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Upper airway collapsibility, dilator muscle activation and resistance in sleep apnoea.

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ABSTRACT.

The calibre of the upper airway is thought dependant upon its passive anatomy/collapsibility and the activation of pharyngeal dilator muscles. Awake, the more collapsible upper airway in obstructive sleep apnea (OSA) increases dilator muscle activity through a negative pressure reflex (NPR).

We hypothesised a direct correlation between the closing pressure of the passive upper airway (Pcrit) as a measure of anatomy/collapsability and EMG activity of genioglossus (GGEMG) and tensor palatini (TPEMG). We also set out to determine the relationship between these indices and pharyngeal resistance (Rphar).

We studied 8 males with OSA aged 48 (46,52) years, [median (IQR)], AHI 75 (65 , 101) / hr on two nights - breathing normally and on nCPAP. Pcrit was measured during NREM sleep on nCPAP using brief, incremental reductions in Pmask. GGEMG and TPEMG were measured breath-by-breath, awake, during sleep onset and on nCPAP. Rphar was measured from airway pressures and flow.

Wakeful GGEMG, early sleep TPEMG and the sleep decrement in TPEMG were directly related to Pcrit. Muscle activation was negatively correlated with Rphar for TPEMG awake and GGEMG early in sleep.

These results confirm that dilator muscle activation is directly related to airway narrowing and reduces resistance across patients with OSA.

INTRODUCTION

The most vulnerable part of the upper airway is that behind the base of the tongue and the soft palate where rigid skeletal support is absent. Here, airway patency is determined by a balance of forces as determined by the interplay of anatomical factors and dilator muscle activation [1] as shown in Figure 1. The neural drive to the upper airway dilator muscles is integrated at the level of brain stem motor nuclei where multiple neural inputs combine to produce a unitary output to the muscles. For example the hypoglossal nucleus which innervates genioglossus (GG) receives multiple inputs from the pre-motor pacemaker neurones of the respiratory pattern generator [2], reflex inputs from the upper airway negative pressure receptors (NPR) [3-7], chemoreceptor inputs [8,9], vagal input due to change in lung volume [10-12], and a wakefulness component from the brainstem reticular formation and other sources [13]. These various neural inputs have been studied in animals [14-16] as well as in humans.

The relative magnitude of each of the components of upper airway muscle activation may vary amongst the sets of upper airway muscles involved and typically some dilators, e.g. genioglossus, have more marked phasic inspiratory activity than others e.g. tensor palatini (TP) the activity of which is less phasically modulated by the respiratory cycle and, in normal subjects at least, is predominantly tonic [17]. Wasicko et al [18] have also shown that added resistive loads lead to differential muscle activation in normals and that these responses are deficient in OSA subjects.

In patients with OSA, anatomical factors resulting in narrowing of the upper airway have been characterised on MRI [19]. These include an increase in lateral wall thickness and fat pad size with reduction in lateral diameter and cross sectional area of the retro-pharyngeal airway. During wakefulness, patency of the narrowed upper airway in OSA is maintained by augmented dilator muscle activation.[20,21]. We have recently shown [22] that this increased activity in OSA patients is largely due to a heightened NPR component as it is markedly reduced by the application of positive airways pressure (CPAP). Even on CPAP however, GG activity (GGEMG) in OSA remains higher during wakefulness and falls further at sleep onset (alpha to theta transitions) than that in normals suggesting that in OSA there is also augmentation of the wakefulness or respiratory pattern generator drives. Furthermore there is recruitment of dilator muscle activation in OSA subjects early after sleep onset suggesting that the NPR remains active to some extent during sleep in this group.

The passive anatomy of the upper airway relates to its structure and collapsibility. This may be assessed during sleep using the passive method for the measurement of critical closing pressure (P_{crit}) described by Schwartz et al [23]. The upper airway dilator muscles remain relatively inactive for several breaths after sudden reduction of continuous positive pressure during sleep and the measured collapsibility reflects the passive anatomical characteristics of the upper airway. This method differs from the earlier method [24] in which airway pressure is gradually reduced and which measures airway collapsibility during sleep with the dilator muscles partially activated by negative pressure. Other methods using imaging techniques for assessing the size, cross-sectional and three dimensional shape of the upper airway with MRI [19] and CT [25,26] have proven useful in identifying differences between OSA patients and normal subjects. These methods however, have not been systematically applied during sleep because of the difficulty in achieving sleep in the scanners or on a breath by breath basis because of the time constraints of image acquisition. In addition, these methods measure anatomical factors with concomitant muscle activation. Philip-Joet et al [27] have demonstrated a linear correlation between muscle activation during sleep and Pcrit measured in the negative pressure activated upper airway of

normal subjects but the relationship between muscle activation and passive collapsibility across sleep onset in OSA has not been previously evaluated.

Our primary aim in this study is to investigate the relationships between passive upper airway collapsibility, dilator muscle activation and airway calibre estimated as pharyngeal resistance across a group of subjects with OSA. We hypothesise that dilator muscle activation is strongly related to passive anatomical calibre estimated as critical closing pressure (Pcrit) and that subjects whose upper airway is more narrow than normal will have greater activation of upper airway muscles. Furthermore, this relationship may be stronger during wakefulness than early after sleep onset when the NPR is less active. We also anticipate a relationship between dilator muscle activation and airway calibre, measured as pharyngeal resistance (R_{phar}), which we predicted would be stronger during wakefulness and weaken during sleep with removal of the wakefulness drive and reduction of the NPR components of muscle activation. We thus set out to measure passive anatomy (P_{crit}), wakeful and sleep muscle activation (GGEMG and TPEMG) and airway calibre as pharyngeal resistance (R_{phar}) and to assess the relationships between these variables across a group of patients with OSA.

METHODS

Subjects

Eight male subjects with OSA were recruited from the sleep clinic. Median (interquartile range) patient characteristics were: - age 48 (46,52) years, BMI 32.8 (29.6, 41.2) kg m^2, and AHI 75 (65, 101) events per hour The 8 subjects undergoing detailed assessment of upper airway mechanics and collapsibility in this report were part of a larger group of OSA subjects in whom the sleep onset effects on dilator muscle activation have recently been published [22]. All 8 subjects in the current study had been on nasal CPAP therapy for at least 3 months and had demonstrated compliance (more than 6 hours per night by history). Nasal CPAP therapy was continued up until the night before these studies. Patients on regular medication other than stable antihypertensive therapy were excluded. Informed consent was obtained from each subject, with the protocol conforming to the principles outlined in the declaration of Helsinki and having the prior approval of the human subjects ethics committee of the Brigham and Womens Hospital.

Protocol

Each subject was studied for 2 nights separated by at least one week. On one night the patient was studied on nasal CPAP for the purpose of measuring airway collapsibility (P_{crit}) during sleep. On the other night spontaneous breathing measures of dilator muscle activation - GGEMG/TPEMG and airway calibre - R_{phar} during wakefulness and sleep were obtained. The

order of the study nights (CPAP or Basal Breathing [BB]) was randomized. Subjects reported to the laboratory at approximately 9 PM, having fasted for at least 4 hours. After obtaining informed consent, the sleep staging electrodes, pressure catheters and intramuscular EMG wires were placed. Subjects then assumed a supine posture in bed, and the nasal mask was attached. Subjects subsequently lay with eyes open in this posture, and were allowed to acclimate to the equipment

Data recording

The laboratory procedures, EMG recordings, and measurement of ventilation and resistance were conducted as previously described [5,17]. In order to assess sleep-wake state, subjects were instrumented with 2 channels of electroencephalography (EEG), 2 channels of electro-oculography (EOG) and chin EMG.

All signals (GGEMG and TPEMG [raw and an electronically derived MTA], airway pressures [mask, choanal and epiglottic], ETCO2, EKG, sleep staging, and inspiratory flow) were recorded on a 16-channel Grass model 78 polygraph (Grass Instruments, Astro-Med, Inc., West Warwick, RI). Certain signals (GGEMG, TPEMG, airway pressures, EEG, inspiratory flow and EKG) were also recorded onto computer using data acquisition software (Spike 2; version 3.17, Cambridge Electronic Design, Ltd, Cambridge, UK).

For each individual, the occipital EEG during each breath was assessed as being predominantly α or θ, as previously described [28,29].

<u>Airway mechanics:</u>

Subjects wore a nasal mask (Respironics, Inc. Murraysville, PA, USA) connected to a non-rebreathing valve. Inspiratory and expiratory airflow were determined with a pneumotachometer (model 3700A, Hans Rudolph Inc. Kansas City, MO) and differential pressure transducer (Validyne Corp., Northridge, CA.), calibrated with a rotameter. Subjects were instructed to breathe exclusively through the nose and were carefully monitored by video camera to ensure that the mouth was completely closed. During sleep, the mouth was taped shut, in order to minimize mouth breathing. Mask leak was detected from a perforated catheter surrounding the mask-face interface, which continuously sampled for CO_2. In addition, end tidal PCO_2 ($PetCO_2$) was monitored from the mask using an infrared analyzer (Capnograph Monitor, BCI, Waukesha, WI).

Pressures were monitored in the mask with an open catheter attached to a pressure transducer (Validyne Corp) and in the airway at the levels of the posterior choanae and the epiglottis using 2 pressure-tipped catheters (MPC-500, Millar, Houston, Tx). One nostril was decongested (oxymetazalone HCl) and anesthetized (lidocaine HCl), and the Millar catheters were inserted

through this nostril and localized at the posterior choanae and the epiglottis. Prior to insertion, the pressure signals were calibrated simultaneously in a rigid cylinder using a standard water manometer. These pressure signals plus flow were demonstrated to be without amplitude or phase lags at up to 2 Hz. Any drift in the pressure catheters was corrected on a breath by breath basis by an automated computer program that defined end-inspiration and end-expiration by identifying the point of zero flow and then correcting any offset in the pressure signals. Minute ventilation and pharyngeal resistance - measured as peak inspiratory resistance (R_{phar}) and at a fixed flow rate [0.2 l/s] were calculated on a breath by breath basis, pharyngeal resistance being the pressure difference between the choanae and the epiglottis related to flow. Our automated analysis program was modified to allow analysis of breaths with complete airway obstruction (as occurred in some OSA patients on the normal breathing night). The algorithm initially used the flow signal to define the beginning and end of each inspiration. However, if respiratory related variations in pressure were detected in the absence of variations in flow, the computer then used the epiglottic pressure signal to define the beginning and end of inspiration. In addition, on breaths with extremely low flow rates, resistance values were set at a maximum of 99 cm H20/l/s.

Muscle activation:

The GGEMG was measured with a pair of unipolar intramuscular electrodes referenced to a single ground, producing a bipolar recording. Two stainless steel Teflon-coated 30-gauge wire electrodes were inserted 15-20 mm into the body of the genioglossal muscle 3 mm lateral to the frenulum on each side, using a 25-gauge needle, which was quickly removed, leaving the wires in place. This technique has been used previously in our laboratory [5, 28-32]. TPEMG was measured in a similar manner to that of the GG muscle, using a pair of referenced unipolar intramuscular electrodes producing a bipolar recording. The tip of the pterygoid hamulus was located at the junction of the hard and soft palate, on each side of the palate. A 25 gauge needle with a 30 gauge stainless steel Teflon-coated wire was then inserted at a 45 degree angle along the lateral surface of the medial pterygoid plate, to a depth of approximately 10-15 mm into the palate. The needle was then removed, leaving the electrode in place. To establish maximal EMG activation and to confirm GG and TP electrode placement, the following respiratory maneuvers, which we have documented previously [33], were performed: maximal tongue protrusion, sucking, blowing and swallowing. These manoeuvres were performed in at least triplicate until reproducible EMG activation by each was obtained.

The raw EMG signal was amplified to provide an easily visible phasic inspiratory signal during quiet breathing (Grass Instruments, Quincy, Ma.), band pass filtered (between 30 and 1000Hz), and stored for subsequent data analysis by computer software. Sections of the recording

containing movement or other artifacts were removed before analysis. The raw EMG signals for the muscles were then integrated using a 100 ms moving time average (MTA). Several values were calculated on a breath by breath basis for each muscle. The first was the *tonic level*. Activity in the expiratory phase was divided into 10 equal segments and the level of tonic activity of the muscle was defined as the activity of the lowest segment. The second was *peak activity*, which was defined as the highest value that occurred during inspiration. The third was *phasic activity,* which was defined as the difference between peak activity and tonic activity. In order to allow comparison between subjects and between the CPAP and control nights, the GGEMG and TPEMG were quantified as a percent of the maximal total activity observed during forced tongue protrusion as previously described [33]. The tonic, phasic, and peak EMG activity for each muscle for each breath were then expressed as a percentage of the maximal values.

Comparison of the magnitude of EMG activation between swallows which remained stable across the night was used to establish that changes in EMG electrode impedance had not occurred.

Basal Breathing Night:

After recording data during stable wakefulness, subjects were allowed to fall asleep (remaining in the supine posture). On the BB night, in order to obtain multiple sleep onsets (alpha – theta transitions) during normal breathing, subjects were awoken if they slept for 5 consecutive minutes without spontaneous awakenings, and were thereafter allowed to fall asleep again. This procedure was repeated until approximately 4 hours of data had been collected.

On the BB night, once each breath had been classified as α or θ, computer software was used to identify sets of consecutive breaths occurring on either side of an α to θ transition. An adequate alpha-theta transition was defined by having at least 3 consecutive alpha breaths followed by at least 2 consecutive theta breaths. Each breath in the transition was then assigned a position relative to the transition from –5 to +3 as previously described [28]. For each subject, the following parameters were averaged at each breath position: V_E, R_{phar}, GGEMG and TPEMG (as % of maximal activity). Waking values for each variable were determined from the initial stable wakeful period at the start of the night and also from the mean alpha level (-5 to –1) immediately before sleep onset. Sleep values were measured.for each theta breath (+1 to +3) following sleep onset and compared with the mean alpha level preceding sleep onset..

Determination of airway collapsibility (P_{crit}): CPAP night

On the CPAP night, subjects were studied using the same nasal mask with CPAP applied. Initially 5 to 10 minutes of data wererecorded during quiet wakefulness lying supine without CPAP in order to estimate baseline GGEMG/TPEMG. Nasal CPAP was then applied. Subjects

breathed through a nasal mask connected to a modified CPAP device incorporating a valve for rapidly switching between 2 preset levels of positive or negative pressure (Respironics, Inc, Murraysville PA, USA). CPAP was initially at the patients previously prescribed pressure and, if flow limitation was noted during sleep, the pressure was increased until this was abolished and the minimum level of GGEMG was obtained.

P_{crit} passive was measured by the method of Schwartz et al [23]. After the patient had been in stable stage 2 sleep for at least 10 minutes without flow limitation, transient decrements in pressure for 3 breaths were applied by switching between breaths to a reduced level of CPAP. Pressure decrements started at 2cmH20 below baseline CPAP and increased progressively by a further 2cm from baseline. These were applied repeatedly after intervals ensuring return of EMG activity to stable baseline until flow limitation to complete cessation of airflow or arousal occurred. When arousal occurred, that pressure decrement series was terminated and the patient was allowed to return to stable stage 2 sleep for at least 5 minutes before another series of progressive pressure decrements was applied. In each patient, data from at least 4 such series was collected.

To measure critical airway closing pressure by the passive method, nasal pressure and maximal flow values for each pressure decrement were plotted for flow limited breaths in each patient. The P_{crit} value was taken as the intercept on the nasal pressure axis at zero maximal flow established as a best fit for all pressure decrement series for that patient .

Statistical Analyses:

All statistical analyses were performed with commercially available software (SigmaStat version 3.0 +, SPSS 12.01, SPSS corp, Chicago). Correlation between passive airway closing pressure (P_{crit}), dilator muscle EMG (GG EMG/TPEMG) and pharyngeal resistance (R_{phar}) was performed using Spearman's rank test. For all analyses, alpha was set at 0.05. Results are presented as Spearman correlation coefficients and p values with lines of best fit determined by linear correlation. The Mann Whitney U test was used to compare wake with sleep data.. Only BB night measures of EMG and R_{phar} were used to assess correlation between collapsibility, muscle activation and resistance.

RESULTS

Patient demographics are presented in table1. Raw data from one subject's CPAP/P_{crit} night showing a run of progressive pressure decrements resulting in flow limitation is shown in figure 2. Note the relatively stable GGEMG recording demonstrating little muscle recruitment during the first few breaths following the pressure decrements.

The determination of the passive airway closing pressure (P_{crit}) is shown in figure 3 for one of the subjects. The intercept on the Pnasal axis at zero flow determined by the line of best fit is taken as that patient's value of P_{crit}.

Median (25, 75 percentile) values for the main study variables awake and for the second and third theta breaths (S2,3) during BB are shown for the 8 OSA subjects in table 1. Data were obtained in all 8 OSA subjects for P_{crit}, GGEMG/TPEMG, V_E, and R_{phar}.

***Sleep onset effects**:*

On the basal breathing night, R_{phar} increased dramatically and significantly between wake and sleep breaths 2 and 3 (both $p<0.05$) during BB. Typically, apnea then arousal occurred within 3-4 breaths after sleep onset in these patients with severe OSA. In 7 of the 8 patients GGEMG and TPEMG either decreased or changed little between wake and the second and third sleep breaths consistent with our previous studies of normal subjects [28,29] and in a larger group of OSA patients which included those in the present study [22]. Tonic activity of both muscles but particularly TPEMG fell at sleep onset (wake v's sleep breaths 2 and 3 on the BB night) but none of these differences between wake and sleep EMG reached statistical significance for either muscle. In one subject there was an unexpected and substantial increase in both GGEMG and TPEMG from wakefulness to sleep although his Rphar increased from wake to sleep as did the other subjects. We could find no explanation to account for the increase in muscle activity in this one subject. Correlation analyses were performed both excluding (n=7) and including (n=8) this subject. The latter yielded slightly lower r values for most of the correlations.

***Relationship between P_{crit} and waking upper airway muscle activation**.*

The results of linear correlation analyses (Spearman rank test) between P_{crit} and BB EMG activation during stable wakefulness, expressed as % maximal activation, are shown for the 7 subjects in whom muscle activation fell at sleep onset in table 2.

Waking peak GGEMG as % maximal showed a significant relationship with P_{crit} ($r=0.79$, $p=0.02$) as shown in Figure 4a. This relationship was also seen for phasic GGEMG ($r=0.82$, $p=0.01$) and a trend for tonic GGEMG ($r=0.68$, $p=0.07$). The relationships of waking TPEMG (% maximal) with P_{crit} were not significant.

***Relationship between P_{crit} and upper airway muscle activation after sleep onset** :*

For GGEMG, the relationship to P_{crit} weakened becoming non-significant during sleep. For TPEMG, activity at the second and third breaths following onset of theta activity (S2, S3) became more strongly related to P_{crit} than during wakefulness and, for peak activity, the relationship was significant at the second breath after sleep onset ($r=0.75$, $p=0.04$) as shown in Fig 4b.

Relationship between P_{crit} and the fall in muscle activation at sleep onset:

The fall in TPEMG activity between wakefulness (breaths –5 to –1) and sleep (breaths +2, +3) was also significantly and positively related to P_{crit}. Correlation coefficients for TPEMG (Wake – S3) v's P_{crit} were significant for peak (r=0.71, p=0.05) and phasic (r=0.89. p=0.01) components. These relationships did not reach significance for GGEMG.

Relationship between upper airway muscle activation, R_{phar} and their changes at sleep onset.:

Waking TPEMG peak (%maximum) activity was significantly and negatively related to wakeful R_{phar} (r = -0.78, p = 0.03) during BB as shown in figure 5a. This relationship was not significant for GGEMG.

During sleep (S3) GGEMG was significantly related to sleep R_{phar} (r=-0.82, p=0.05) as shown in Fig 5b whereas for TP these relationships were not significant.

The rise in R_{phar} at sleep onset (Wake – S3) was strongly related to wakeful baseline TPEMG for both peak and tonic activity (r=0.94, p=0.01). It was not significantly related to the fall in muscle activation at sleep onset.

Relationship between muscle activation and other factors:

The results of the correlations between GGEMG and TPEMG with body mass index are also shown in table 2. Generally, waking GGEMG showed a weaker relationship with BMI than with P_{crit}. Waking TPEMG was not significantly related to BMI and, for GGEMG, the relationship with BMI weakened during sleep.

Other Relationships

R_{phar} measured during BB was not significantly related to P_{crit} either awake (r=-0.21, p=0.60) or after sleep onset (S2: r=-0.46, p=0.25, S3: r=-0.60, p=0.24). Similarly, R_{phar} was not significantly related to BMI in wakefulness or sleep across the group.

DISCUSSION

In this study we set out to determine the relationship between upper airway calibre evaluated as pharyngeal resistance and its two principal determinants – 1) the passive anatomical structure / collapsibility of the upper airway and 2) the activation of upper airway dilator muscles across a group of patients with OSA.. We have observed significant direct relationships with P_{crit} for both waking GGEMG and early sleep TPEMG. Waking TPEMG was also inversely related to R_{phar} across the group. R_{phar} itself was not correlated with P_{crit}. These findings taken together are consistent with the hypothesis that narrowing of a more collapsible upper airway augments dilator muscle activation via the negative pressure reflex to widen the upper airway and that this upper airway muscle response reduces pharyngeal resistance most in those with most active dilator activation.

We have previously shown in OSA patients (31) and in normal subjects (31,34)that increasing negative pressure in the upper airway with the addition of an inspiratory resistive load or using a negative pressure ventilator] causes an increase in GGEMG within the individual that is proportional to both the increased epiglottic pressure and the pharyngeal resistance that develops. The current findings suggest that this negative pressure reflex is consistent between patients with sleep apnoea as well as within the individual patient.

The correlation between GG muscle activation and upper airway collapsability (P_{crit}) during wakefulness is explained by a narrower, more collapsible upper airway (high Pcrit) experiencing more negative inspiratory pressure and activation of the NPR to augment dilator muscle activation. This relationship weakens in the early breaths after sleep onset in this study. We have previously demonstrated a loss of dilator muscle activation for GG (and for TP) at sleep-onset in normal subjects and in patients with OSA .[28,29,32,35]. These findings are consistent with diminution of both the augmented wakeful NPR and the phasic central pattern generator components of GG activation at the point of sleep onset when the wakefulness drive is lost.

In the current study of patients with OSA, the tonic activation of both muscles but particularly that of the predominantly tonic TPEMG tends to diminish at sleep onset. This diminution in tone at sleep onset has been observed in our previous studies of normal subjects (29) and OSA patients (22) and would likely have reached significance in this study given a larger number of subjects. The relationship between TP muscle activation and P_{crit} is weak before but becomes more strongly positive after sleep onset and is significant by the second sleep breath. This suggests that the NPR is still present during early sleep. Reduction of phasic GG activity at sleep onset may result in the relationship between activation of the more tonic TP and airway collapsibility (via negative pressure in the airway lumen) becoming more clearly apparent.

The direct influence of upper airway dilator muscle activation on airway calibre is seen in the negative correlations between wakeful TPEMG and R_{phar} and between GGEMG after sleep onset (S3) and R_{phar}. These are consistent with NPR-augmented activation of TP during wakefulness in OSA subjects serving to decrease upper airway resistance in compensation for the airway narrowing. If this compensatory mechanism were completely efficient one would expect no correlation across subjects between these two variables. The presence of a significant inter-individual relationship between GGEMG and R_{phar} during the early sleep breaths is also consistent with higher activation of GG early after sleep onset in those subjects with a narrower upper airway. We have previously documented the NPR as an intra-individual muscle activation / epiglottic pressure (dEMG/dPepi) relationship in normal subjects (31,34) and OSA patients (31). The current study suggests that amongst OSA patients higher muscle activation results in better preserved airway calibre.

The magnitude of the increase in R_{phar} at sleep onset was also positively correlated with wakeful TPEMG activity. This is consistent with the greatest sleep onset related rise in airway resistance occurring in those subjects with the most NPR-activated tonic muscle activity during wakefulness. The lack of a significant relationship between TPEMG and R_{phar} during sleep in this study is also consistent with our previous finding of lesser recruitment of TP than of GG by the NPR during sleep in OSA [35] and in normals [28]. The lack of a correlation between P_{crit} and R_{phar} during wakefulness is not unexpected given the intermediate influence of dilator muscle activation to reduce Rphar below its passive value during wakefulness.

This study is the first to document the relationship between muscle activation and passive airway collapsibility across sleep onset in patients with OSA. One previous study in a group of normal subjects by Philip-Joet et al [27] demonstrated that genioglossal activation during stable sleep is positively correlated with Pcrit measured with the upper airway muscles activated by continuous negative airway pressure but there have been noprevious studies relating muscle activation and resistance both awake and at sleep onset to measures of the passive upper airway.

We have also demonstrated that dilator muscle activation correlates less well with body mass index than with passive P_{crit}. The strength of this correlation would not be expected to be as high as that between muscle activation and P_{crit} because BMI is a general anthropometric measure rather than a specific measure of upper airway calibre. Craniofacial anatomical factors independent of body weight and body fat distribution are also important in determining upper airway anatomy. The influence of craniofacial characteristics on closing pressure and site of collapse of the passive upper airway in OSA has previously been demonstrated in anaesthetised humans [36] . This is separate from the lateral pharyngeal wall fat deposition effect of obesity

and the lung volume effect of reduction in functional residual capacity leading to reduced caudal traction on the upper airway thus increasing its resistance [37]. All of the subjects in this study were overweight or obese with their mean BMI clearly in the obese range at 32.8. Our findings may thus not be generalisable to non-obese OSA subjects in whom other mechanisms such as cranio-facial abnormalities or respiratory control factors are primarily responsible for the sleep disordered breathing.

One limitation of the current study is that pharyngeal resistance increases substantially in the first few breaths after sleep onset often precipitating apnoea which then results in arousal making it difficult to measure sleep effects on R_{phar} in severe OSA patients. In this study, there was frequent rapid cycling between wakefulness and sleep with flow limitation or apnoea repeatedly resulting in arousal within 3-4 breaths of sleep onset. It was thus not possible to study muscle activation and upper airway mechanics deeper than 3-4 breaths into sleep. Also, when airflow limitation develops, inspiratory flow becomes independent of downstream (epiglottic) pressure upon which our R_{phar} measure depends. In the present study, correlations with pharyngeal resistance measured at low flow (200ml/se) were of similar strength to those with peak inspiratory resistance ($R_{phar)}$ suggesting that our data is not seriously affected by this consideration. Our use of rank correlation analysis is also appropriate in this regard.

It is also important to note that the OSA subjects in this study had all been on nasal CPAP therapy for at least 3 months with good compliance. It is possible that treatment may have improved upper airway muscle function and reflexes so that the results may not be fully applicable to untreated OSA patients. Another potential limitation of this study is the confounding effect of the instrumentation required to measure airway mechanics (Millar catheters at posterior choanae and epiglottis via the nasal cavity) and muscle activation (intramuscular wire electrodes) on arousal threshold. These may make arousal more likely to occur during the airway pressure decrements used to measure the passive P_{crit} (breaths in which arousal is observed need to be excluded from analysis). This effect appears to be less of a problem in OSA patients than in similarly instrumented normal subjects in whom we have found it more difficult to reliably obtain consistent flow limitation without arousal as needed for P_{crit} determination. This is clearly less of a problem when P_{crit} measurements are made using nasal pressure without instrumentation of the upper airway as originally described by Schwartz et al [23,24].

In summary we have shown that dilator muscle activation relates both to anatomical airway narrowing / collapsibility (Pcrit) and to airway patency (Rphar) across a group of subjects with OSA. These findings are consistent with the model of passive anatomical factors and muscle

activation (in part driven by the NPR) being the principal determinants of pharyngeal patency. The relationship between Pcrit and GG muscle activation weakens when dilator tone diminishes with loss of the wakefulness drive at sleep-onset but, for the activity of TP, it becomes stronger suggesting persistence of the NPR during early sleep. Upper airway muscle activation is more strongly related to airway collapsibility (Pcrit) than to BMI, reflecting the fact that obesity is only one of several factors narrowing the airway in patients with OSA.

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Table 1: DEMOGRAPHICS AND STUDY VARIABLES FOR THE 8 OSA PATIENTS

Median (25,75 percentiles)

Age	48(46,52) years		
BMI	32.8 (29.6, 41.2) kg/m^2		
AHI	75/ (65, 101) / hr		
Pcrit	2,4 (0.6, 3.6) cmH20		
	Wake	Sleep, Breath 2	Sleep, Breath 3
GG EMG %			
Max	7.6 (5.6, 9.8)	8.4 (7.6, 10.7)	10.9 (8.2, 13.2)
Peak	4.0 (2.9, 4.5)	5.4 (4.6, 5.8)	6.3 (4.9, 7.8)
Phasic	3.9 (3.0, 5.2)	3.0 (2.8, 5.5)	3.7 (3.5, 5.7)
Tonic			
TP EMG %	9.4 (4.5, 21.4)	6.0 (4.3, 10.4)	5.9 (4.1, 10.2)
Max	1.5 (0.7, 3.9)	3.4 (1.8, 6.5)	3.7 (1.9, 5.8)
Peak	6.8 (3.8, 14.5)	2.6 (2.1, 4.1)	2.6 (2.1, 4.4)
Phasic			
Tonic			
R phar	1.7 (0.9, 2.6)	5.6 (3.2, 7.1) *	4.1 (3.4 , 6.2) *

* = P< 0.05, Mann-Whitney test: v's BB wake

** = P< 0.05, Mann-Whitney test: v's CPAP wake

Table 2: SPEARMAN RANK CORRELATIONS BETWEEN STUDY VARIABLES (n=7)

		Pcrit		R phar wake		BMI	
		r	p	r	p	r	p
AWAKE							
GGEMG % max	peak	0.79	0.02	0.00	0.97	0.66	0.07
	phasic	0.82	0.01	0.00	0.97	0.68	0.07
	tonic	0.68	0.07	-0.29	0.49	0.39	0.34
TPEMG % max	peak	-0.04	0.91	-0.78	0.03	0.10	0.78
	phasic	0.46	0.25	0.18	0.66	0.50	0.21
	tonic	-0.14	0.72	-0.64	0.09	0.17	0.66
SLEEP Breath 2				Rphar Sleep, Br2			
GGEMG % max	peak	0.07	0.84	043	0.30	-0.29	0.49
	phasic	0.32	0.43	0.11	0.78	-0.07	0.84
	tonic	0.001	0.97	0.34	099	-0.21	0.60
TPEMG % max	peak	0.75	0.04	-0.50	0.21	0.53	0.18
	phasic	0.64	0.09	-0.39	0.34	0.50	0.21
	tonic	0.42	0.30	-0.54	0.18	0.64	0.09
SLEEP Breath 3				Rphar Sleep, Br3			
GGEMG % max	peak	0.32	0.44	-0.82	0.05	0.17	0.66
	phasic	0.64	0.09	-0.82	0.05	0.11	0.78
	tonic	-0.14	0.72	-0.60	0.24	-0.14	0.72
TPEMG % max							
	peak	0.64	0.09	-0.02	1.00	0.55	0.22
	phasic	0.61	0.12	-0.02	1.00	0.39	0.34
	tonic	0.42	0.30	0.14	0.80	0.64	0.09

Fig 1

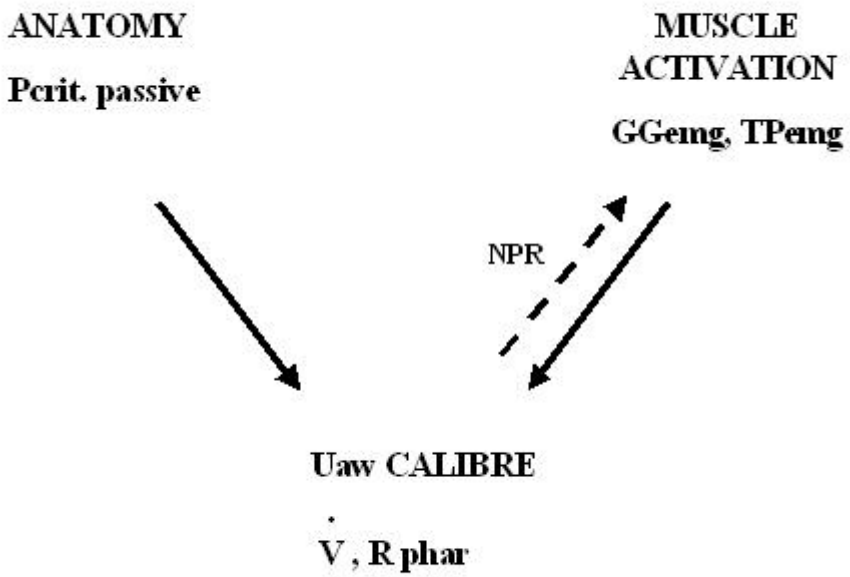


Fig 2

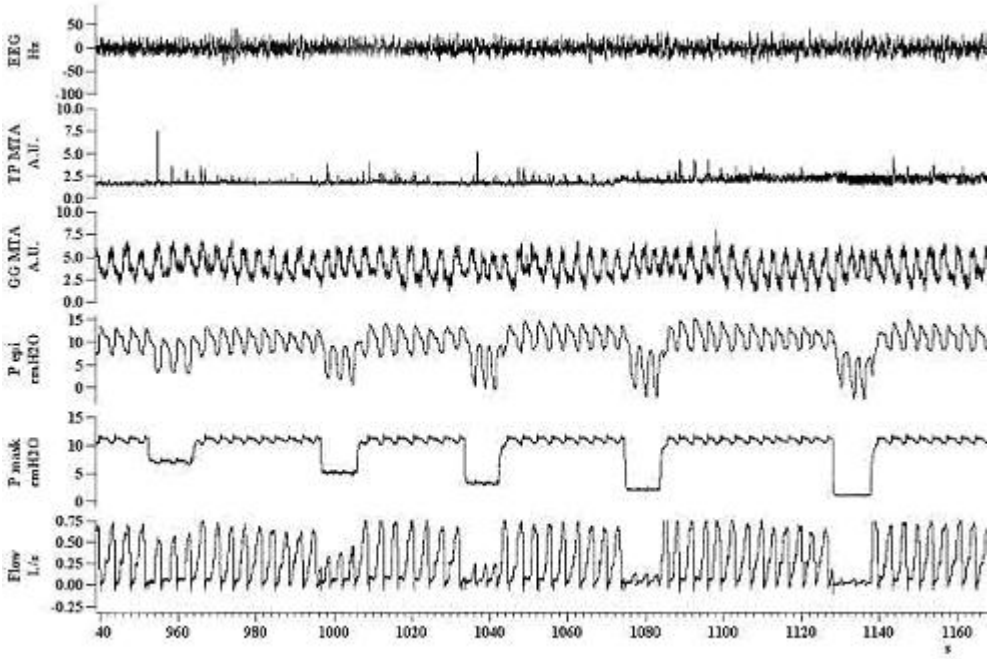


Fig 3

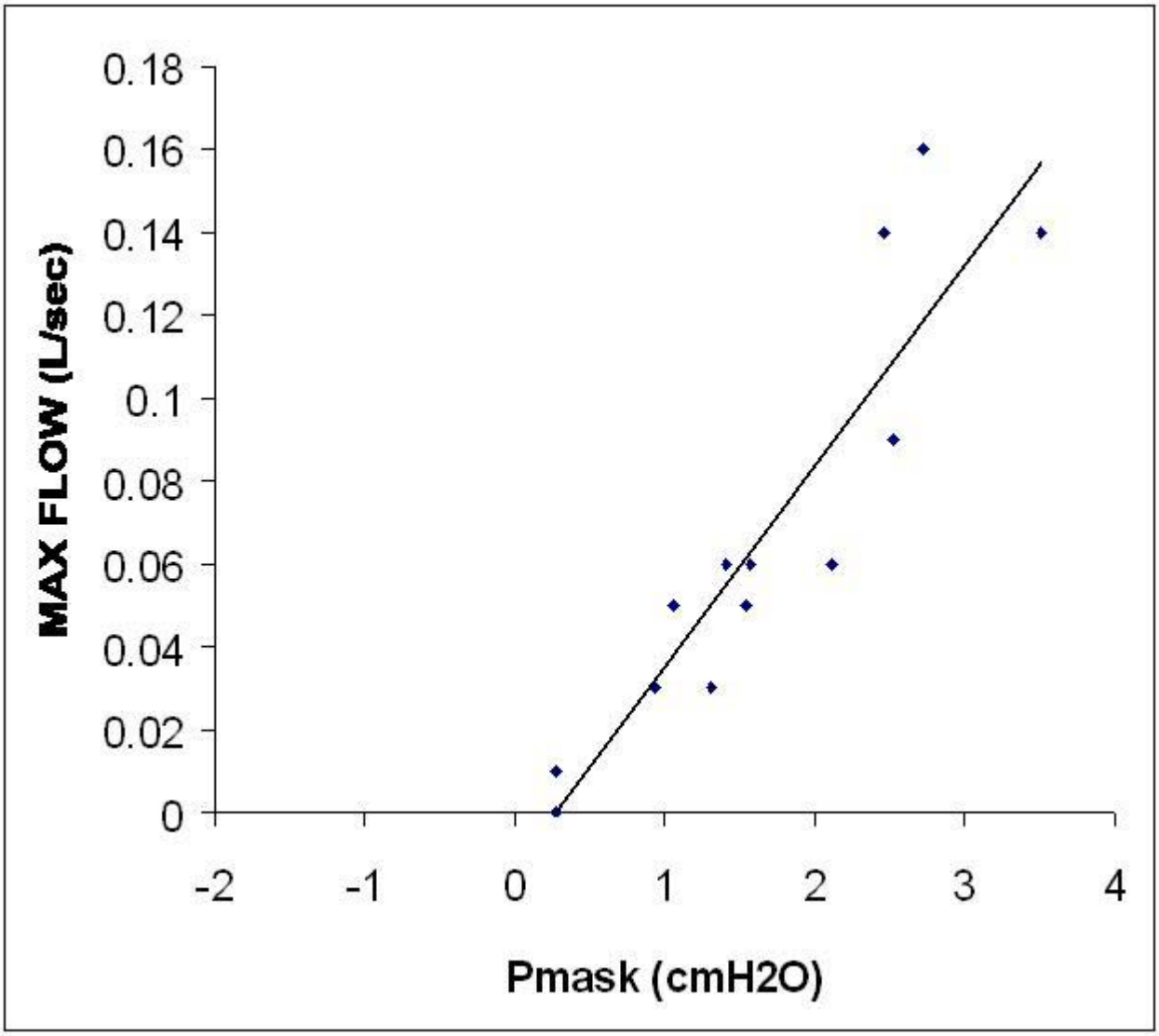

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
MAX FLOW (L/sec)
-2
-1
0
1
2
3
4
Pmask (cmH2O)

Fig 4

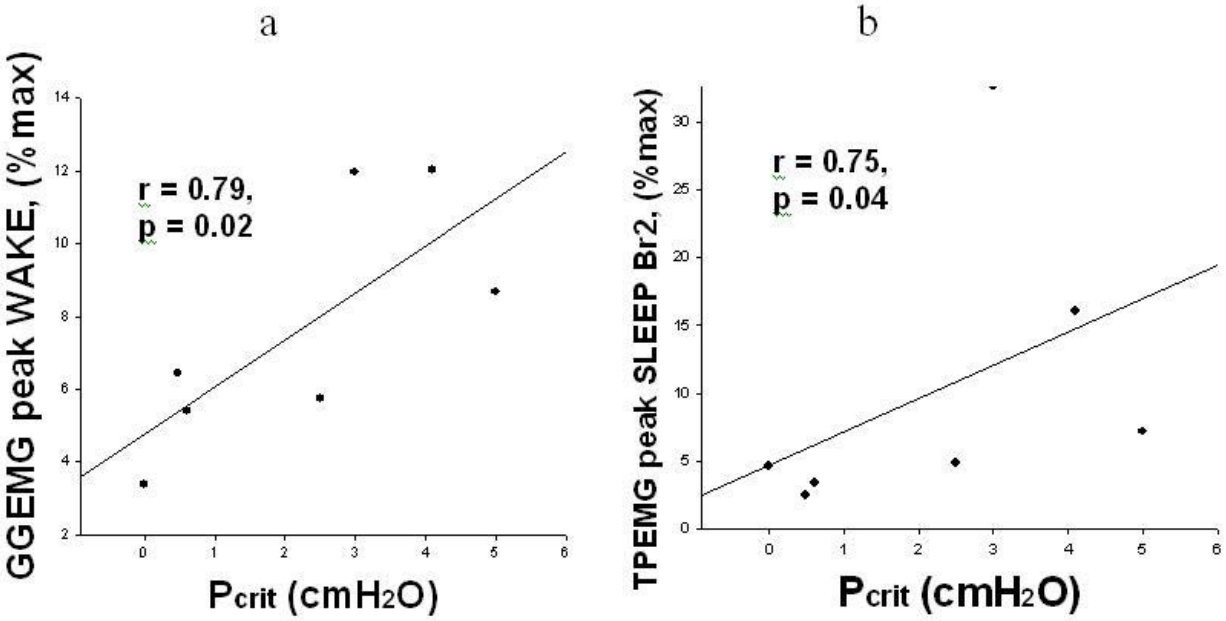
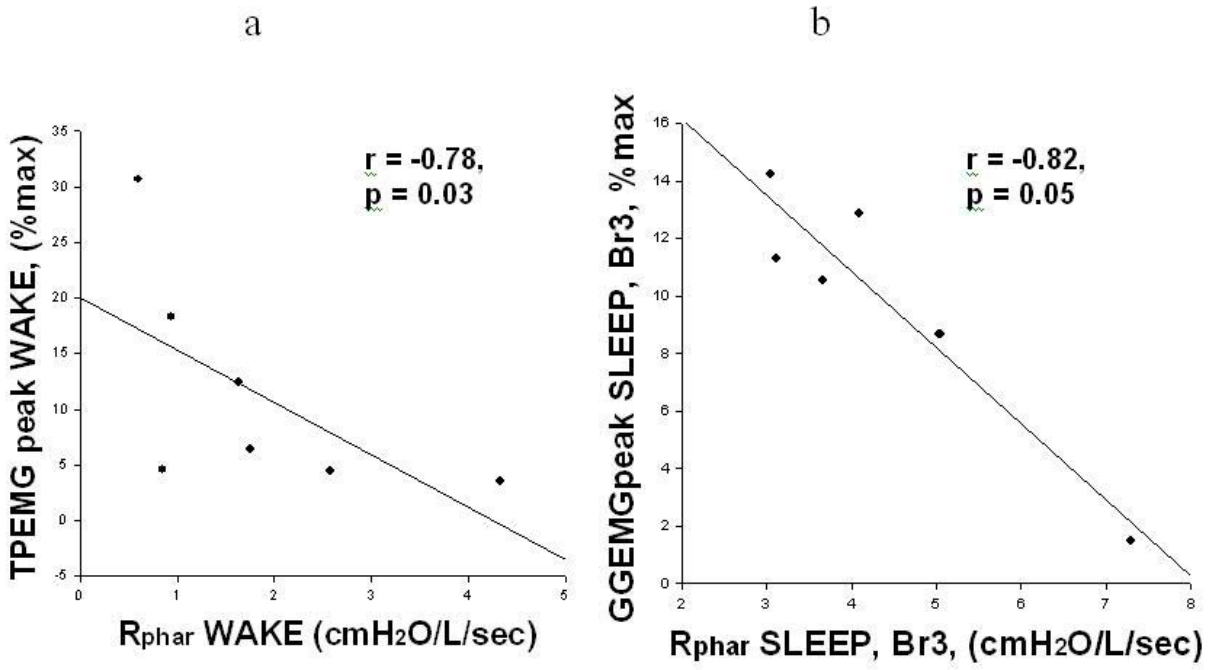

a
b
GGEMG peak WAKE, (% max)
r = 0.79,
p = 0.02
Pcrit (cmH2O)
TPEMG peak SLEEP Br2, (%max)
r = 0.75,
p = 0.04
Pcrit (cmH2O)

Fig 5


a
b
TPEMG peak WAKE, (%max)
r = -0.78,
p = 0.03
Rphar WAKE (cmH2O/L/sec)
GGEMGpeak SLEEP, Br3, % max
r = -0.82,
p = 0.05
Rphar SLEEP, Br3, (cmH2O/L/sec)