthpiece on the upper airways measured though acoustic pharyngometry

Sponsored by:

NAME: Philips Respironics
Respironics New Jersey, Inc.

ADDRESS: 5 Wood Holow Road
Parsippany, NJ 07054

UNCONDITIONAL APPROVAL: The research project, including any revisions, modifications or corrections arising from the initial review (if any), are hereby unconditionally approved as described in this section.

Final Protocol Number and Date: RDD-2009-001 dated September 29, 2009

Final Protocol Title: Assessment of the impact of a stepped mouthpiece on the upper airways measured though acoustic pharyngometry

Informed Consent Version: 2009-DEC-04

IRB SERVICES DEC 11 2009 APPROVED

Qualified Investigator Name/Site Address: Dr. John S. Viviano, 4099 Erin Mills Parkway, Suite 1, Mississauga, ON

Other Investigator(s) at the site: None

COMPLIANCE STATEMENT / ATTESTATION: The membership of this IRB complies with the requirements defined in Health Canada regulations, 21 CFR parts 56 and 312.3 and 45 CFR 46. The IRB carries out its functions in accordance with good clinical practices (e.g., ICH GCP Guidelines) and Health Canada regulations and in compliance with FDA 21 CFR parts 50 and 56, *for US federally funded research* DHHS 45 CFR part 46, *for Canadian federally funded research* - and the Tri-Council Policy Statement for Ethical Conduct of Research Involving Humans.

Allan Knight, MD FRCP(C) FACP
Chairperson, ON Institutional Review Board

div. 1373737 Ont. Ltd. *address* 372 Hollandview Trail, Suite 300, Aurora, Ontario, Canada L4G 0A5
tel. (905) 727-7989 *fax* (905) 727-7990 *e-mail* info@irbservices.com *web* www.irbservices.com

Subject Information and Consent Form

Study Title: Assessment of the impact of a stepped mouthpiece on the upper airways measured through acoustic pharyngometry

Protocol Number: RDD-2009-001

Study Sponsor: Philips Respironics, 5 Wood Hollow Road, Parsippany, NJ 07054, USA

Study Doctor: John Viviano

Address: 4099 Erin Mills Parkway, Mississauga, ON, L5L 3P9, Canada

Telephone: 905 820 3200

You are invited to consider taking part in a medical device research study involving persons with or without sleep apnea. This information and consent form describes the study. It may contain words that you do not understand. If you have any questions, or do not understand anything in this form, please ask the study doctor to explain. Please take the time to read this form thoroughly, and discuss it with your family, friends and/or regular doctor.

Your participation in this study is entirely voluntary. If you decide to take part in the study, you will be asked to sign at the end of this form. Even if you decide to participate in the study, you may withdraw at any time.

Study Overview:

The purpose of this study is to measure in subjects with or without sleep apnea their upper airways to try to understand whether the design of a new stepped mouthpiece for nebulizers is functional or not. The measurement will be performed with an acoustic pharyngometer, and the measurements are completely non-invasive. The stepped mouthpiece is thought to expand the upper airways by moving the lower jaw slightly forward. The stepped mouthpiece is investigational, which means it has not been approved by Health Canada for use outside of research studies like this one. This study is expected to involve 60 subjects in Canada.

This study will not involve any treatment with an investigational drug.

Study Procedures:

This study consists of one (1) visit to Dr Viviano's office.

If you enter the study you will be required to take a screening questionnaire which will take approximately 15 minutes. If you qualify and agree to participate in this study, your age, gender, height, weight, collar size and possible diagnosis of sleep apnea will be recorded. Following that, Dr Viviano will use a device called Eccovision – an acoustic pharyngometer - in a non-invasive way to measure the size, structure and behaviour of the upper airways, whilst you breathe through the mouthpiece (Figure 1).

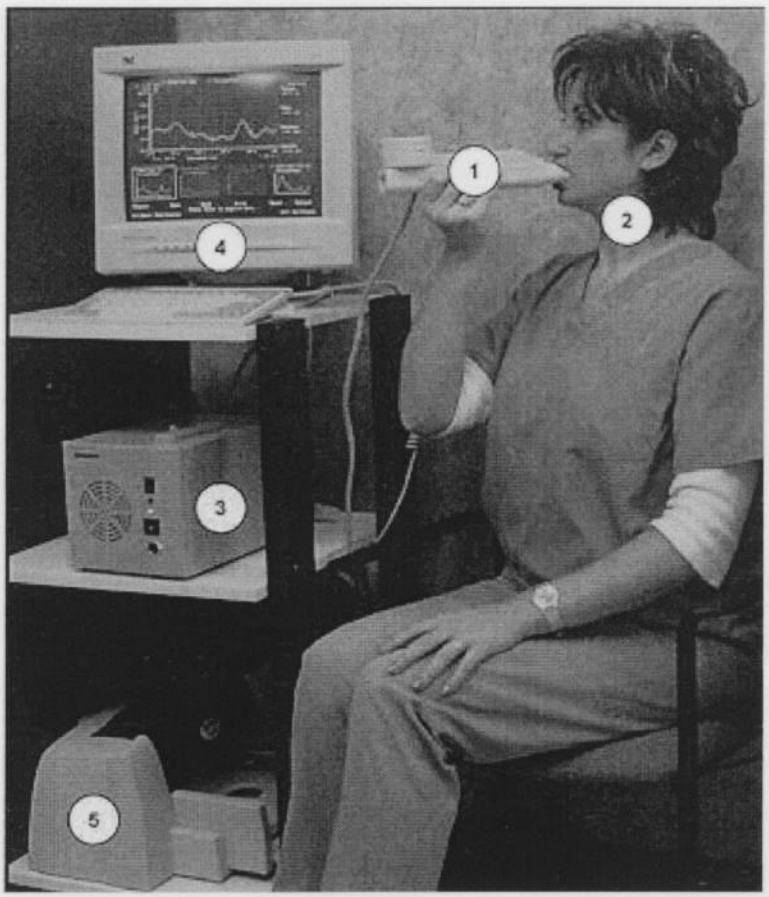


Figure 1. Sound is generated in the wave tube (1), reflected in the upper airways (2), and recorded by two microphones in the wave tube. The unit (3) processes the acquired information and displays a cross-sectional area of the upper airway on the monitor (4). A report is printed by the printer (5) for hard copy archiving.

The stepped mouthpiece looks as follows, and will during the study be connected to one end of the acoustic pharyngometry wave tube (#1 in Figure 1):

Figure 2. The stepped mouthpiece is ~75 mm long including the tongue depressor, ~34 mm wide, and ~24 mm high.

You will be asked to hold the first part of the mouthpiece in the mouth. This part is ~33 mm long, ~34 mm wide and ~18 mm high. The stepped mouthpiece has been designed with 6 steps of 1 mm pitch each with a total horizontal movement of 6 mm. You will also be asked to keep your teeth around the end of the stepped mouthpiece with the tongue depressor against the tongue.

For the acoustic pharyngometry measurements you will be asked to do the following:

1. Sit in a chair and hold a wand with the stepped mouthpiece on it.
2. Place the mouthpiece in your mouth and do various breathing maneuvers on the mouthpiece while in varying jaw positions as instructed by the Dr Viviano.
3. Breath through your mouth normally for 10 to 12 seconds.
4. Breath through your nose normally for 10 to 12 seconds.

A member of staff will instruct you on how to perform the test and coach and encourage you to do your best. The whole procedure should take approximately 1 hour. The stepped mouthpiece will not be available for use after the study. After this visit your participation in the study will be over.

If you decide to leave the study before all measurements have been made you will not be asked to participate again.

Subject Responsibilities:

During the study, you will need to do the following:

- Follow the instructions of the study doctor and the study staff.
- Tell the study doctor about any side effects that you experience.

Risks and Side Effects:

The stepped mouthpiece is an investigational mouthpiece and is not in clinical use right now. It is made of transparent plastic which is approved for medical devices. It should be used as any other nebulizer mouthpiece for inhalation of aerosols. If any new information about this study becomes available that may affect your decision to continue your participation, you will be informed as soon as possible.

Possible Benefits:

No direct benefit is guaranteed to you from taking part in this study. You may benefit from the screening of you upper airways that is part of the study. The information gained from this study may help the development of better ways to treat patients with respiratory disorders.

Voluntary Participation:

Your participation in this study is completely voluntary. You have the right to withdraw from the study at any time, without giving a reason, even if you have signed this consent form. If you decide not to take part, or to withdraw, it will not harm your relationship with the study doctor. If you do not take part or withdraw from the study, you will not be penalized nor lose any benefits to which you are entitled.

The study doctor may also withdraw you from the study if your condition worsens, if you do not follow the doctor's instructions, if the doctor feels it is in your best interests to be withdrawn, if the study sponsor discontinues the study, or for administrative reasons. You can be withdrawn without your consent, but the study doctor will tell you why.

Confidentiality:

Your identity will be kept confidential at all times, except where disclosure is required by law. As part of this research, the study doctor will collect the results of your study-related tests and procedures. Information from this study will be submitted to the sponsor, and to Health Canada and possibly to governmental agencies in other countries (e.g. US Food and Drug Administration) where the study device may be considered for approval. Information sent from the study site will contain a code number, but will not contain your name. The results of this research study may be presented at meetings or in publications but your identity will not be disclosed.

Your study records, which include your name, may be inspected at the study site by representatives of the Sponsor, the research ethics board – IRB Services (an independent committee that reviewed the ethical aspects of this study to help protect the rights and welfare of study participants), government regulatory authorities (e.g. Health Canada, the US Food and Drug Administration - FDA) and other foreign regulatory agencies. This inspection is to check the accuracy of study records.

Your study records including confidential information about you collected during the study will be kept at a secure location for 25 years after study completion, as required by Canadian clinical trial regulations.

You have the right to check your study records and request changes if the information is not correct.

While every effort will be made to protect the privacy of your information, absolute confidentiality cannot be guaranteed. This does not limit the duty of the researchers and others to protect your privacy.

By signing this information and consent form, you consent to the collection, access, use and disclosure of your information as described above.

Study Costs/Reimbursement:

There is no cost to you, your private medical insurance (if any), or the public health insurance plan, for study procedures. This clinic provides reimbursement for expenses, such as travel, parking or babysitting. You will be reimbursed $200 for the study visit.

The sponsor is paying your study doctor for the time, effort and expenses to conduct this study.

Compensation for Injury:

In case of an injury or illness suffered by participating in this study, you will receive appropriate medical care. The sponsor will cover necessary medical costs not covered by the provincial health plan or your private medical insurance (if any). By signing this form, you are not giving up your legal rights, nor releasing the study doctor or sponsors from their legal and professional obligations.

If You Have Questions:

If you have questions regarding this study, you should contact Dr. John Viviano at 905 820 3200.

If you have any questions about your rights as a research subject, please contact the committee that reviewed the ethical aspects of this study at: The Director, Human Research Protection Program, IRB Services, 372 Hollandview Trail, Suite 300, Aurora, ON L4G 0A5. You may also call IRB Services, at 1-866-449-8591, or contact IRB Services by email at subjectinquiries@irbservices.com.

Consent and Signature:

I have been given enough time to read this form and to ask questions. All of my questions, if any, have been answered to my satisfaction. I freely volunteer to take part in this study. By signing this form, I am not giving up my legal rights or releasing the study doctor or sponsors from their legal and professional obligations. I will be given a signed copy of this form to take home with me.

I have not participated in another research study or taken an investigational medication within the last 30 days.

The study doctor has my permission to tell my regular doctor about my being in this study:
❒ YES ❒ NO

_____	_____	_____
Patient Name (printed)	Date	Signature

_____	_____	_____
Name of Person Administering Consent	Date	Signature

STATEMENT OF INVESTIGATOR:

(Investigator preferably to sign the consent form on the same date as the subject, but prior to subject visit to the clinic).

I acknowledge my responsibility for the care and well being of the above subject, to respect the rights and wishes of the subject, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

_____	_____	_____
Investigator Name	Date	Signature

INVESTIGATOR PROGRESS REPORT

IRB INSTITUTIONAL REVIEW BOARD SERVICES

Under ICH GCP, it is the investigator's responsibility to conduct the study with a valid ethics approval and to file progress reports on time. FAILURE TO DO SO IS A SERIOUS GCP VIOLATION THAT MAY LEAD TO IMMEDIATE SUSPENSION OF THE RESEARCH AT YOUR CENTRE AND REGULATORY INSPECTION.

Please complete all fields below and attach the first page of the consent form in use at your site.

INVESTIGATOR NAME & CENTRE:	Dr. John S. Viviano, 4099 Erin Mills Parkway, Suite 1, Mississauga, ON
Sponsor: Philips Respironics	
Initial Approved Final Protocol No.: RDD-2009-001 dated September 29, 2009	
Initial Approved Final Protocol Title: Assessment of the impact of a stepped mouthpiece on the upper airways measured though acoustic pharyngometry	
Initial Approved ICF Ver.: 2009-DEC-04	
Initial IRB Approval Date: 2009-DEC-09 **Current Approval Expiry:** 2010-DEC-08	
TYPE OF REPORT	❑ **End of Study / Site Closure** ❑ **Progress Report, Extension to IRB Approval Required**
Reason Extension Required:	

Site Initiated:	YYYY	MM	DD	Last Subject Seen:	YYYY	MM	DD	Site Closed Out:	YYYY	MM	DD

DESCRIPTION OF SUBJECTS		**WITHDRAWAL REASONS**			
# of Subjects Initiated/Randomised:		# of Withdrawals:			
# of Males:		# Medically-related:			
# of Females:		Description:			
# Minors:		# Reason not given by subject:		# Moved:	
# Age of majority – 65:		# Other:			
# Age > 65:		Explanation:			

EXPERIENCES

Observed Benefits (if any):

Research results obtained thus far (if known):

Current Risk/Benefit Assessment based on results to date:	❑ Acceptable	❑ Not Acceptable	❑ Unknown, presumed acceptable
# of major protocol deviations since last Progress Report		If any **unreported** major deviations, attach relevant documentation.	

ANY NEW INFORMATION since last Progress Report and not previously reported? *(Refer also to General Guidance: Investigator Reporting Requirements)*

❑ No
❑ Yes. If there is information **not** previously reported, check boxes as applicable. Please attach relevant supporting documentation.

Amendments:			❑ New ICF Version:	❑ Safety Information ❑ Unanticipated Problems	❑ New Co-I/Sub-I ❑ Address Change ❑ Other:
❑ 1	❑ 2	❑ 3	❑ New Assent Version:		
❑ 4	❑ 5	❑ 6	❑ ICF Addendum Version:		

❑ Regulatory authority inspection (describe any significant problems identified and actions taken to prevent re-occurrence)
❑ Professional license suspended, or been debarred by any government agency or licensing body (explain)
❑ Any IRB/REB suspended or terminated approval of your site (describe the circumstances, including time frame and reasons)

HAS YOUR SITE BEEN MONITORED?	❑ No	❑ Yes	# of visits by Study Monitor since last report:

***CERTIFICATION (must be signed by investigator)**: I certify that the above information is accurate and truthful to the best of my ability. I also certify that I have conducted the Study in accordance with the IRB approved protocol and consent document(s), and that I have not charged the Public Health System or third party private payers for any costs which are to be paid by the Sponsor.*

Investigator Name (print or type)	**Signature**	**Valid License No.**	**Date (yy/mm/dd)**

FOR IRB USE ONLY:
Reviewer Name: Signature: Date:

div. 1373737 Ont. Ltd. *address* 372 Hollandview Trail, Suite 300, Aurora, Ontario, Canada L4G 0A5
tel. (905) 727-7989 *fax* (905) 727-7990 *e-mail* info@irbservices.com *web* www.irbservices.com

CHANGE OF SITE INFORMATION FORM

IRB
INSTITUTIONAL REVIEW BOARD SERVICES

This form is used for informing IRB Services of changes to site information including, but not limited to addition of Sub-Investigators, change of address or change of Study Coordinator.

Qualified/Principal Investigator Contact Information For IRB Services Use:							
Title:		First Name:		Initial:		Last name:	
Clinic Name:						Telephone:	
Address:						Fax:	
Suite/Room/Floor:						Email:	
City:						Postal/Zip:	
Province/State:						Country:	

Changes to Sub-Investigator Information:			
Sub-Investigator:	❑ N/A / No Change		
	❑ Addition:	for Study # _____	
Please complete the following information: (include an attachment if you need additional space)			
	Sub-investigator 1		Sub-investigator 2
Title, Name			
Address where subjects will be seen (if different than QI/PI)			
Earned Degrees and, if applicable, License Numbers			
Delegated Duties			
	Sub-investigator 3		Sub-investigator 4
Title, Name			
Address where subjects will be seen (if different than QI/PI)			
Earned Degrees and, if applicable, License Numbers			
Delegated Duties			
	❑ Deletion:	for Study # _____	
Dr.			
Dr.			

Changes to Study Coordinator Information:		
Study Coordinator:	❑ N/A / No Change	
	❑ Addition:	for Study # _____
Coordinator's Name:		
Coordinator's Tel:		
	❑ Deletion:	for Study # _____
Coordinator's Name		
Coordinator's Name		

div. 1373737 Ont. Ltd. *address* 372 Hollandview Trail, Suite 300, Aurora, Ontario, Canada L4G 0A5
tel. (905) 727-7989 *fax* (905) 727-7990 *e-mail* info@irbservices.com *web* www.irbservices.com

CHANGE OF SITE INFORMATION FORM

IRB
INSTITUTIONAL REVIEW BOARD SERVICES

Changes to Site Address/Contact Information:			
Applicable:			❑ Yes ❑ No
Clinic Name:		Telephone:	
		24Hr. /Emergency Tel:	
Address:		Fax:	
Suite/Room/Floor:		Email:	
City:		Postal/Zip:	
Province/State:		Country:	

Changes to On-Site Facilities:				
Applicable:				❑ Yes ❑ No
❑ Lab / Collection Station	❑ X-Ray	❑ ECG	❑Endoscopy/Gastroscopy	❑ Lung Function
❑ EEG	❑ CT Scan	❑ MRI	❑ CV Stress Testing	❑ Ophthalmic
❑ Crash Cart / Emergency Equipment		❑ Other:		

Other Changes:		
Applicable:	❑ Yes	❑ No
Please describe:		

List of Studies Affected by the Above Changes*:	
Sponsor	
Study #	
Sponsor	
Study #	
Sponsor	
Study #	
Sponsor	
Study #	
Sponsor	
Study #	
Sponsor	
Study #	

*Attach additional sheet(s) if necessary

Qualified/Principal Investigator/Designate Name (printed)	Qualified/Principal Investigator/Designate Signature	Date (YYYY-MMM-DD)

NB. Information provided on this form is considered to be confidential and is for internal IRB/IRBS use only.

div. 1373737 Ont. Ltd. *address* 372 Hollandview Trail, Suite 300, Aurora, Ontario, Canada L4G 0A5
tel. (905) 727-7989 *fax* (905) 727-7990 *e-mail* info@irbservices.com *web* www.irbservices.com

APPENDIX C-2

5.7.2. Landmarks

For each subject the position of the oropharyngeal junction (OPJ), the epiglottis (EPI) and the glottis (GLO) was determined by the Investigator. This was performed before saving the four baseline measurements performed while the subjects exhaled on the hard disk of the acoustic pharyngometer. In Table 5.11 the individual landmarks for OPJ, EPI and GLO for all 60 subjects are presented as distance from the teeth.

Table 5.11: Individual landmarks given as centimeters from the teeth for the 60 subjects.

Subject #	OPJ	EPI	GLO
1	8.88	12.31	20.88
2	8.88	12.31	20.88
3	7.16	11.88	20.45
4	9.31	12.74	18.74
5	6.73	10.16	20.45
6	6.30	9.31	19.60
7	9.31	12.74	20.02
8	9.31	12.31	20.02
9	9.31	12.31	19.17
10	9.31	13.16	20.45
11	9.31	12.74	20.45
12	9.31	13.16	21.74
13	6.30	9.31	20.02

14	8.88	12.74	20.88
15	7.16	9.73	20.02
16	8.45	12.31	21.31
17	8.45	12.31	20.45
18	8.88	12.74	23.02
19	6.30	9.31	20.45
20	9.31	12.74	20.45
21	6.73	11.02	22.60
22	8.45	12.31	20.88
23	9.31	13.16	20.45
24	9.31	13.59	23.45
25	8.45	13.59	23.45
26	9.31	12.74	22.60
27	6.73	9.73	19.60
28	8.88	13.16	19.17
29	8.45	13.16	19.60
30	8.88	12.31	18.74
31	9.31	12.74	19.17
32	9.31	13.59	23.02
33	8.45	12.31	19.17
34	8.88	12.74	22.17
35	6.30	10.16	21.74
36	8.88	12.74	20.45
37	8.88	12.74	22.60

38	7.16	12.31	19.60
39	8.02	12.31	23.02
40	8.88	12.31	20.88
41	8.88	12.31	19.17
42	9.31	13.16	20.02
43	9.31	12.74	19.17
44	8.02	12.74	20.02
45	8.88	12.74	22.17
46	6.30	10.16	20.02
47	8.02	12.31	20.45
48	8.45	12.74	22.60
49	8.88	11.88	19.17
50	8.88	12.74	22.60
51	8.88	11.88	19.60
52	8.88	12.31	20.88
53	8.88	12.31	20.02
54	8.45	12.74	19.60
55	8.45	12.31	22.17
56	8.88	12.74	22.60
57	9.31	12.74	22.60
58	8.88	12.74	20.02
59	8.88	12.74	19.60
60	8.88	12.31	19.17

Mean	8.48	12.22	20.72
SD	0.95	1.08	1.34
Minimum	6.30	9.31	18.74
Maximum	9.31	13.59	23.45
Median	8.88	12.53	20.45
RSD	11.20	8.85	6.45

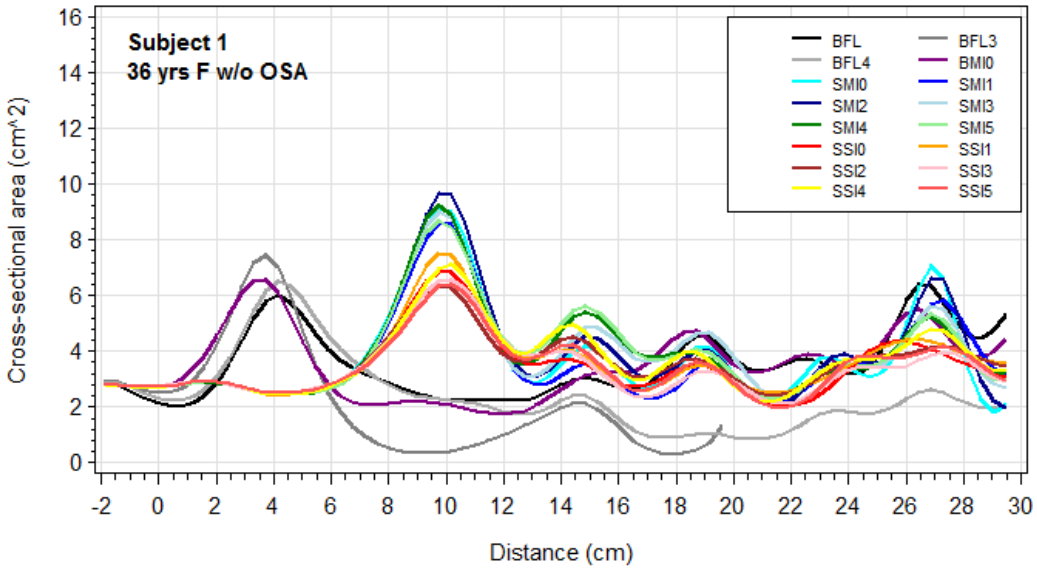
APPENDIX C-3

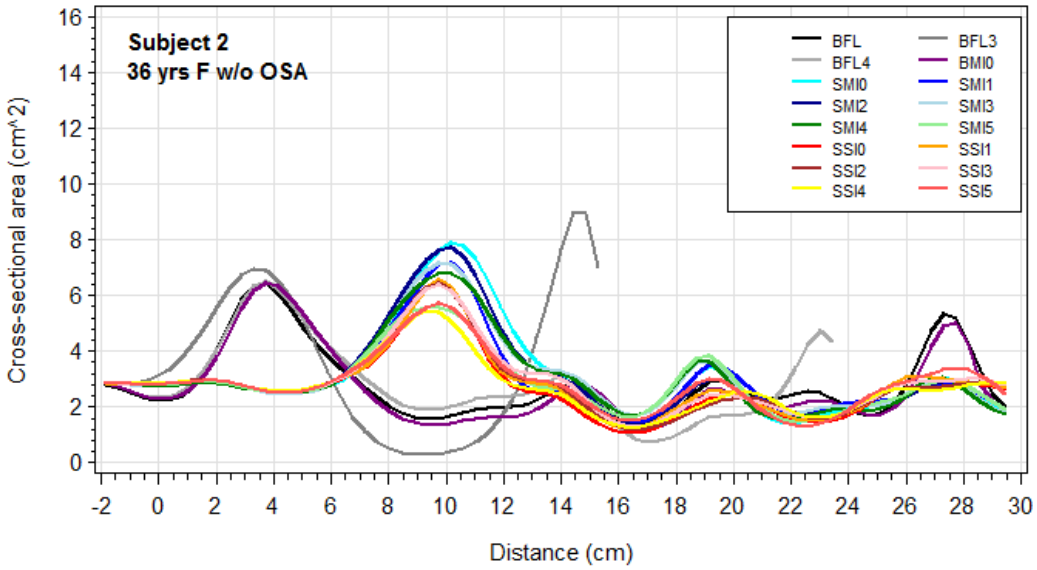
5.7.3. Individual acoustic pharyngograms

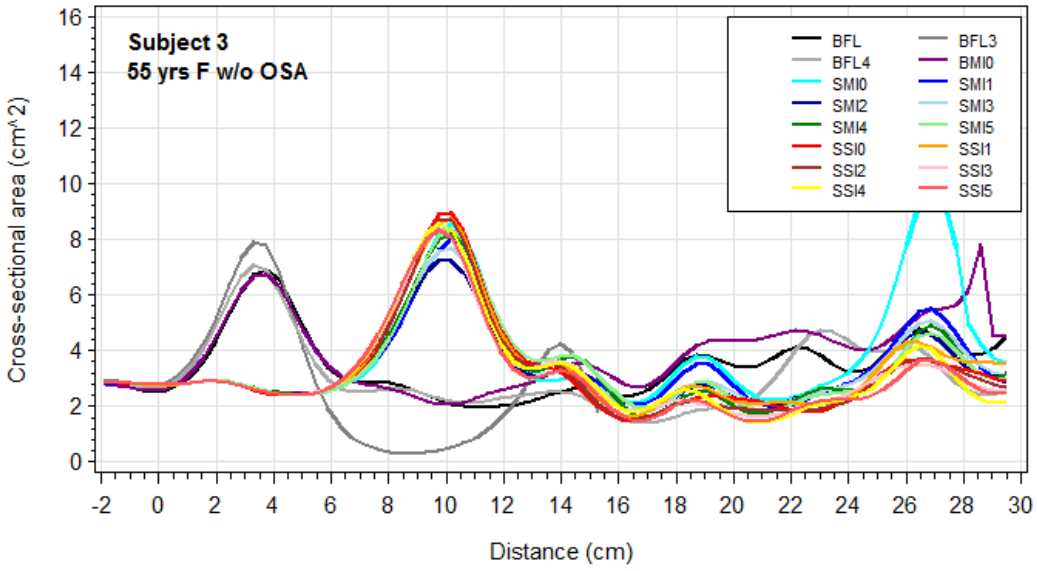
The individual acoustic pharyngograms are coded in each figure as outlined in Table 5.2 (from 5.6.1. Data files).

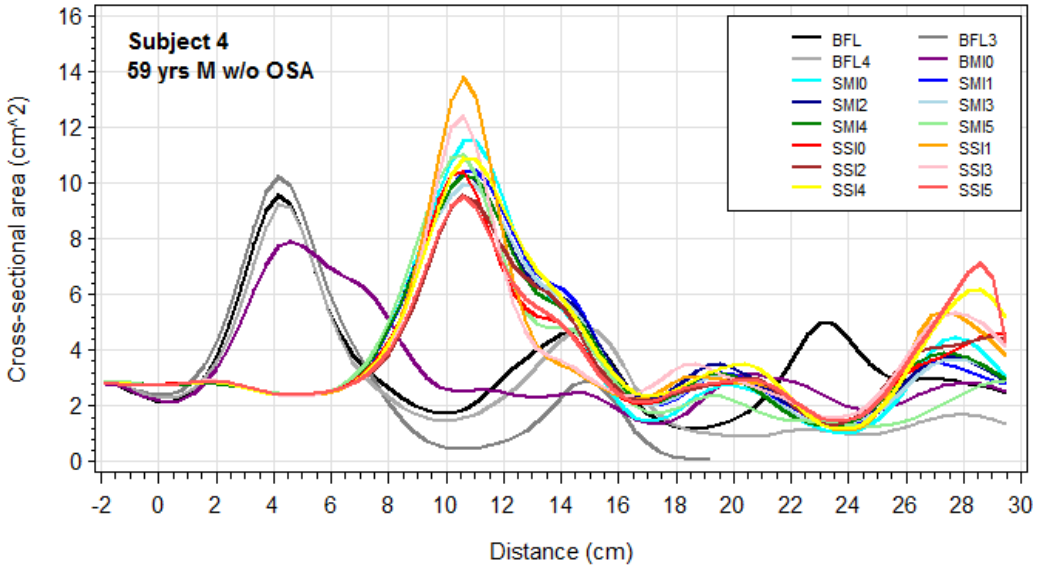
Table 5.2: Data files recorded in the study acoustic pharyngometer.

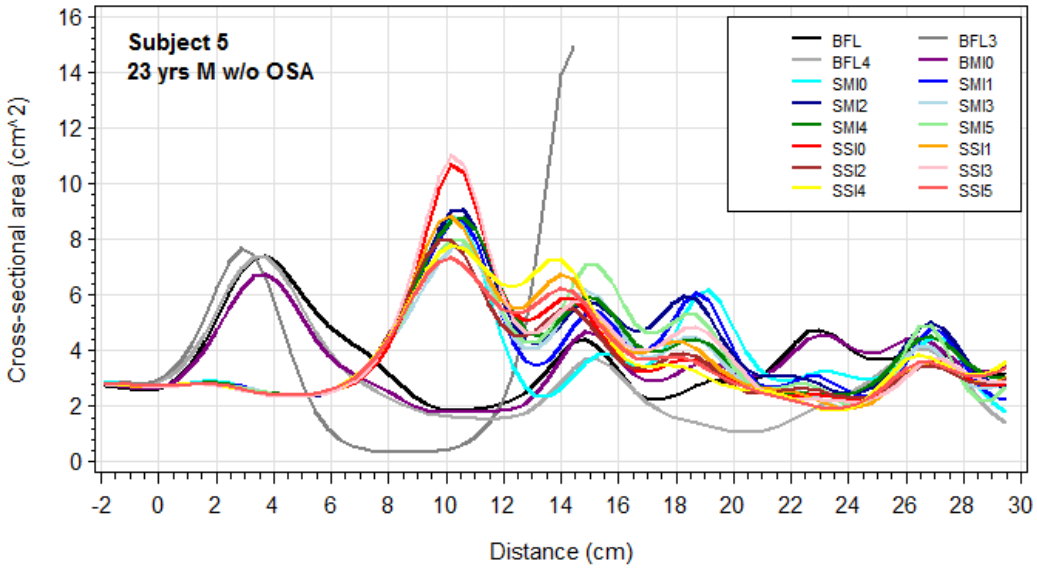
Data files	Explanations
BFL0	BFL, in which B = Baseline, FRC = Functional Residual Capacity and L = Landmarks. Each BFL file contained four acoustic readings as follows: • 1 – Functional Residual Capacity • 2 - Functional Residual Capacity • 3 - Nasal breathing • 4 - Coaching for glottal closure In the figures the following short forms were used: BFL = two measurements from FRC, BFL3 = one measurement during nasal breathing, and BFL4 = one measurement during glottal closure.
BMI0	BMI, in which B = Baseline, M = Mid tidal inhalation and I = Inhalation.
SMI0	SMI0, in which S = Stepped mouthpiece, M = Measurement at mid tidal inhalation, I = Inhalation and 0 = no advancement with stepped mouthpiece (baseline).
SMI1	SMI1, as above with 1 = 1 mm advancement with stepped mouthpiece.
SMI2	SMI2, as above with 2 = 2 mm advancement with stepped mouthpiece.
SMI3	SMI3, as above with 3 = 3 mm advancement with stepped mouthpiece.
SMI4	SMI4, as above with 4 = 4 mm advancement with stepped mouthpiece.
SMI5	SMI5, as above with 5 = 5 mm advancement with stepped mouthpiece.
SSI0	SSI0, in which S = Stepped mouthpiece, S = Measurement during slow prolonged inhalation, I = Inhalation and 0 = 0 mm advancement with stepped mouthpiece (baseline).
SSI1	SSI1, as above with 1 = 1 mm advancement with stepped mouthpiece.
SSI2	SSI2, as above with 2 = 2 mm advancement with stepped mouthpiece.
SSI3	SSI3, as above with 3 = 3 mm advancement with stepped mouthpiece.
SSI4	SSI4, as above with 4 = 4 mm advancement with stepped mouthpiece.
SSI5	SSI5, as above with 5 = 5 mm advancement with stepped mouthpiece.

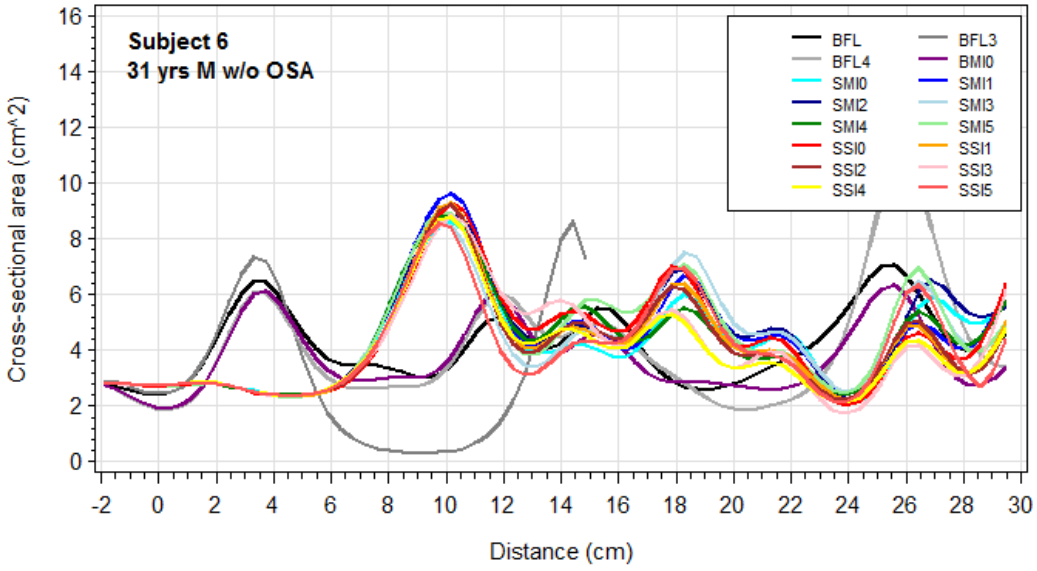

Subject 1
36 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

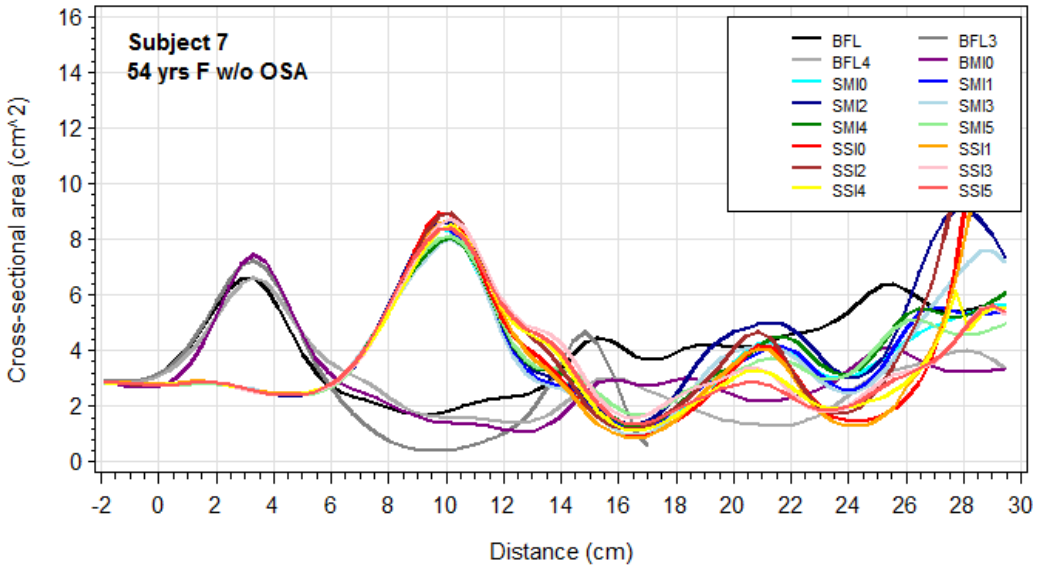

Subject 2
36 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

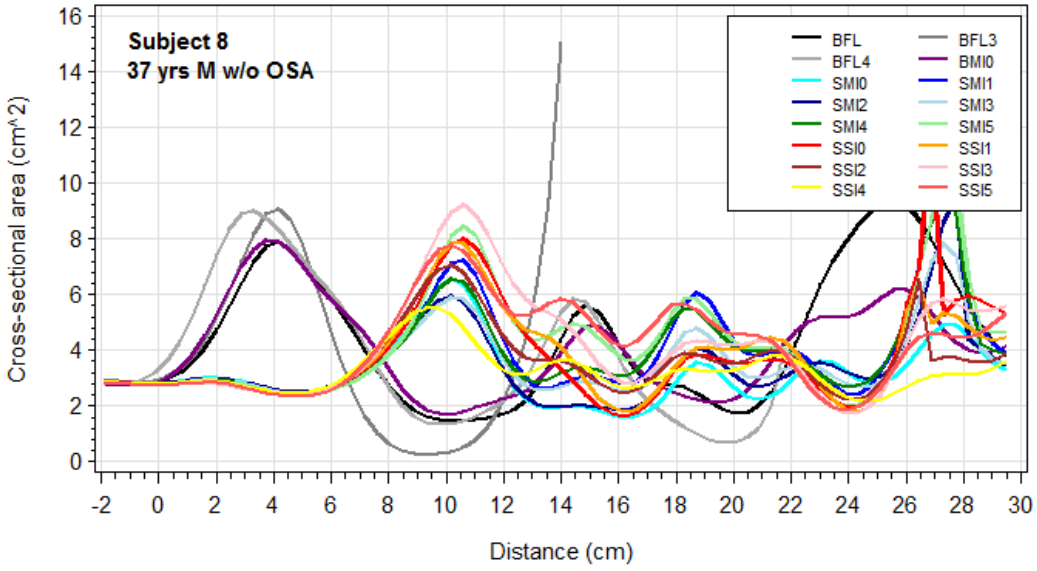

Subject 3
55 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

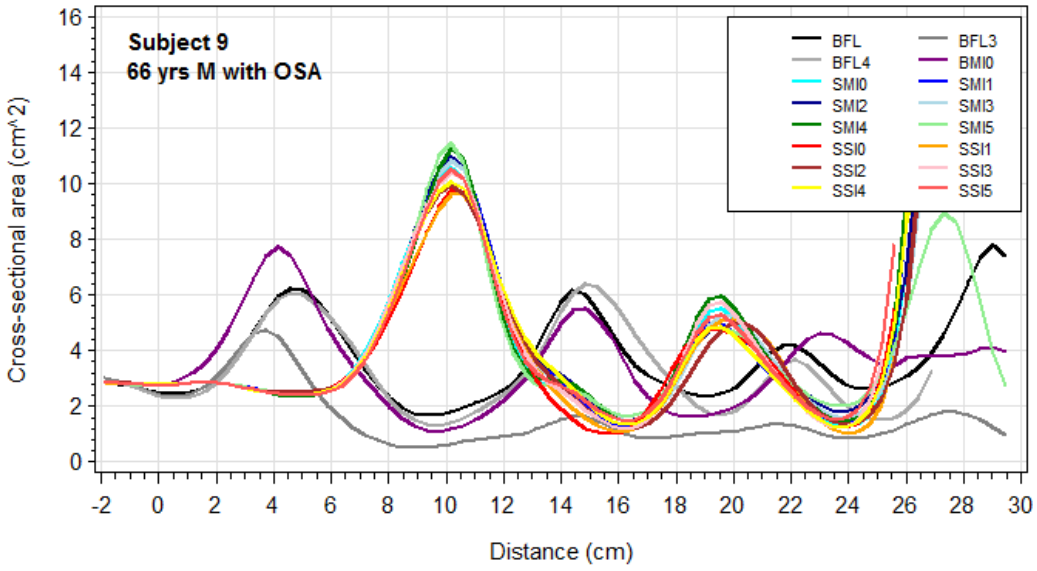

Subject 4
59 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

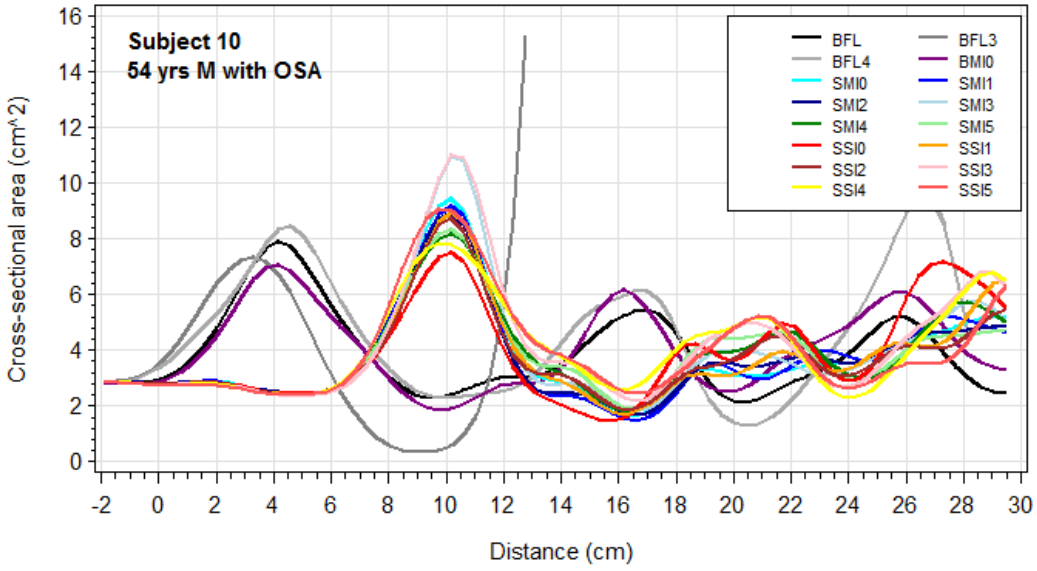

Subject 5
23 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

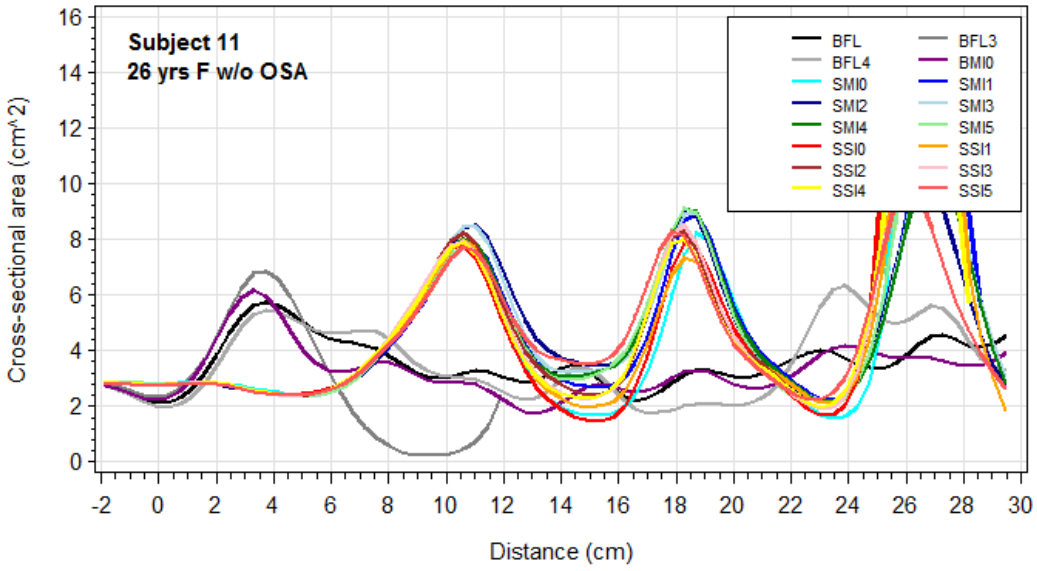

Subject 6
31 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

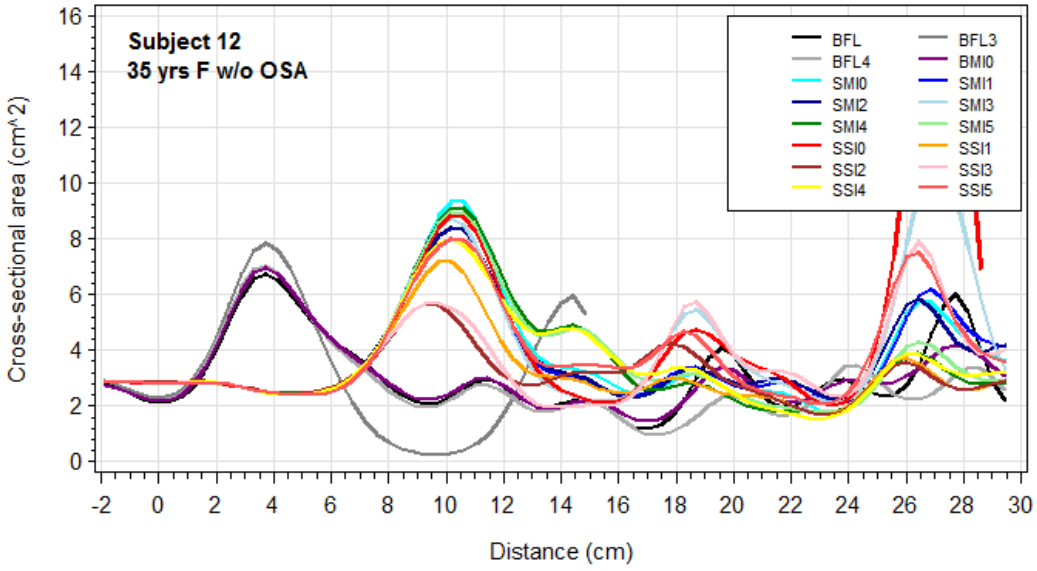

Subject 7
54 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

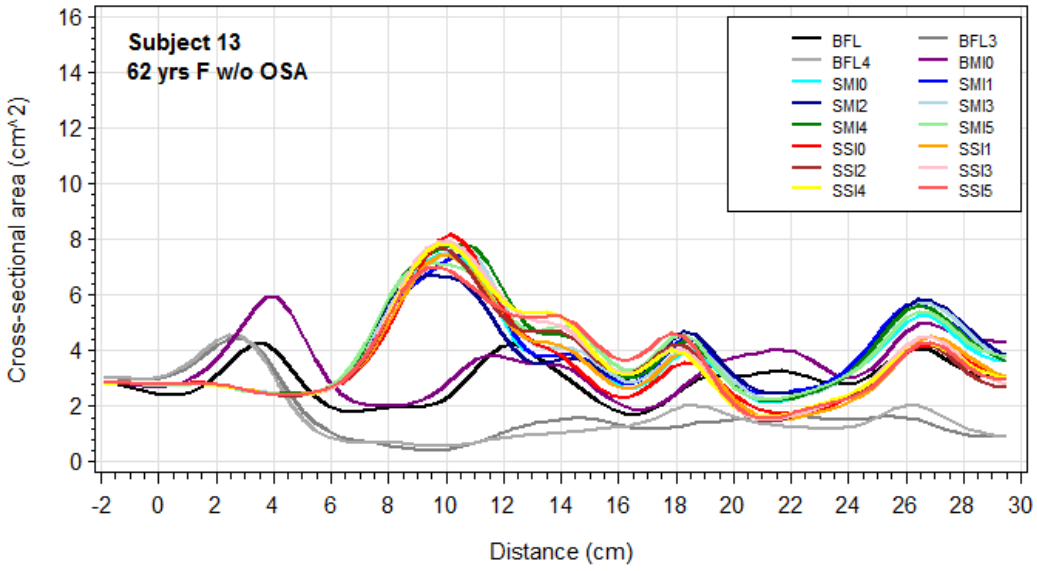

Subject 8
37 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

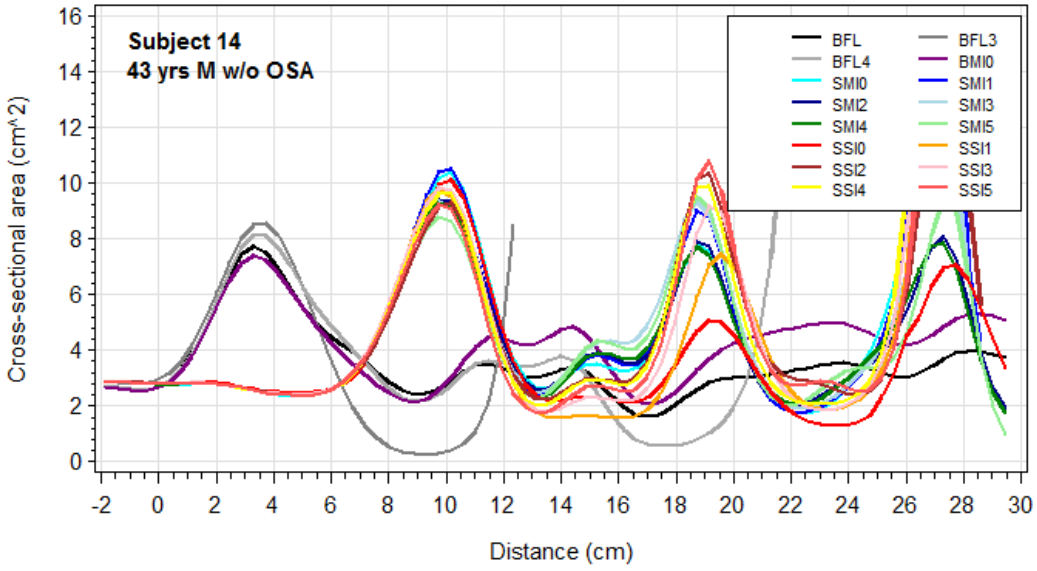

Subject 9
66 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

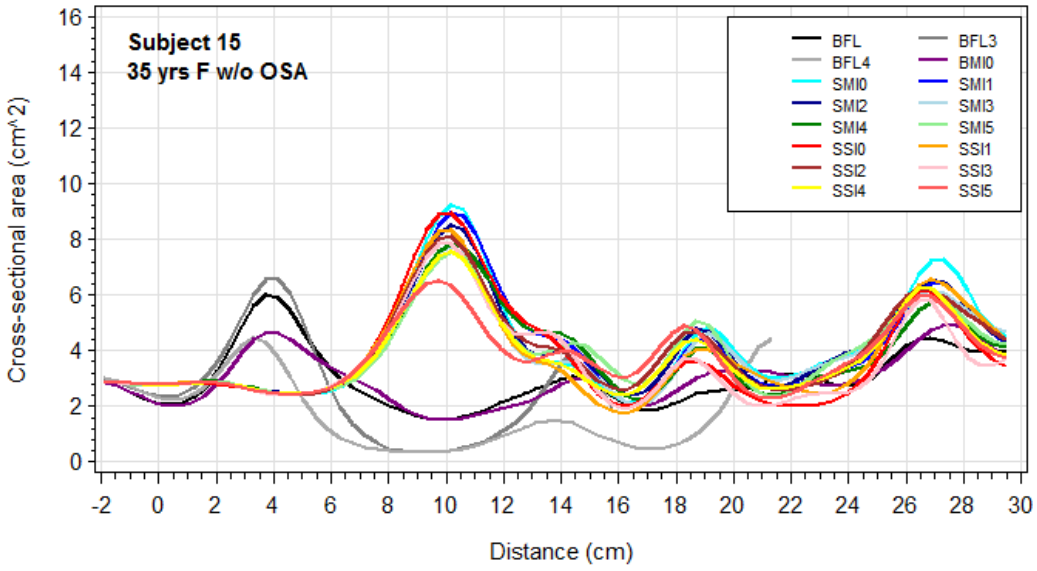

Subject 10
54 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

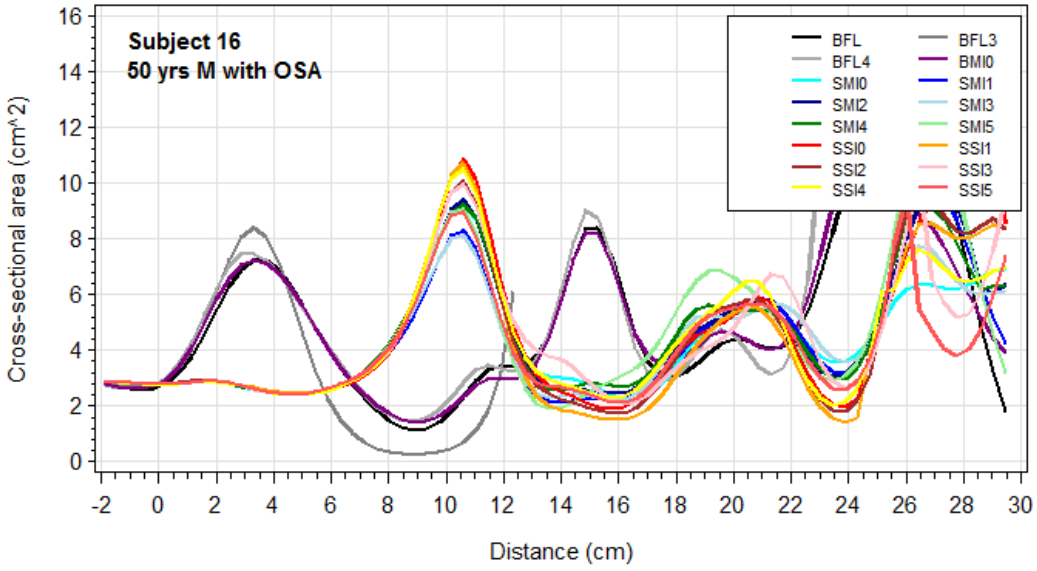

Subject 11
26 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

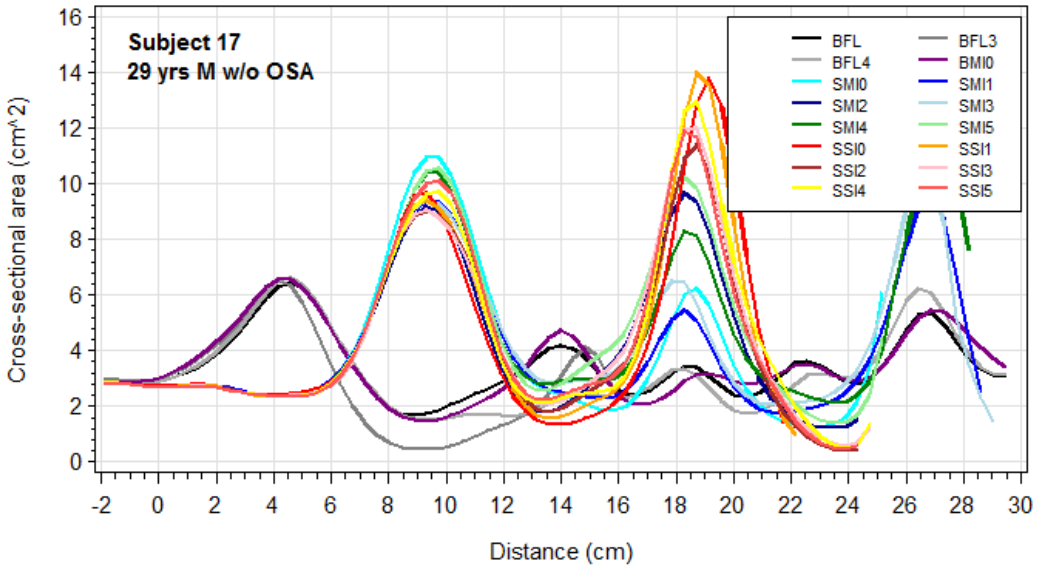

Subject 12
35 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

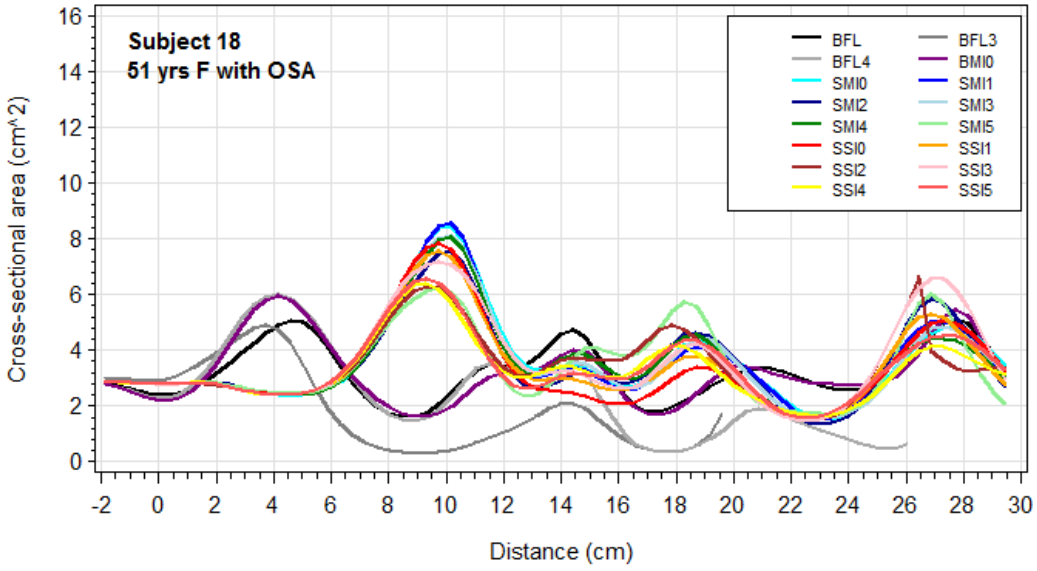

Subject 13
62 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

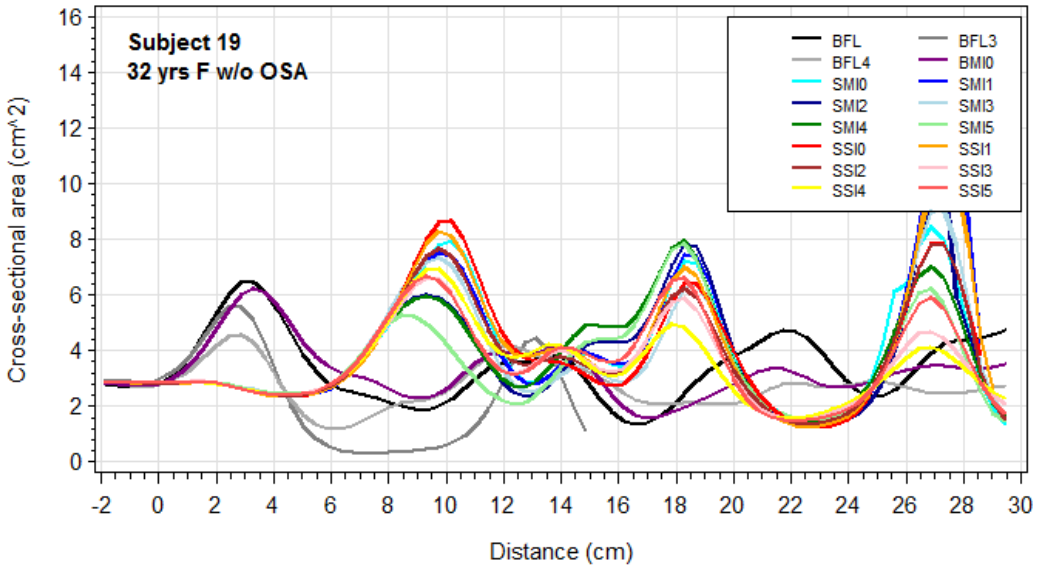

Subject 14
43 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

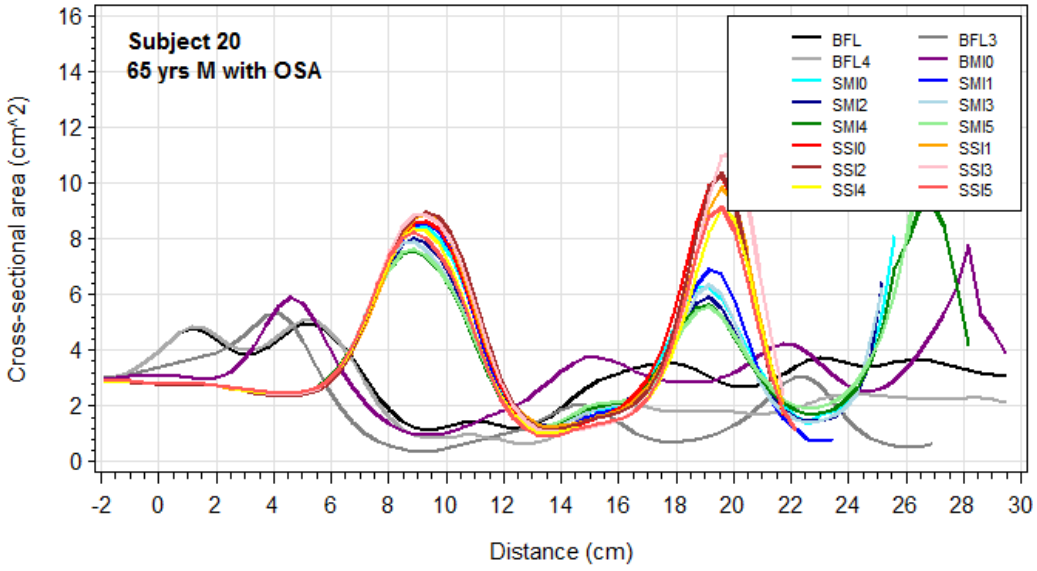

Subject 15
35 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

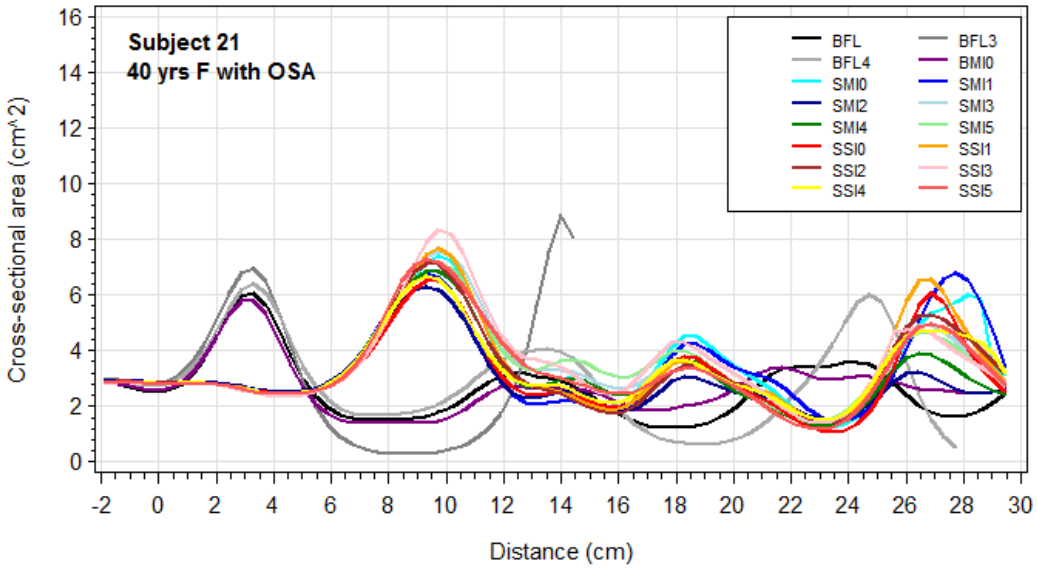

Subject 16
50 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

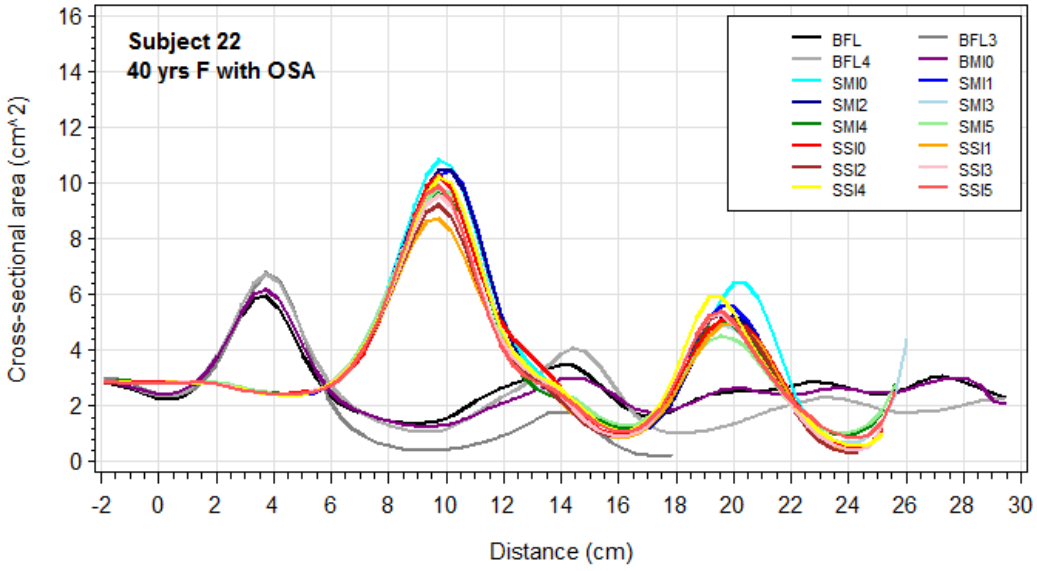

Subject 17
29 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

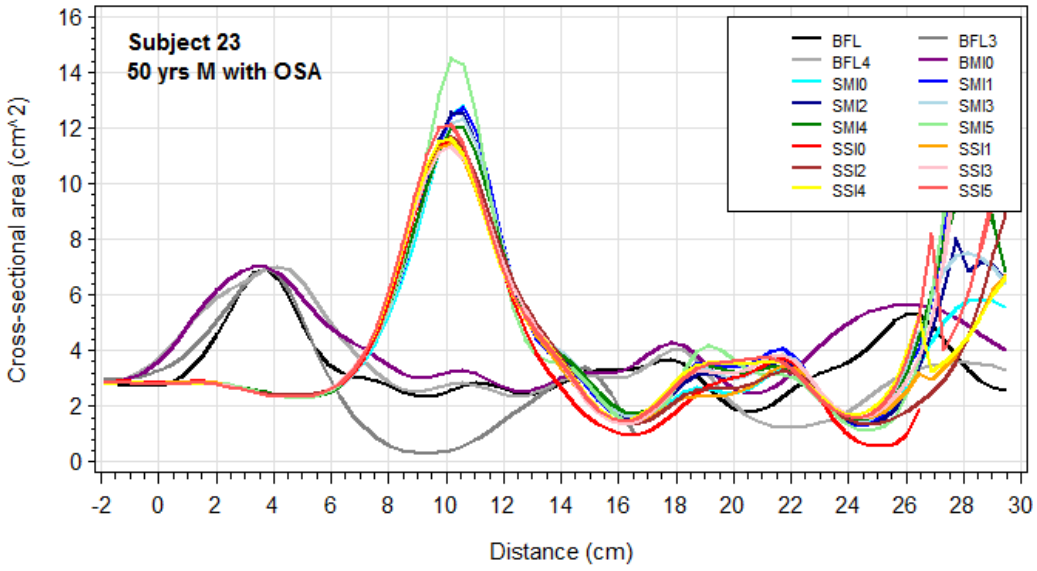

Subject 18
51 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

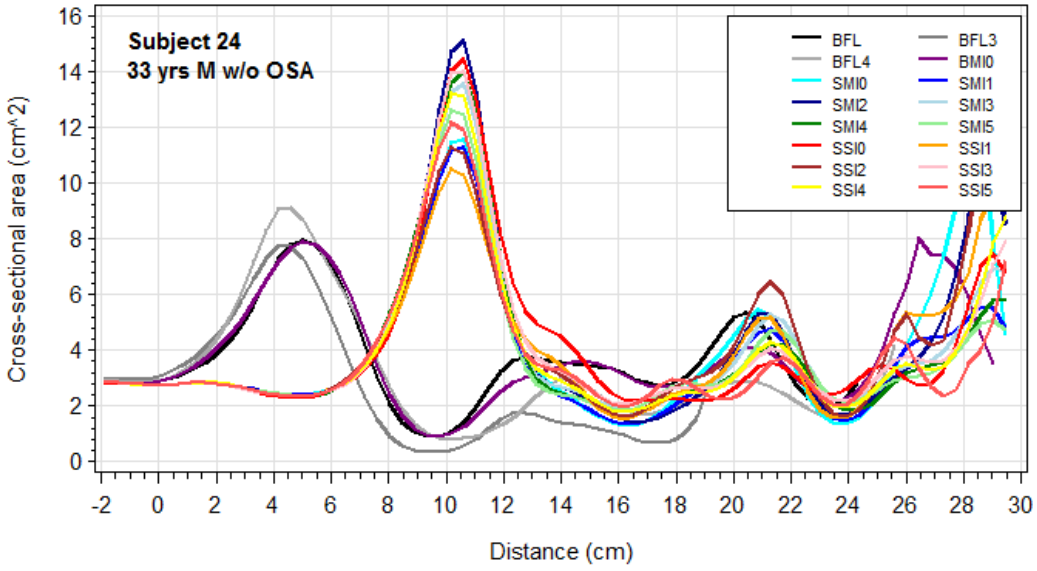

Subject 19
32 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

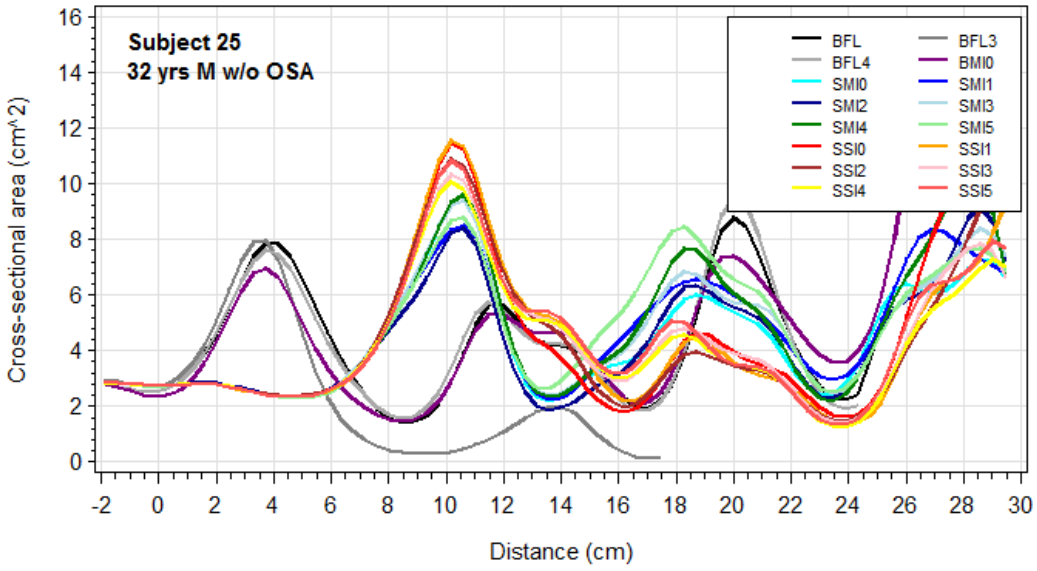

Subject 20
65 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

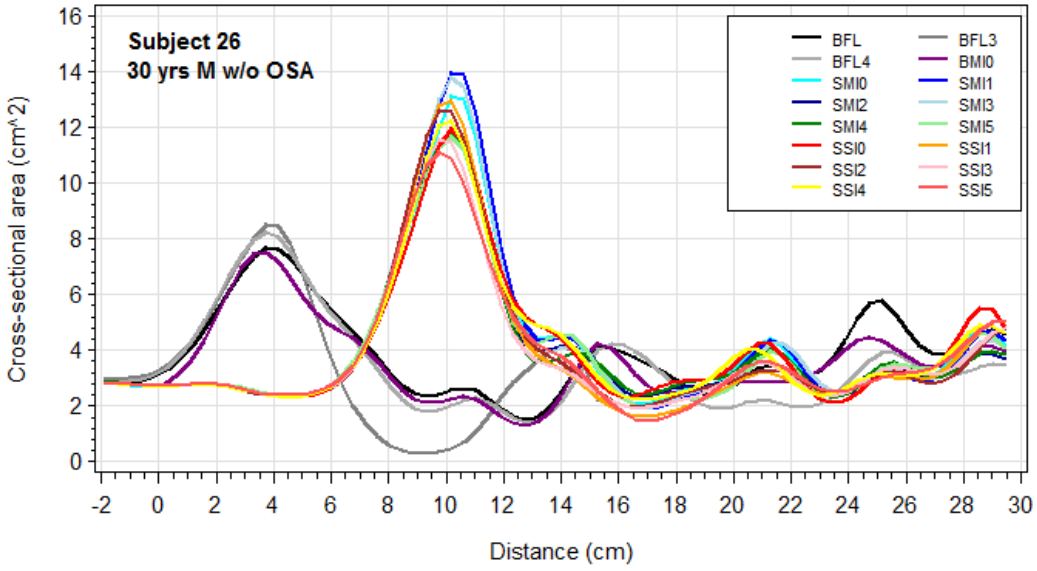

Subject 21
40 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

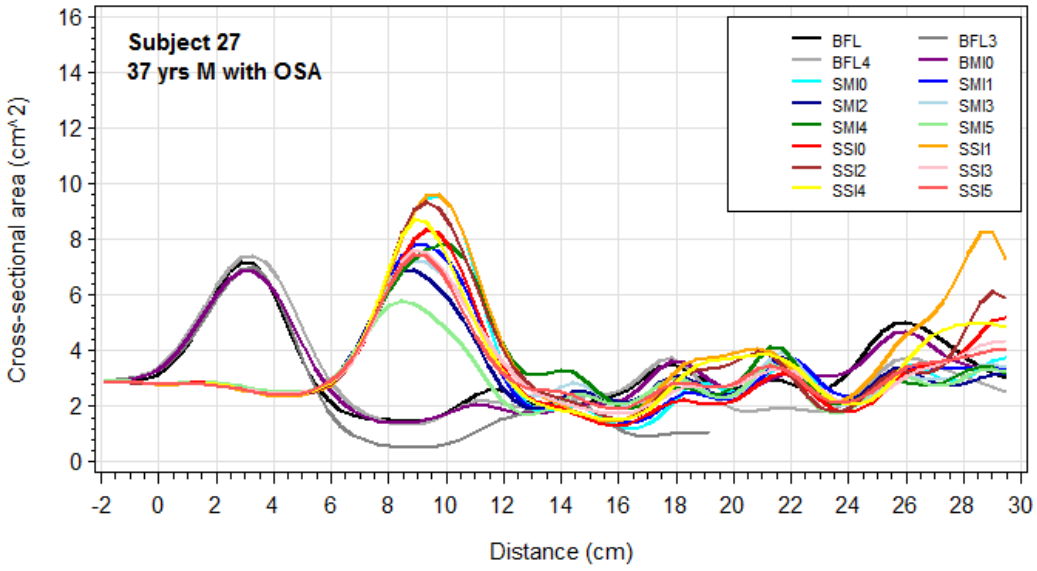

Subject 22
40 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

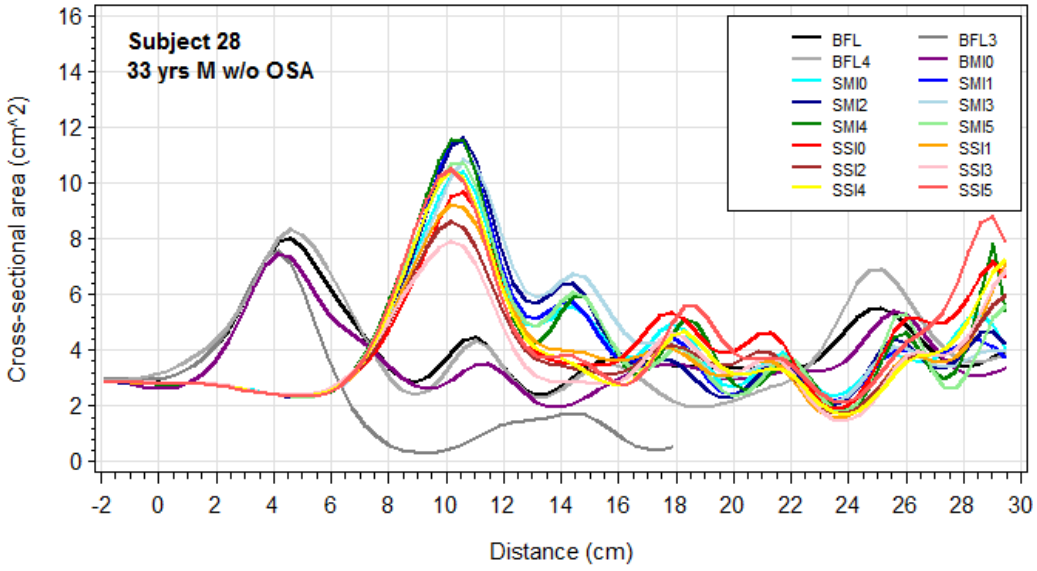

Subject 23
50 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

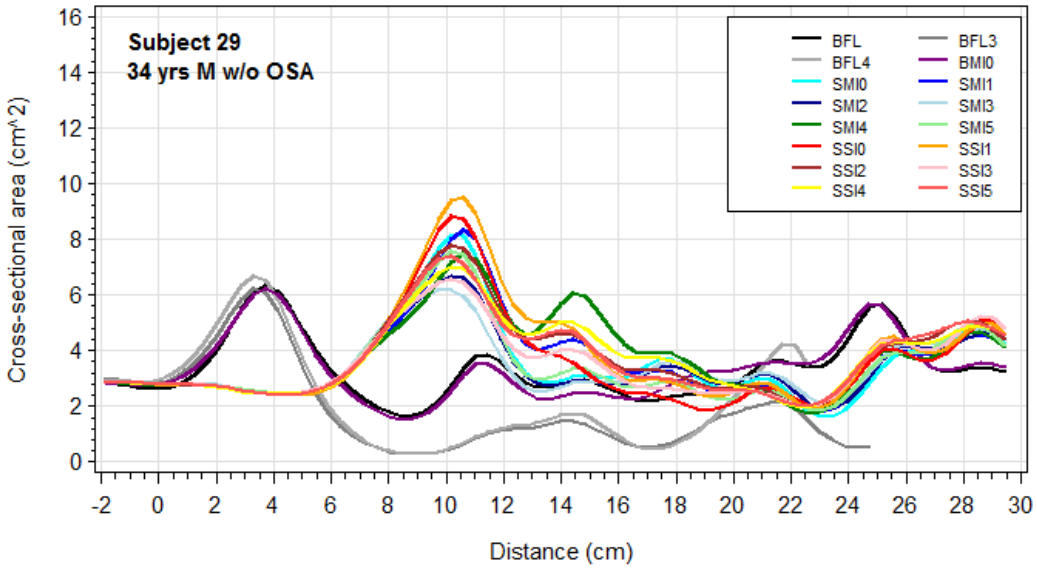

Subject 24
33 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

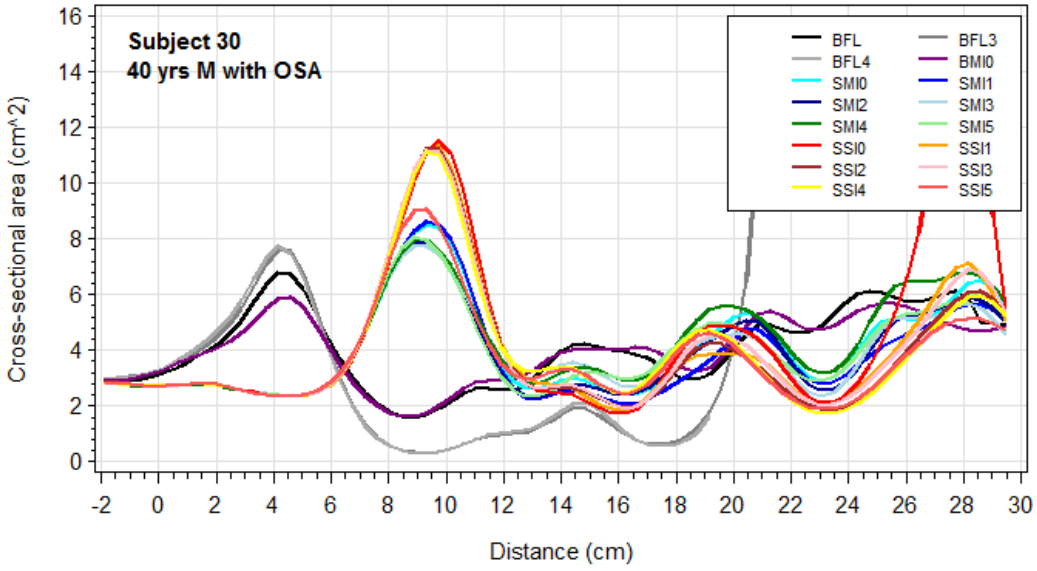

Subject 25
32 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

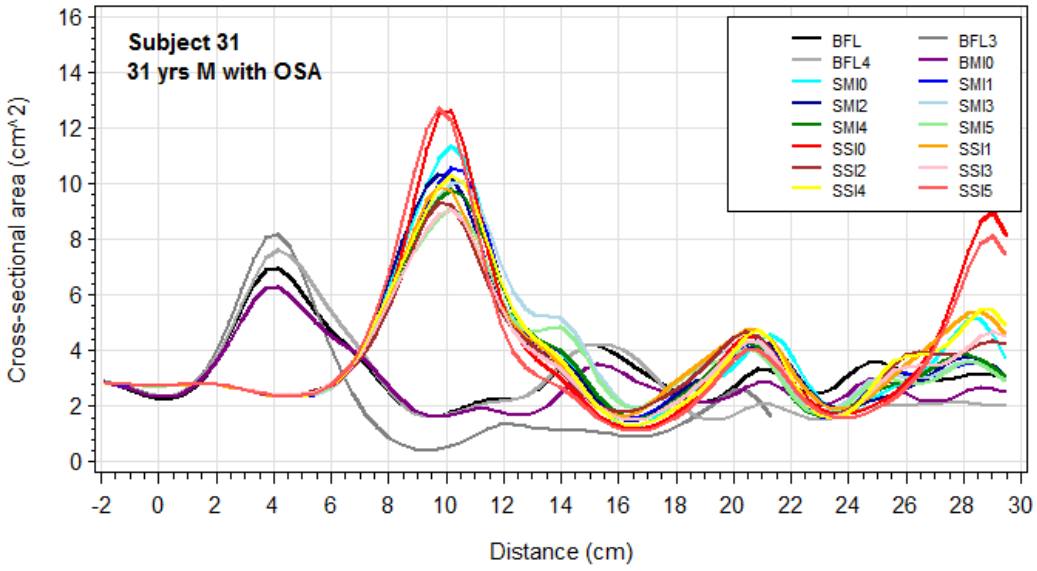

Subject 26
30 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

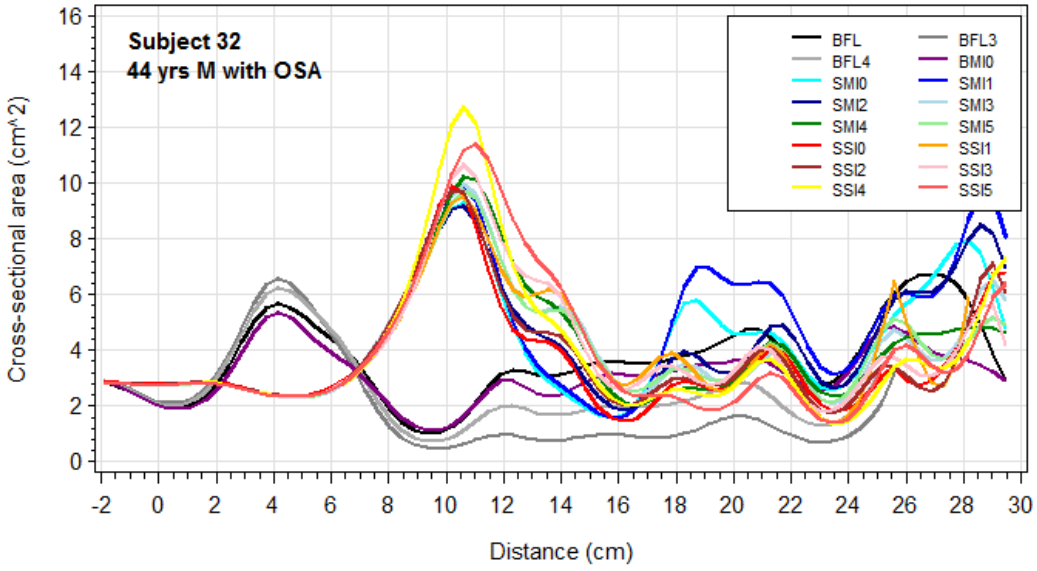

Subject 27
37 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

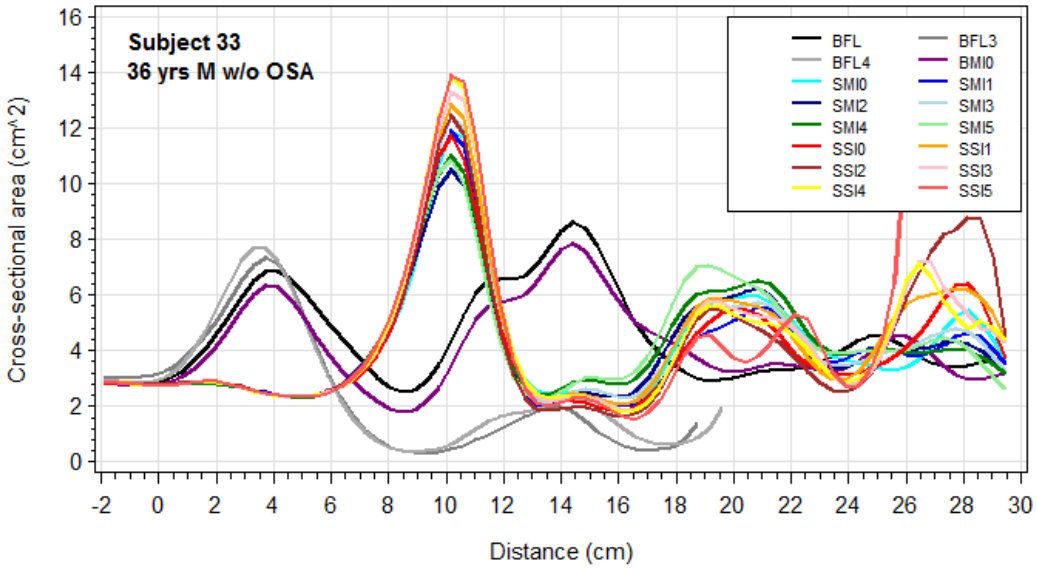

Subject 28
33 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

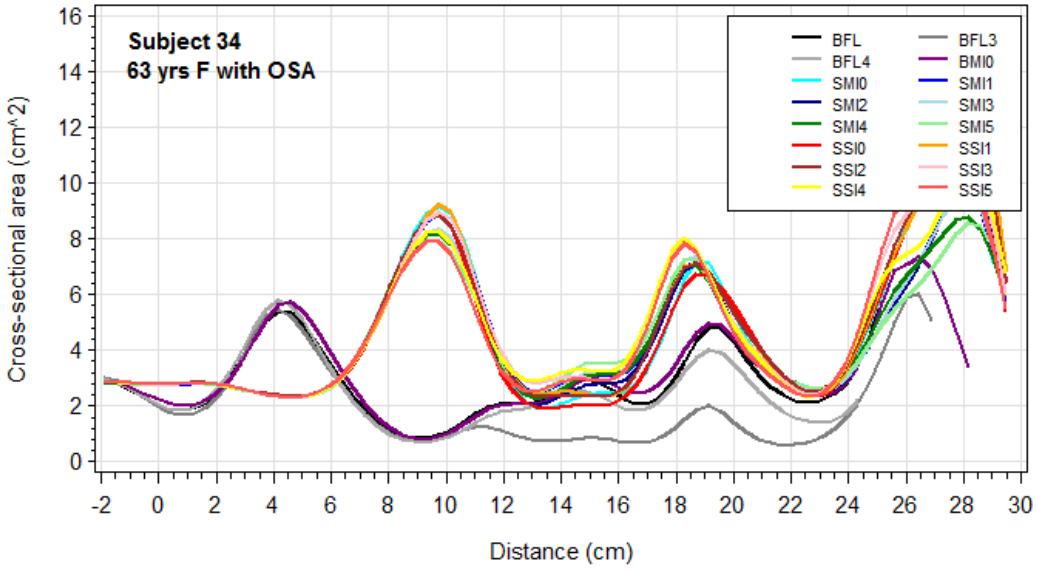

Subject 29
34 yrs M w/o OSA
BFL
BFL4
SMI0
SMI2
SMI4
SSI0
SSI2
SSI4
BFL3
BMI0
SMI1
SMI3
SMI5
SSI1
SSI3
SSI5
Cross-sectional area (cm^2)
Distance (cm)

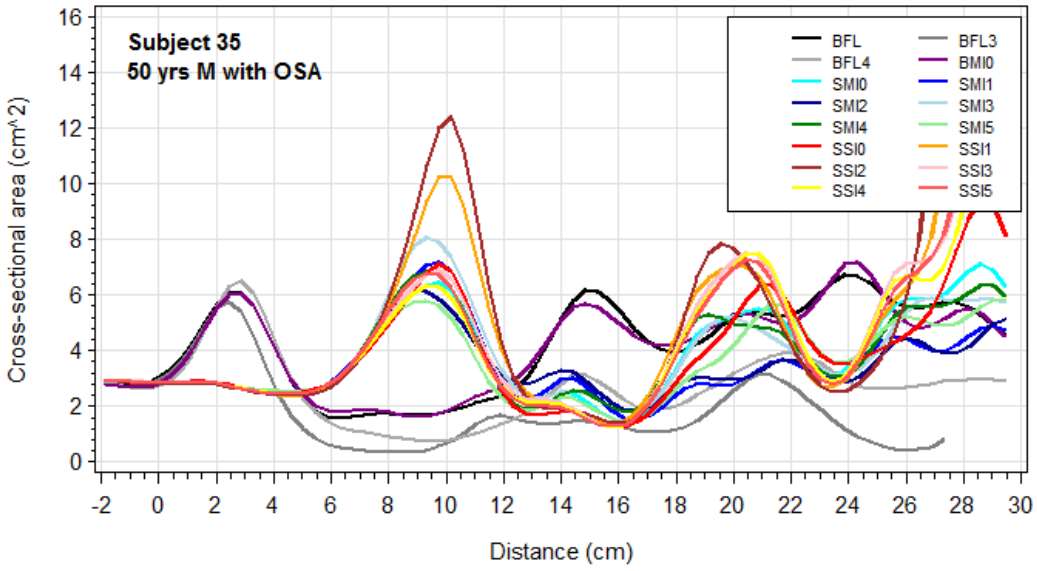

Subject 30
40 yrs M with OSA
BFL
BFL4
SMI0
SMI2
SMI4
SSI0
SSI2
SSI4
BFL3
BMI0
SMI1
SMI3
SMI5
SSI1
SSI3
SSI5
Cross-sectional area (cm^2)
Distance (cm)

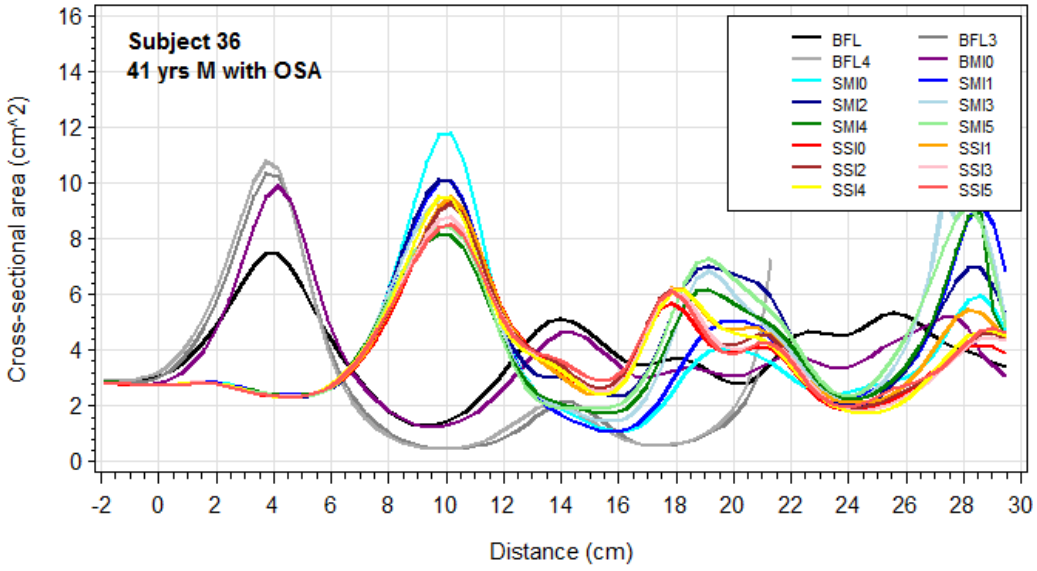

Subject 31
31 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

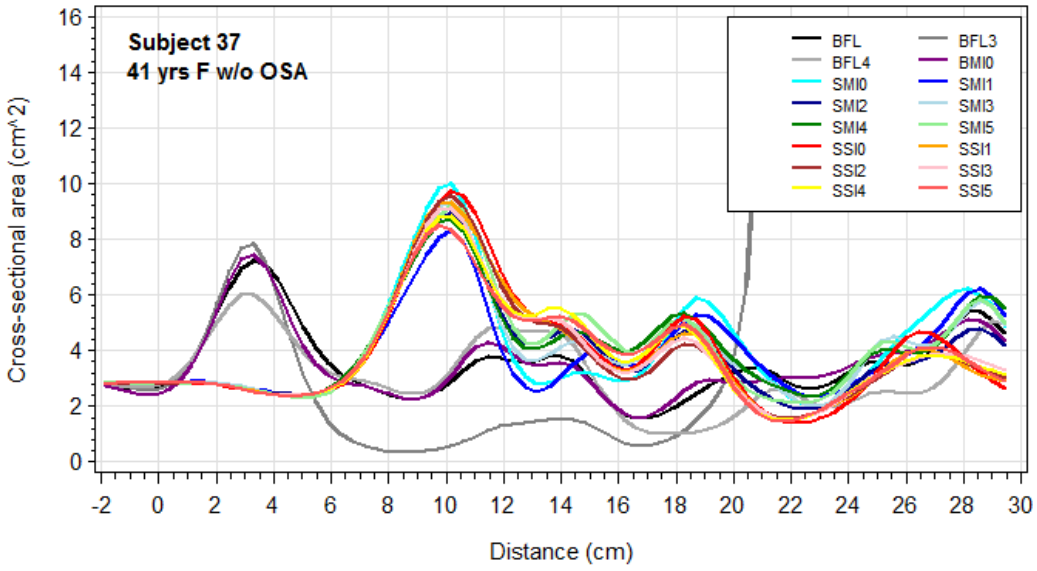

Subject 32
44 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

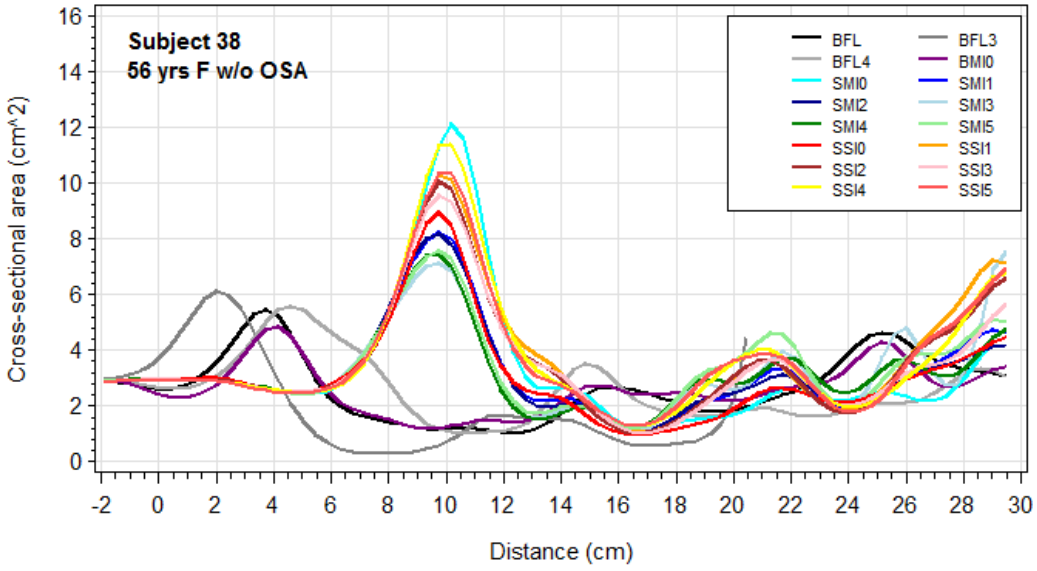

Subject 33
36 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

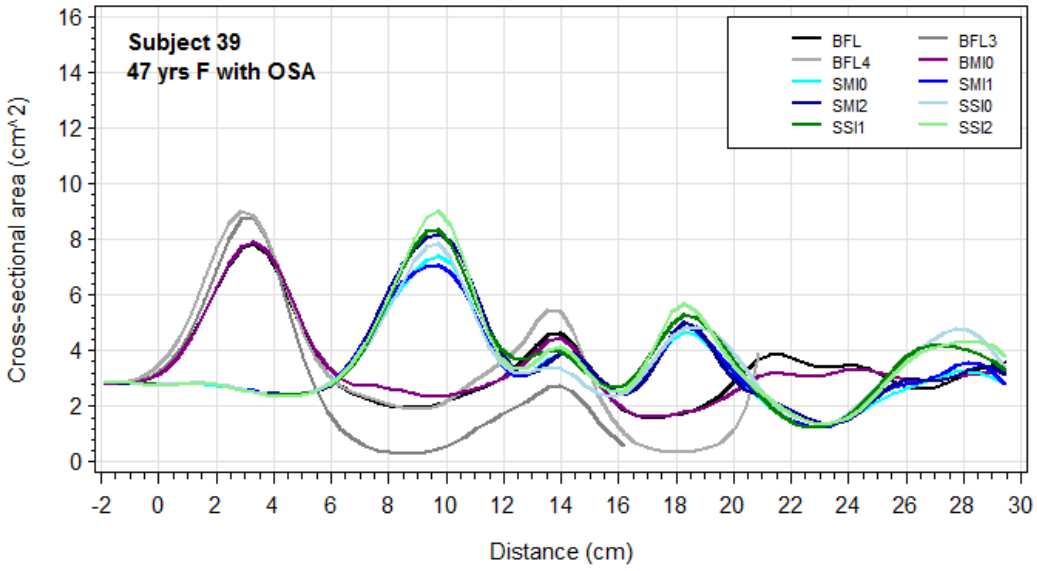

Subject 34
63 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

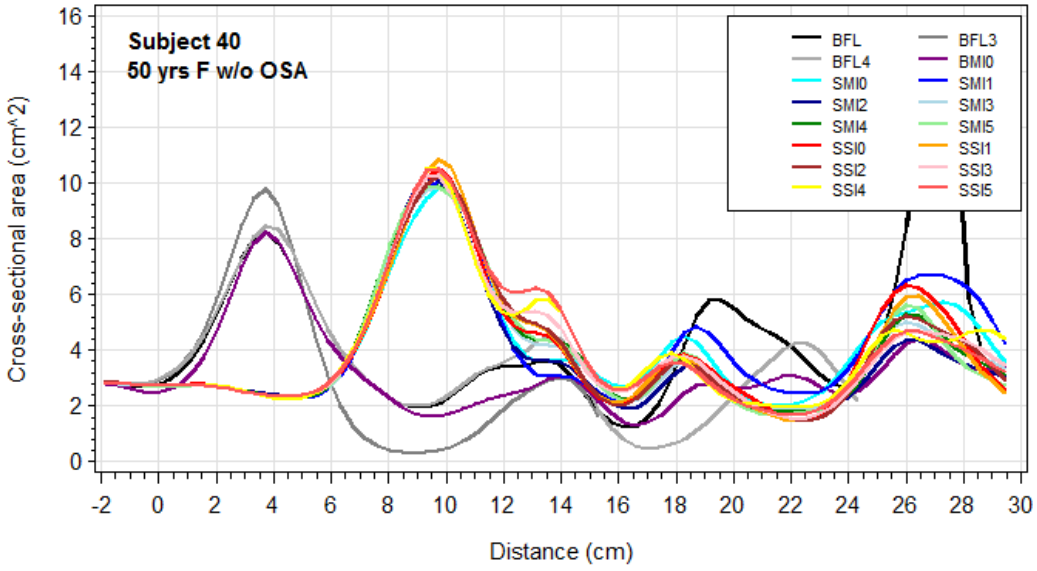

Subject 35
50 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

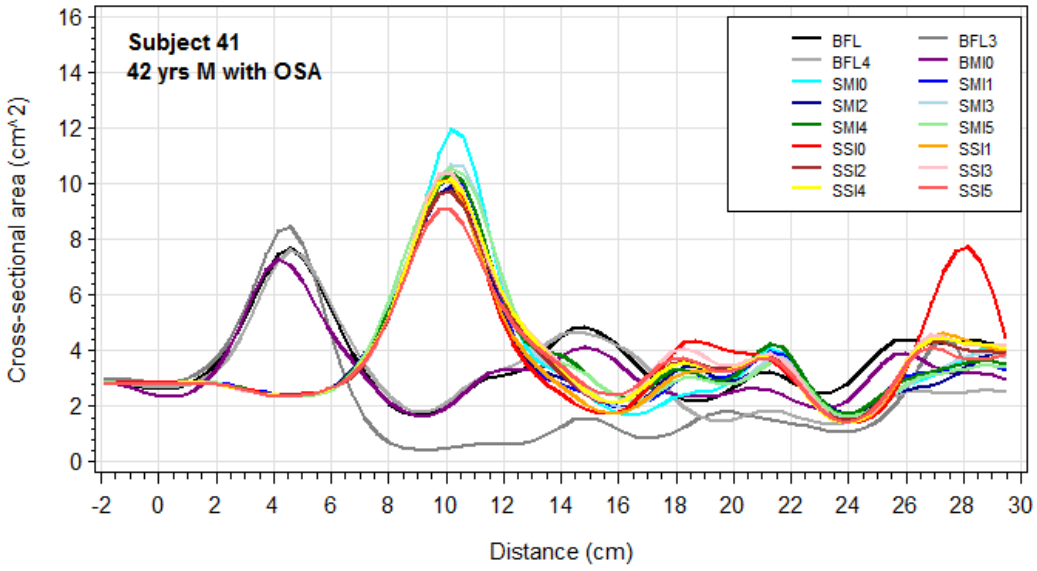

Subject 36
41 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

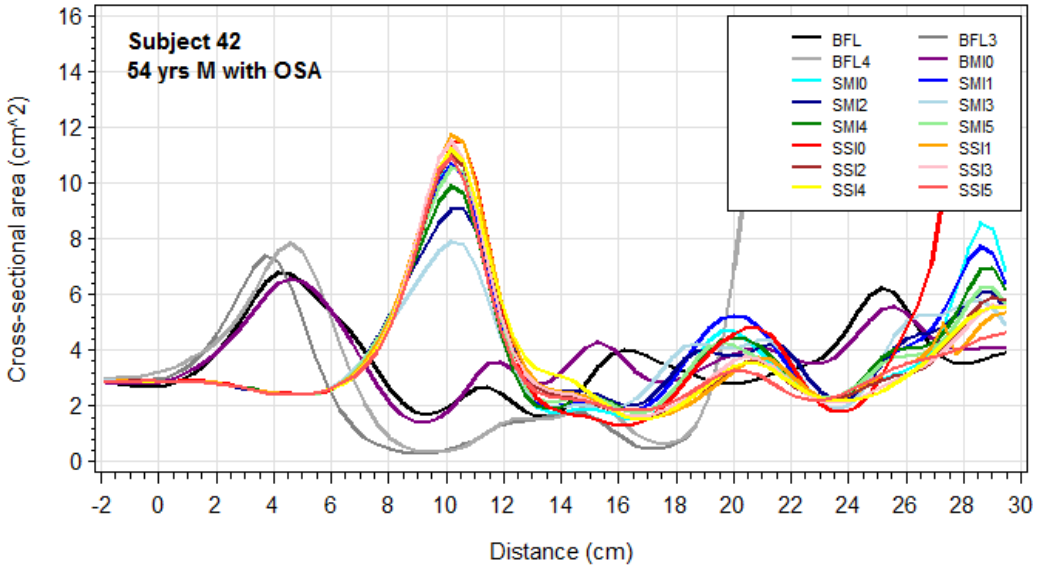

Subject 37
41 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

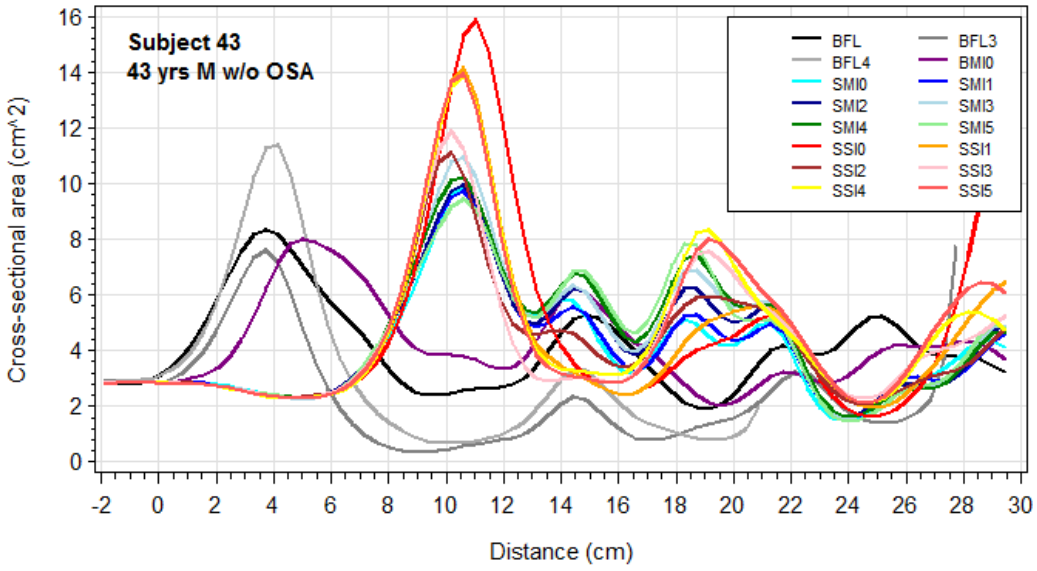

Subject 38
56 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

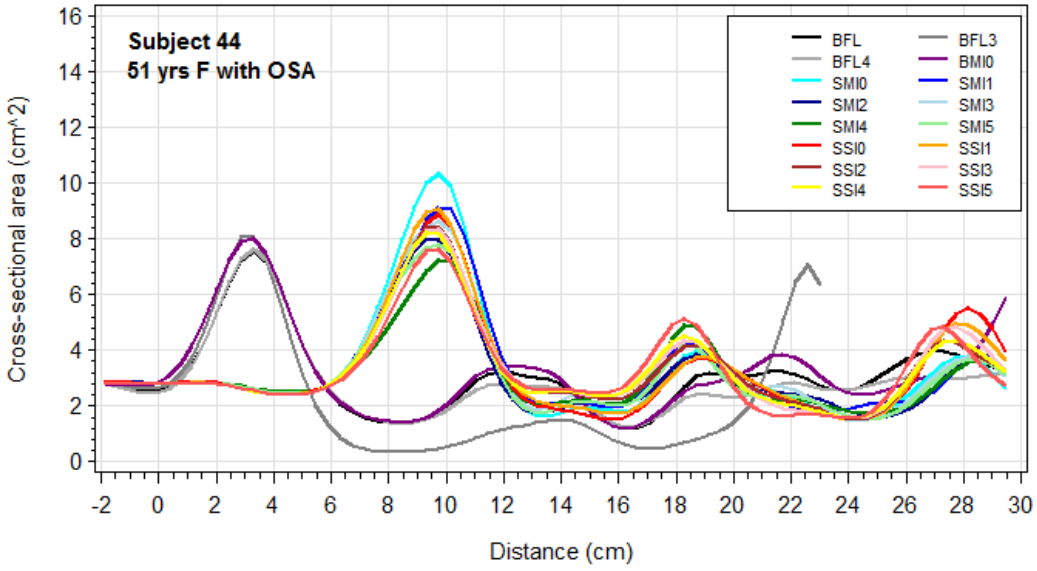

Subject 39
47 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SSI0
SSI1
SSI2
Cross-sectional area (cm^2)
Distance (cm)

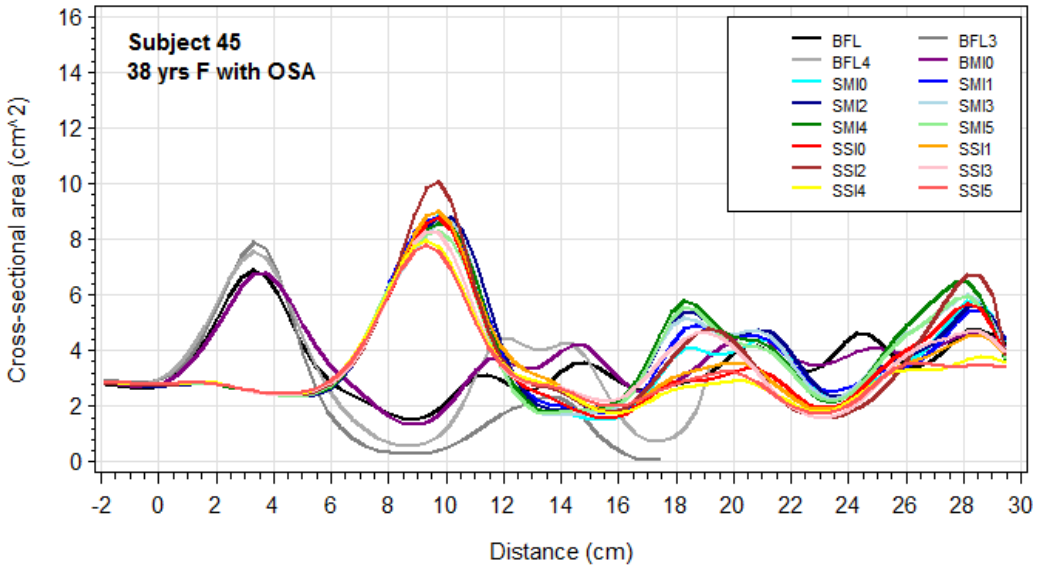

Subject 40
50 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

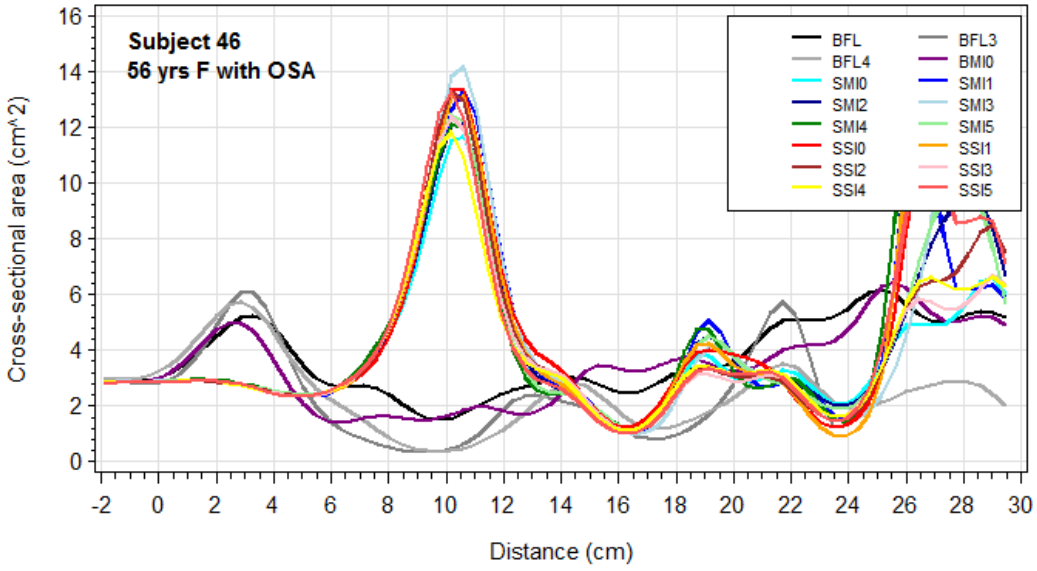

Subject 41
42 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

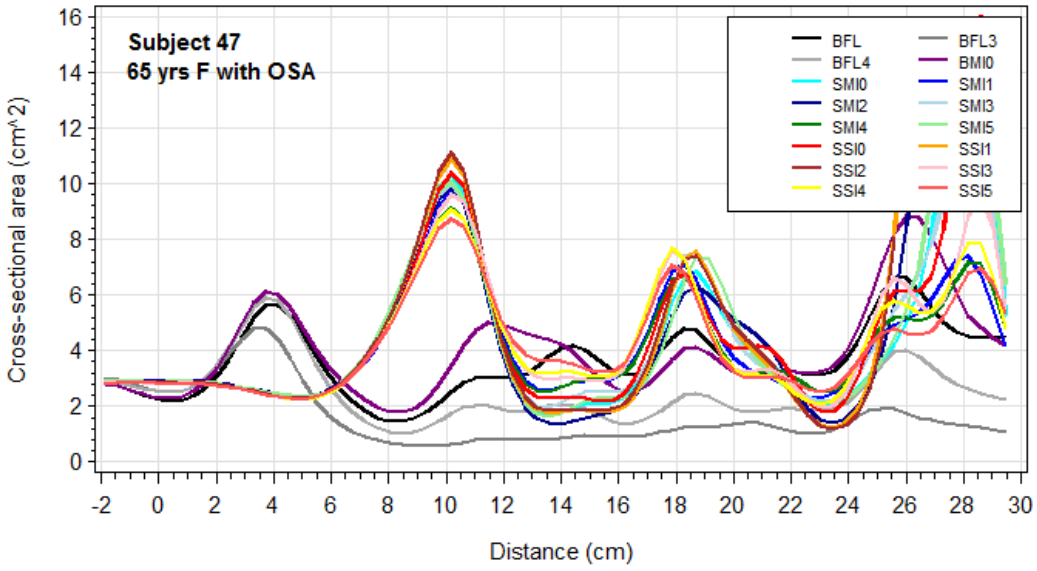

Subject 42
54 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

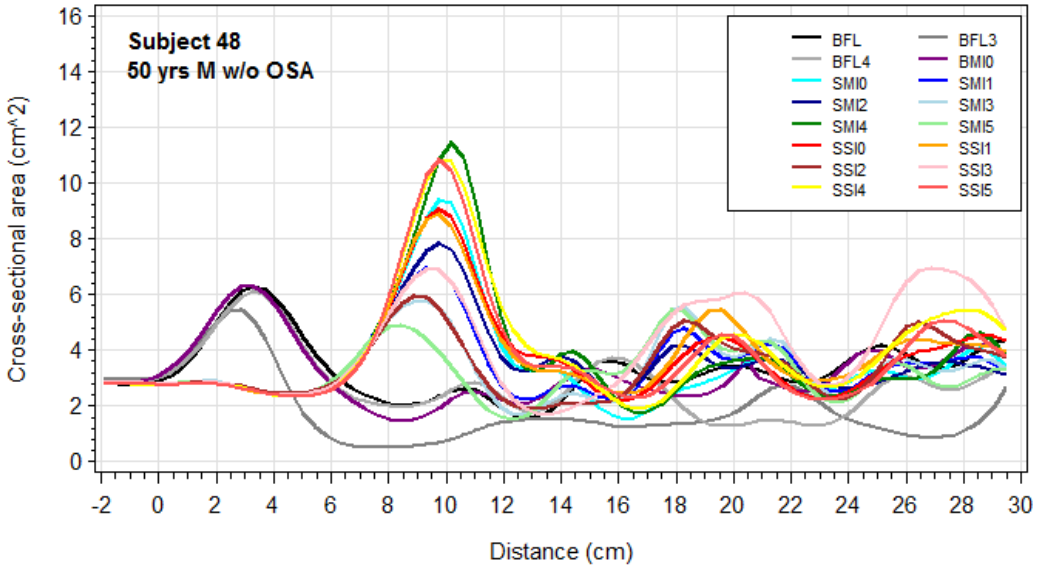

Subject 43
43 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

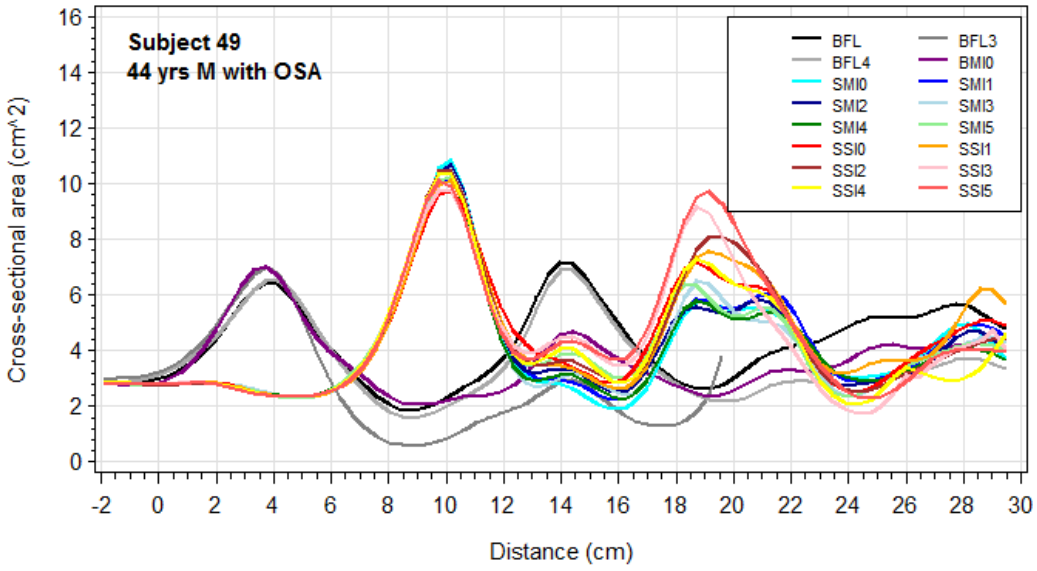

Subject 44
51 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

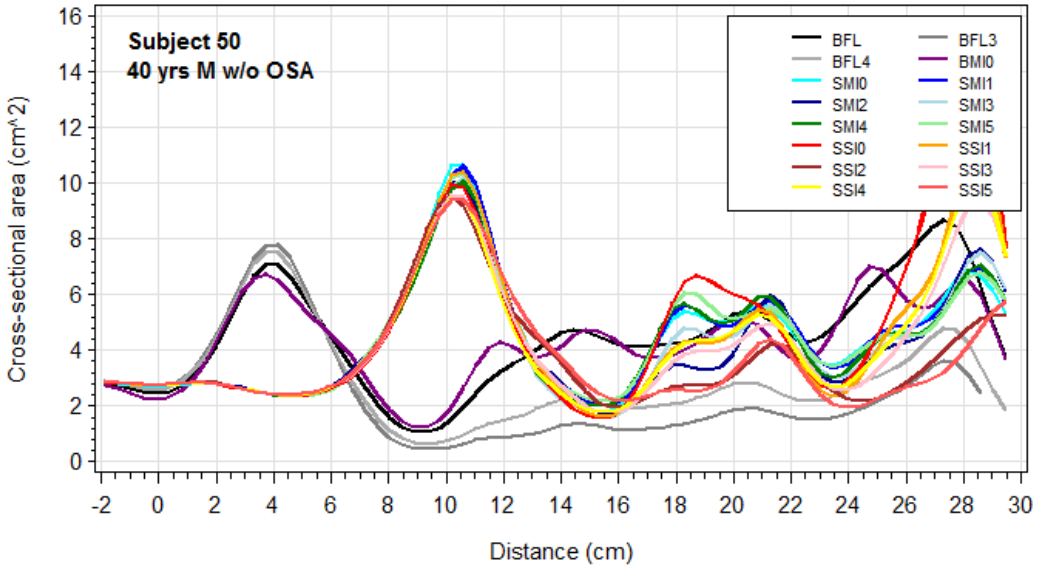

Subject 45
38 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

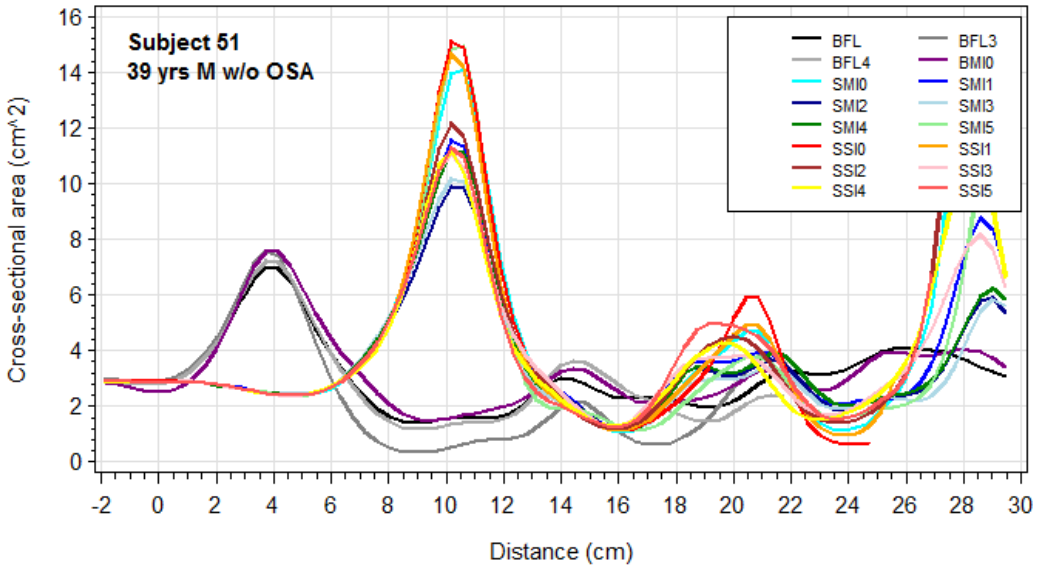

Subject 46
56 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

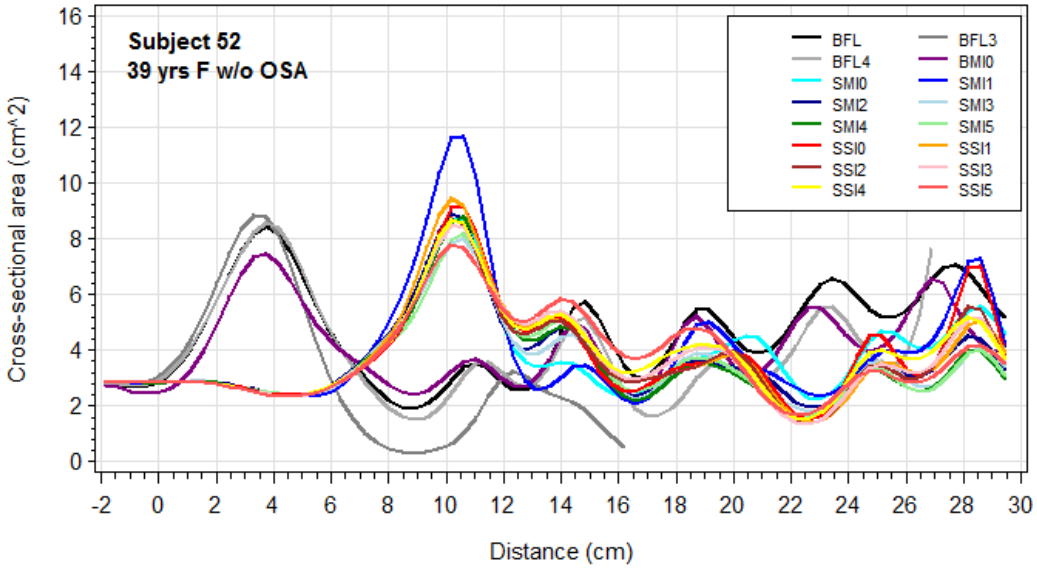

Subject 47
65 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

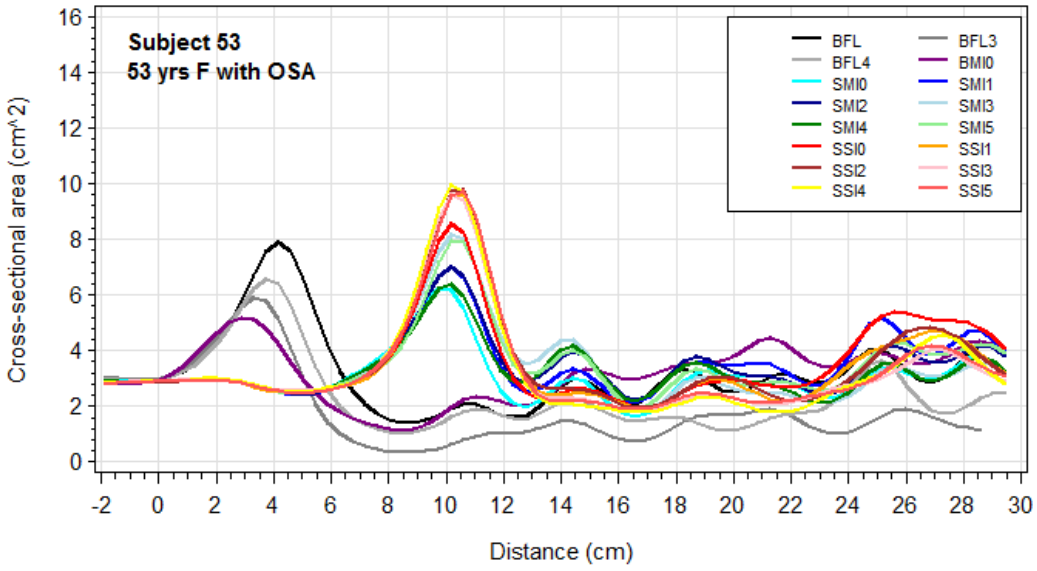

Subject 48
50 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

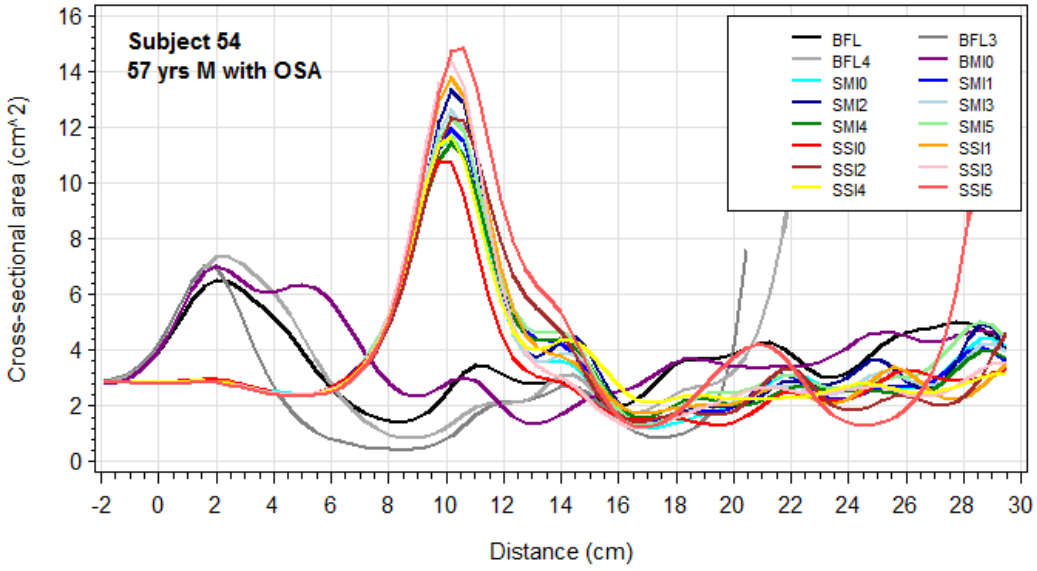

Subject 49
44 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

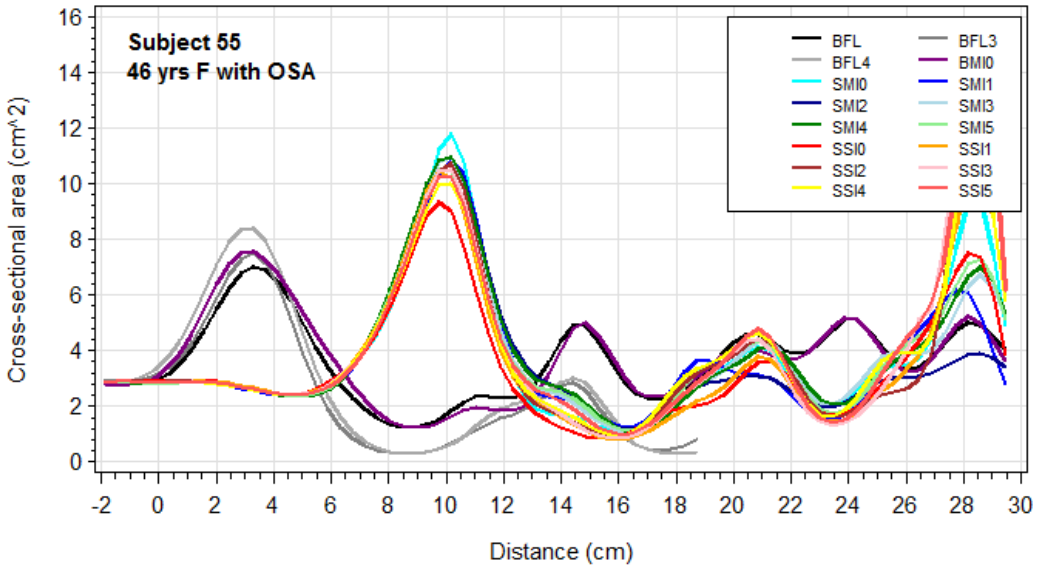

Subject 50
40 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

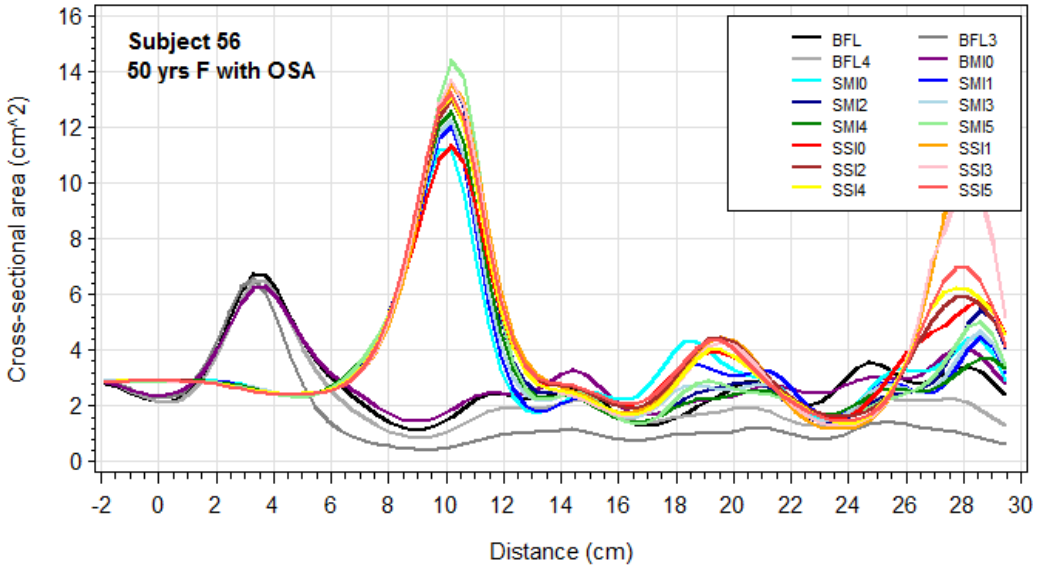

Subject 51
39 yrs M w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

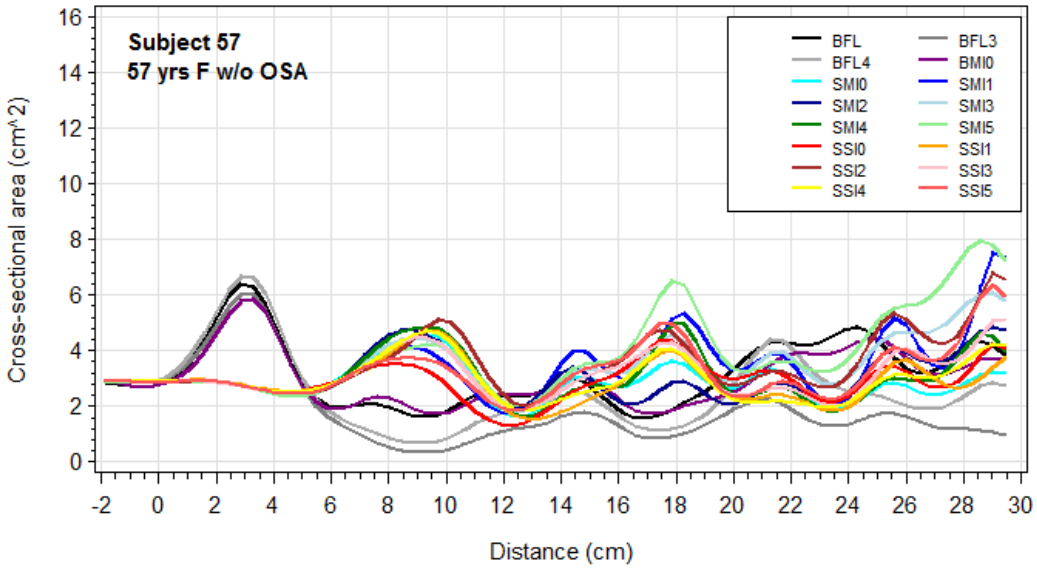

Subject 52
39 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

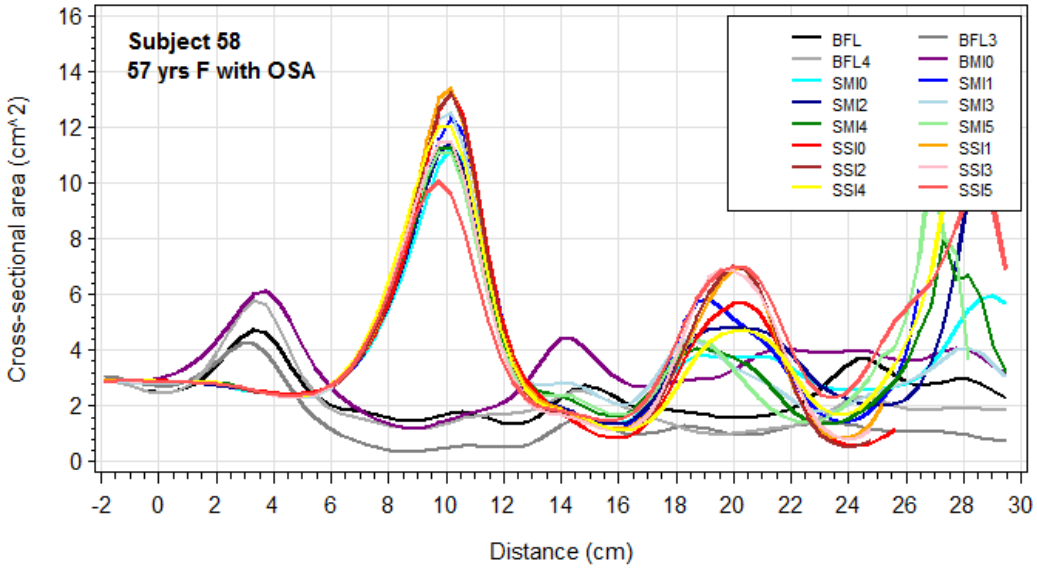

Subject 53
53 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

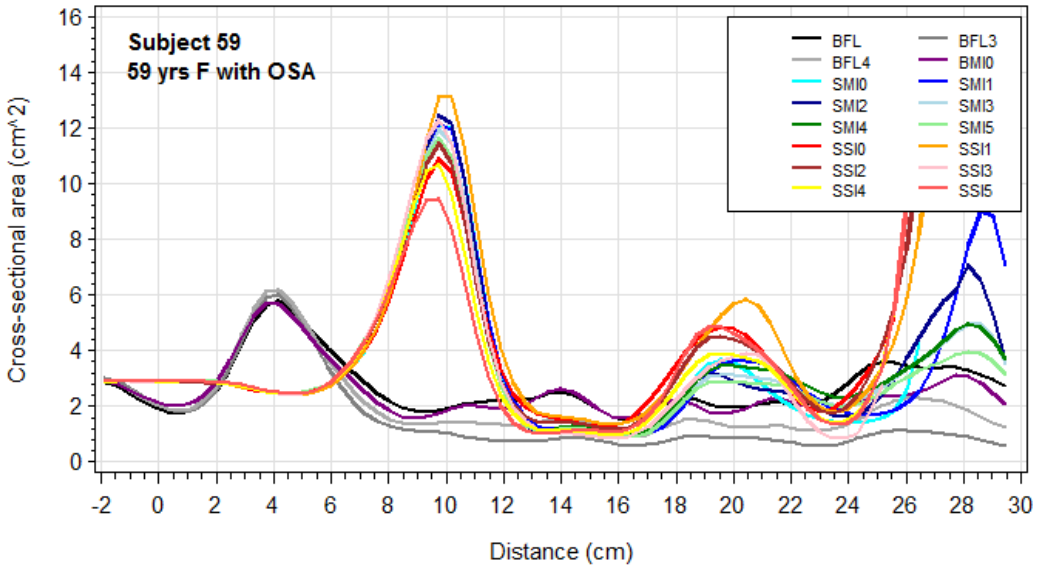

Subject 54
57 yrs M with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)

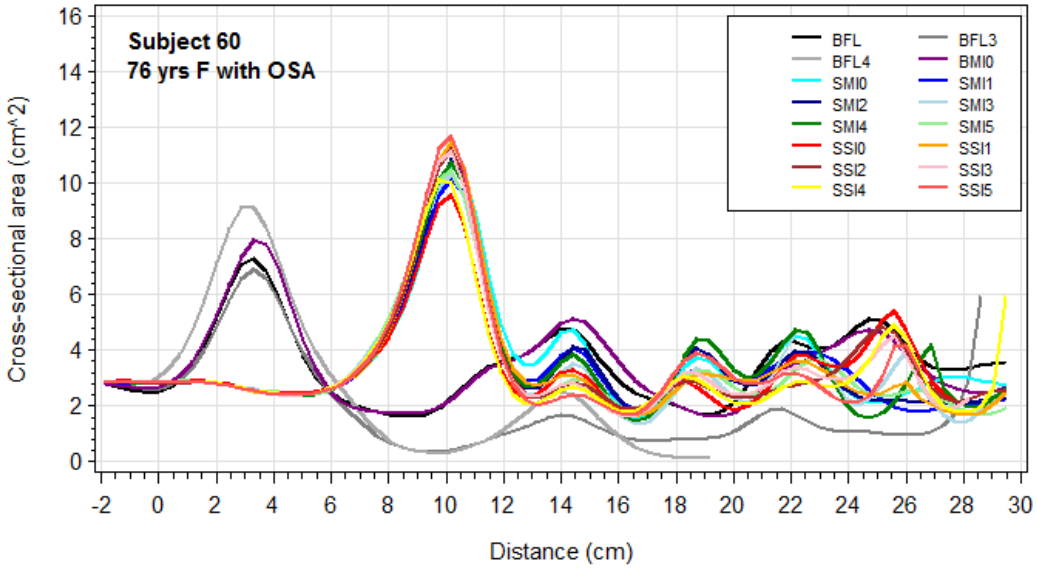

Subject 55
46 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)


Subject 56
50 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)


Subject 57
57 yrs F w/o OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)


Subject 58
57 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)


Subject 59
59 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)


Subject 60
76 yrs F with OSA
BFL
BFL3
BFL4
BMI0
SMI0
SMI1
SMI2
SMI3
SMI4
SMI5
SSI0
SSI1
SSI2
SSI3
SSI4
SSI5
Cross-sectional area (cm^2)
Distance (cm)