Online supplement

Detailed Methods

Subjects

The Repatriation General Hospital Research and Ethics committee approved the study and 14 patients with moderate to severe OSA participated after providing written informed consent. Retrospective review of diagnostic polysomnography studies was used to select patients with predominantly (>85%) obstructive events who showed significant improvement in SWS. Patients were considered eligible if they had a clinical diagnosis of OSA with an apnoea-hypopnoea index (AHI) $\geq 30hr^{-1}$, showed at least a 40% improvement in AHI during SWS compared to stages 1-2 non-REM in the same posture and with at least 5 minutes of SWS for assessment. OSA was diagnosed according to American Academy of Sleep Medicine 1999 criteria.[E1] Patients were required to have been on CPAP therapy for a minimum of 3 months, with minimum compliance of at least 4 hours usage every night. Patients were excluded from participation if they were on medications potentially affecting respiration and/or cause sedation such as benzodiazepines or opiates. Pre-menopausal women and post-menopausal women on hormone replacement therapy were excluded, given possible effects on ventilation and upper airway function by the menstrual cycle and hormone replacement.[E2] Patients with other sleep disorders and circadian rhythm abnormalities were also excluded.

Equipment

A P_{CRIT} research system (Philips-Respironics, Murrysville, PA) able to switch rapidly between 2 different airway pressures was attached via T-piece to the inspiratory and expiratory limbs of the breathing circuit. A computer controlled rapidly inflatable balloon occlusion valve was placed upstream from a low resistance pneumotachograph (Jaeger PT36) on the inspiratory limb and was attached via a 2 way inspiratory-expiratory breathing valve (Hans Rudolph, Series 2600) connected to a nasal mask (ComfortGel^{TM}, Philips-Respironics, Murrysville, PA) via large bore tubing (34mm ID Clean Bor, Vacu Med, Ventura, Ca). On the expiratory side, corrugated CPAP tubing (ID 20.5mm) was connected to an expiratory port connected to the T piece. Consequently, the inspiratory and expiratory limbs of the circuit received the same delivered pressure and the upper airway could be occluded under positive pressure conditions.

Sleep signals consisted of EEG recorded at C3/A2, C4/A1 scalp locations, bilateral EOG, submental EMG, ECG, and SaO_2 measured via finger pulse-oximetry (Novametrix Oxypleth, Soma Technology, CT). The expirate was sampled at the mask to determine the end-tidal CO_2 ($ETCO_2$ Capstar-100, CWE Inc, PA) via tubing incorporating 30cm of Nafion tubing (Permapure, NJ) to prevent condensation from blocking the sample line. A perforated tube was threaded around the nasal mask and connected to a CO_2 analyser to act as a qualitative leak detector (POET II 602-3, Criticare Systems, Waukesha, WI, USA). Epiglottic pressure was measured by a catheter (2.1mm OD, Microtube Extensions, Sydney, Australia) that was advanced under direct visualisation 1-2 cm below the tongue base, after decongestion of both nostrils (Oxymetazoline 0.05%) and lubrication with 2% Lignocaine gel. Approximately 10, 1 mm diameter holes were cut radially around the distal 1 cm tip of the catheter. Catheter patency was maintained using low flow (1-2 ml/min) air

perfusion. Pressure transducers (MP45, Validyne Engineering, Northridge, CA) were used to measure mask and epiglottic pressures.

Data were acquired using 2 recording systems. One system (Compumedics S series, Abbottsford, Victoria, Australia) was used for recording EEG, EOG, submental EMG and body position. Sleep staging and arousal scoring was performed using this system. A second system (WinDaq, DataQ instruments inc, OH) was used to record inspiratory and expiratory flow, epiglottic and mask pressures, $ETCO_2$, SaO_2, the mask leak signal and ECG, all sampled at 200Hz. An event mark generated from a common source was placed simultaneously on both systems prior to each intervention to link both systems in time.

Protocol

Patients were asked to attend the laboratory 1 hour prior to their usual bedtime, having abstained from alcohol and caffeine for 24 hours. Patients were instrumented as described above and asked to sleep only in the supine posture, with 1 pillow. The mouth was taped to prevent mouth leak.

CPAP was commenced initially at the patient's documented therapeutic pressure and increased if required, 1-2 cmH_2O above the point where visible inspiratory flow limitation was noted. This pressure was maintained as the baseline pressure for the duration of the study. 3 subtherapeutic pressures were determined during a brief assessment period prior to commencement of the study proper. These pressures were chosen as approximately 75, 50 and 25% on a scale from flow limitation first being observed, to the development of frank apnoeas. Once determined, these dialdown pressures remained fixed throughout the remaining study. Brief upper airway occlusion was performed by inflation of the balloon valve during stable baseline pressure conditions.

Interventions were grouped into blocks of 4 (25, 50, 75, occlusion) with the order of interventions randomized within each block. A 30 second baseline period of arousal free sleep was required prior to each intervention. Both dialdowns and occlusions were performed until EEG/EMG evidence of arousal was observed, or for a maximum of 2 minutes (dialdowns only), after which CPAP was returned to the baseline pressure. Intervention blocks continued throughout the night.

Data analysis

An experienced technician blinded to all other channels except EEG, EMG and EOG performed sleep staging and arousal scoring according to conventional standards.[E3,E4] Interventions scored to have commenced following at least 30-sec stable stage 2 or SWS without arousal underwent further analysis. The remainder (stage 1 or REM, or with arousal within the baseline period) were excluded.

Breath timing (inspiratory, TI; expiratory, TE; and total breath time, TTot), inspiratory tidal volume (V_{Ti}), minute ventilation (VI), peak inspiratory flow (PIF), CPAP level (mask pressure at end expiration) and $ETCO_2$ were determined breath-by-breath throughout the 30-sec baseline before each intervention and up to the onset of EEG defined arousal or 120-sec (whichever came first), using custom software developed in our laboratory. The ΔPepi (a measure of inspiratory drive) was

determined as the difference between the epiglottic pressure at breath onset and the nadir of epiglottic pressure for each breath as previously described.[E5] For upper airway occlusions, time to arousal (TTA) was determined as the time from the first negative deflection in epiglottic pressure up to the point of EEG defined arousal. Maximum ΔPepi was defined as the ΔPepi for the last completed inspiratory effort prior to arousal and was used to assess ventilatory arousal threshold.[E6]

Breath-by-breath measurements in each 30-sec baseline period were averaged within each trial and averaged across all replicate trials within each patient for each intervention and sleep stage. Throughout each intervention, measurements were obtained from all completed inspiratory efforts commencing after the onset of the intervention and up to the point of EEG defined arousal. Consequently, breaths with an arousal occurring within the inspiratory time were excluded.

To summarise the overall pattern of ventilatory response to dialdown interventions, ventilatory measurements from the first 5 and last 3 arousal free inspiratory efforts were averaged across all replicate trials within each patient for each dial-down pressure in stage 2 and SWS. Given a variable and often short latency to arousal, particularly under the more severe dialdown conditions, breaths potentially contributed to both the first and last breath periods. Early dialdown and pre-arousal upper airway function was assessed from the PIF vs CPAP relationship on the third and second to last dial-down breath respectively. P_{CRIT}, or the estimated airway pressure at which no flow occurs was calculated by performing linear regression of PIF vs CPAP levels as previously described.[E7] The ventilatory drive response to occlusion was assessed from the linear regression slope of the ΔPepi versus the corresponding time relationship across each post occlusion effort before arousal.

Sample size and statistical analysis

Based on the study design and an anticipated within-subject coefficient of variation in peak flow and minute ventilation of the order of 25% during replicate CPAP dialdowns to the same pressure, we estimated that differences between sleep stages in the order of 10% of baseline (or 4.5 l/min peak flow and 1.3 l/min minute ventilation) could be detected with a sample size of 10 patients, 80% power and a two-tailed significance level of 0.05.

Differences in ventilatory parameters at baseline and between breaths, sleep stages and dialdown pressures were examined via mixed model analysis, using an autoregressive covariance structure and separate random effects intercept for each patient (SPSS version 14, SPSS Inc, Illinois). Mixed model analysis was also used to examine sleep stage and intervention effects on TTA, arousal threshold (Maximum ΔPepi), the PIF vs CPAP relationship (CPAP as a covariate) on the third and second to last pre-arousal dialdown breath, and the ventilatory drive response to occlusion. Arousal free survival time was examined using Cox regression with robust standard errors, with sleep stage and dialdown level as covariates (Stata version 10, StataCorp, Texas). All data and figures are presented as mean ± SEM, unless otherwise specified. $p<0.05$ was considered statistically significant.

References for online supplement

E1. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. Sleep 1999; 22: 667-89.
E2. Popovic RM, White DP. Upper airway muscle activity in normal women: influence of hormonal status. J Appl Physiol 1998; 84: 1055-62.
E3. Rechtschaffen A, Kales A. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects, Bethsda, MD: National Institutes of Health; 1968.
E4. EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. Sleep 1992; 15: 173-84.
E5. Berry RB, Bonnet MH, Light RW. Effect of ethanol on the arousal response to airway occlusion during sleep in normal subjects. Am Rev Respir Dis 1992; 145: 445-52.
E6. Gleeson K, Zwillich CW, White DP. The influence of increasing ventilatory effort on arousal from sleep. Am Rev Respir Dis 1990; 142: 295-300.
E7. Penzel T, Moller M, Becker HF, Knaack L, Peter JH. Effect of sleep position and sleep stage on the collapsibility of the upper airways in patients with sleep apnea. Sleep 2001; 24: 90-5.