Expiratory Pharyngeal Narrowing during Central Hypocapnic Hypopnea

On-Line Supplement: Detailed Methods and Supplemental Tables

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On-line detailed Methods:

Subjects

The Human Investigation Committee of the Wayne State University School of Medicine and the John D. Dingell Veterans Affairs Medical Center approved the experimental protocol. Informed written consent was obtained from 20 healthy subjects. Subjects were screened for sleep-disordered breathing by a baseline polysomnography performed at a separate visit. Female subjects were not pregnant nor on birth control pills. Subjects were asked to restrict their sleep the night before the study to total sleep of 4–6 hours, and study was done under spontaneous natural sleep.

Equipment and measurements

The subjects were connected to the circuit with an airtight silicone rubber mask strapped to the face to prevent leaks. The mask was connected to a Plateau Exhalation Valve (Respironics, Inc, Pittsburgh, PA) via a heated pneumotachometer. The mouth was taped tight to ensure nasal breathing. Arterial O_2 saturation (SaO_2) was measured by a pulse oximeter (Biox 3700, Ohmeda). Ventilation and timing was measured by a facemask pneumotachometer. End-tidal PCO_2 was measured at the nasal orifice using a tube connected to a mass spectrometry. All signals were displayed on a polygraph recorder (Digital Comet ®, West Warwick, RI). The signals were recorded using POWER LAB ® digital data acquisition/analysis software. Electroencephalograms (EEG), electrooculograms (EOG) and chin

electromyograms (EMG) were recorded using the international 10-20 system of electrode placement. Every subject was studied with an enhanced screening polysomnography including quantitative flow measurement using a pneumotachometer. Sleep staging was performed according to the criteria of Rechtschaffen and Kales in 30 sec epochs (1). Conventional arousals were also defined according to the American Sleep Disorders Association definition (2). Subjects included in this analysis were in stable stage-2 or stage-3 (slow wave=20-50%) sleep during the hypocapnic exposures and data collection. Breaths for analysis were selected during a period of stable sleep with no evidence of an arousal.

In the first analysis, pharyngeal pressure (P_{ph}) was measured in 9 subjects at the palatal rim. A pediatric fiberoptic bronchoscope was used to visualize the pharyngeal airway during NREM sleep. The scope was inserted via the nares after lubrication and local anesthesia. Topical anesthesia was applied as follows: 2% lidocaine jelly was applied to each nostril followed by 4% liquid lidocaine to anaesthetize the pharynx; 2% lidocaine jelly was used to lubricate the nostril during advancing the scope. A continuous image of the retro palatal lumen was obtained from a video camera connected to the scope (Endovision 3000, Pentax Precision Instrument). The video image and respiratory signals was digitalized at 10 frames/sec and 25 Hz, respectively, by using specially developed software. In the second analysis, upper airway pressure was measured in 11 subjects at the supraglottic level (P_{sg}) using transducer tipped catheter (Millar, Inc).

To avoid nasal congestion and excessive secretions from obscuring the video recording during sleep, oxymetazoline hydrochloride 0.05% dose was given before the start of the study. Sleep staging electrodes were attached, and the subjects then lay supine in the bed. Local anesthesia was given, and the pressure catheter was passed through one nostril. The fiberoptic scope was then passed through the opposite nostril and positioned as described above. The nasal mask was then carefully lowered onto the face and secured. At this point, the exact position of the fiber-optic scope was adjusted and the scope plus the attached video camera were placed in a clamp suspended above the subject's head. The mask was carefully sealed, including the hole through which the scope was inserted. A check for air leakage around the mask was made by occluding the airflow during an attempted inspiration and expiration. The remaining transducers were then attached, and further fine adjustments to the orientation of the scope were made, the subjects were allowed to go to sleep. Subjects were not allowed to change body position during the trials.

Induction of hypocapnic hypopnea

We induced hypocapnic hypopnea during NREM sleep as previously described (3). Noninvasive positive pressure ventilation (NPPV) was applied for three minutes then terminated abruptly, with the expiratory pressure set at its lowest pressure (EPAP= 2 cmH_2O) throughout the study. The inspiratory positive airway pressure (IPAP) was increased gradually in 1-2 cmH_2O increments at the beginning of each mechanical hyperventilation trial and was reduced to 2 cmH_2O at the end of each three minutes of NPPV. The hyperventilation trials were

repeated at higher IPAP (1-2 cmH_2O) Hypocapnic hypopnea was defined as a reduction in flow of at least 30% from control without inspiratory effort (Figure 1). Spontaneous breathing for 5 minutes followed each mechanical ventilation trial to restore chemical stimuli to baseline levels.

Study Design and Data Analysis

The study was divided into two analyses. For both analysis, standard ventilatory parameters were measured breath by breath and analysis was conducted on the subjects' means. The ventilatory parameters included inspired tidal volume (V_T), inspiratory time (T_I), total breath time (T_{TOT}), breathing frequency (F_b), and $P_{ET}CO_2$.

Analysis 1: Nine subjects participated in this protocol that aimed to measure CSA, pharyngeal pressure (P_{ph}) and flow in different stages of respiratory cycle from beginning of inspiration (BI: after which flow crossed from negative to positive) to end expiration (EI: before which flow crossed from negative to positive). In each hypopnea trial, we compared the three breaths of hypocapnic hypopnea with the three control breaths preceding mechanical ventilation (see figure E1).

The absolute value of retro palatal CSA was obtained from five different phases of the respiratory cycle (BI: Beginning inspiration, PI: peak inspiration, EI: end inspiration, BE: beginning expiration, PE: peak expiration, EE: end expiration) by manually outlining the retro palatal lumen using computer software

(Sigma Scan, Jandel) in mm^2 unit. The reproducibility of this technique has been previously validated by our laboratory (5, 6). For each image, the scanning software provided an area in pixels. We converted these relative areas to absolute areas by using the dimensions of the pressure catheter as a reference (5). During this process, the investigator was blinded to the phase of respiration. For each trial the retro palatal CSA measurements were expressed as a percentage of the CSA that occurred at beginning of inspiration (BI) for the preceding control breath.

To assess the dynamic changes in pharyngeal compliance within the respiratory cycle, the CSA-P_{ph} relationship was calculated as Δ CSA/Δ P_{ph} for three different phases,, first from beginning of inspiration to nadir CSA (BI-nadir) at peak inspiration, from nadir to maximal CSA (CSA_{nadir} –CSA_{max}) at peak expiration, and from maximal CSA to end expiration. These three phases allowed us to predict the dynamic changes in upper airway compliance (C_{UA}) throughout a breath.

Analysis 2: Eleven subjects participated in this protocol that aimed to measure inspiratory and expiratory upper airway resistance (R_{UA}). R_{UA} was measured on a breath-by-breath basis as a numeric representation of the maximal linear part of the pressure-flow loop during inspiratory and expiratory phases (See figure E1). Specifically, R_{UA} was calculated as flow/P_{SG} at the maximal flow. We compared, five eupneic control breaths preceding mechanical ventilation to the first three-hypopnea breaths following termination of three

minutes of MV. Each breath was analyzed for inspiratory flow limitation (IFL), P_{sg}, and flow. IFL was defined as the dissociation in pressure-flow linear relationship using a previously validated mathematical model (4). To measure the changes of lung volumes at end expiration between the control and hypopnea breaths, we measured the end expiratory supraglottic pressure (EEP) at 0 flow for three consecutive control and hypopnea breaths.

Statistical analysis

A commercially available computer statistical package was used to analyze the data (Sigma Stat 3.5, Jandel Scientific). A paired two tails t-test was used to compare the mean values of each ventilatory parameter between control and hypopnea breaths.

For analysis 1, a two-way repeated-measures analysis of variance (ANOVA) was used to compare each dependent variable (CSA, P_{ph} and flow). The two factors for each dependent variable were: control vs. hypocapnic hypopnea and respiratory cycle phases from beginning of inspiration to end expiration (five phases). Holm-Sidak method was used for all pairwise multiple comparison procedures. A paired two-tailed t-test was used to compare the mean values of $\Delta CSA/\Delta P_{ph}$ between eupnea and hypopnea breaths. The overall significance level was considered at 0.05.

A Pearson's correlation was used to determine the association between the retropalatal airway CSA at peak inspiration and expiration during eupnea and

hypopnea. Physiologic and demographic markers were used such as body mass index (BMI), age, neck circumference (NC), pharyngeal pressure at peak Inspiration and expiration or inspiratory flow limitation during eupnea (IFL).

A forward stepwise regression analysis was used to ascertain potential determinants of retro palatal airway expiratory narrowing during hypocapnic hypopnea using physiologic and demographic markers such as body mass index (BMI), age, NC, IFL during eupnea and the change in pharyngeal pressure from peak inspiration to beginning of expiration $\Delta \dot{P}_{ph}$ (PI-BE) during control. For analysis 2, A paired two tails t-test was used to compare each dependent variable (inspiratory R_{UA}, expiratory $R_{UA,}$ and EEP) between control and hypopnea breaths. The overall significance level was considered at 0.05.

References

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E(3) Zhou, XS, Rowley, JA, Demirovic, F Diamond, MP and Badr MS. Effect of testosterone on the apneic threshold in women during NREM sleep. *J Appl Physiol* 2003, 94: 101 - 107.

E(4) Mansour KF, Rowley JA, Meshenish AA, Shkoukani MA, and Badr MS. A mathematical model to detect inspiratory flow limitation during sleep. *J Appl Physiol* 2002, 93: 1084 - 1092.

E(5) Rowley JA, Zahn BK, Babcock MA, and Badr MS. The effect of rapid eye movement (REM) sleep on upper airway mechanics in normal human subjects. *J Physiol* 1998, 510: 963–976.

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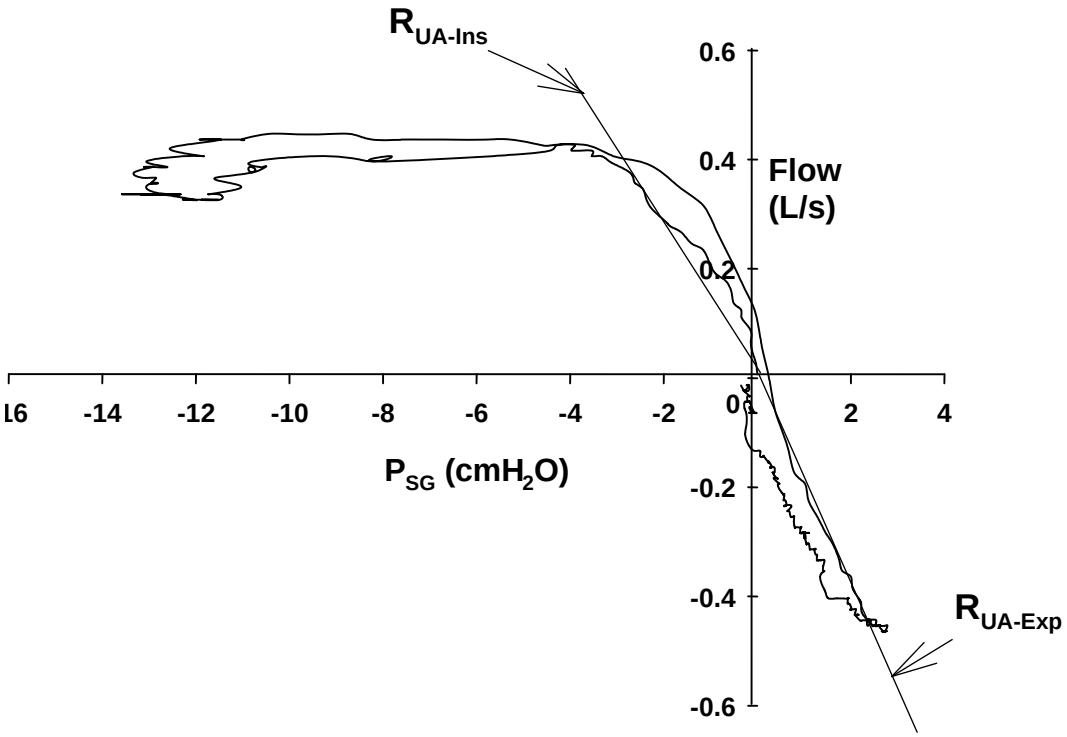
Supplemental figures:

Figure E1: Figure E1: The pressure-flow loop for one breath. The upper airway resistance was represented as the numeric representation of the inverse of the slope at the maximal linear part of the pressure-flow loop during inspiratory and expiratory phases; R_{UA} ($cmH_2O/L/s$).



Supplemental Tables:

Table E1: Results of forward stepwise regression for determinants of retro palatal airway expiratory narrowing during hypocapnic hypopnea

	ΔCSA_{BE}			ΔPph_{PI-PE}		
	r^2	Coefficient	p value	r^2	Coefficient	p value
Age (year)			0.83			0.16
BMI (Kg/m^2)			0.99	0.74	0.34	0.005
NC (cm)			0.46	0.92	0.47	0.02
ΔPph_{PI-PE} (cmH2O)	0.59	7.07	0.02			
$IFL_{-eupnea}$			0.47			0.99

BMI, body mass index; NC, neck circumference; $IFL_{-eupnea}$, inspiratory flow limitation during eupnea; ΔCSA_{BE}, change in cross-sectional area between eupnea and hypopnea at the beginning of expiration; ΔPph_{-PI-PE}, change in pharyngeal pressure from peak inspiration to peak expiration.

Table E2: Results of Pearson's correlation for determinants of retro palatal airway patency during eupnea

	CSA_{PI}		CSA_{PE}	
	Correlation Coefficient	p value	Correlation Coefficient	p value
Age (year)	0.19	0.63	0.09	0.80
BMI (Kg/m^2)	-0.42	0.26	0.73	0.02
NC (cm)	0.24	0.56	0.62	0.10
$IFL_{-eupnea}$	-0.61	0.08	-0.37	0.32
Pph_{-PI}	-0.12	0.73		
Pph_{-PE}			0.73	0.03

BMI, body mass index; NC, neck circumference; $IFL_{-eupnea}$, inspiratory flow limitation during eupnea; Pph_{-PI} , pharyngeal pressure at peak inspiration; $Pph_{-PE,}$ pharyngeal pressure at peak expiration; $CSA_{PI,}$ cross-sectional area at peak inspiration; $CSA_{PE,}$ cross-sectional area at peak expiration.

Table E3: Results of Pearson's correlation for determinants of retro palatal airway narrowing during hypopnea

	CSA_{PI}		CSA_{PE}	
	Correlation Coefficient	p value	Correlation Coefficient	p value
Age (year)	0.09	0.82	0.09	0.83
BMI (Kg/m^2)	0.02	0.95	0.31	0.42
NC (cm)	0.36	0.37	0.47	0.25
$IFL_{-eupnea}$	-0.84	0.005	-0.77	0.02
Pph_{-PI}	0.07	0.82		
Pph_{-PE}			0.38	0.32

BMI, body mass index; NC, neck circumference; $IFL_{-eupnea}$, inspiratory flow limitation during eupnea; Pph_{-PI} , pharyngeal pressure at peak inspiration; $Pph_{-PE,}$ pharyngeal pressure at peak expiration; $CSA_{PI,}$ cross-sectional area at peak inspiration; $CSA_{PE,}$ cross-sectional area at peak expiration.

Supplemental Video: Fiber-optic video recording of the retro palatal airway from a representative subject for control (Video E1) and hypocapnic (Video E2) hypopnea conditions. Note the smaller pharyngeal size and the dampening of pharyngeal wall motion in hypopnea compared to control.