# Increased Propensity for Central Apnea in Patients with Obstructive Sleep Apnea: Effect of nCPAP

## On-line Data Supplement

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## Detailed Methods

The experimental protocol was approved by the Human Investigation Committee of the Wayne State University School Medicine and the John D. Dingell Veterans Affairs Medical Center. Informed written consent was obtained from all subjects. We studied 14 subjects with documented obstructive sleep apnea and 16 healthy non-snoring subjects without documented OSA; 15 of the control subjects controls were originally studied for other investigations while one was recruited specifically to match a middle-aged OSA subject. However, the raw tracings of the 15 control subjects were re-analyzed to ensure that similar methodology was used for all subjects. All the OSA subjects had the presence of obstructive events based upon full polysomnography. For the 16 control subjects, the absence of sleep-disordered breathing was confirmed by either full polysomnography (6 subjects) or by the absence of sleep-disordered breathing events during non-intervention portions of research studies (10 subjects, approximately 100 minutes of monitoring per subject). All subjects were free of heart disease. Demographics of the subjects included in each analysis are provided in Tables E1-3. All OSA subjects were naïve to nasal continuous positive airway pressure (CPAP) therapy. All subjects with OSA who completed the first night of apneic threshold determination were offered CPAP and invited to return approximately six weeks later for a repeat apneic threshold determination study. Six subjects returned for the second study. Objective compliance with CPAP, measured by number of days used and number of hours on days used, was downloaded from the CPAP unit at the time of the second study.

## Measurements

Electroencephalograms (EEG), electrooculograms (EOG), and chin EMG, were attached using the international 10-20 system of electrode placement (EEG: C3-A2, C4-A1, Oz-A2; EOG: F7-A2 and F8-A1). Data were logged to a polygraph recorder (Grass Inc.) and sleep stage was scored according to standard methods.[1] An appropriate sized tight fitting nasal CPAP mask was glued to the face with liquid latex, to prevent mask leaks and was connected to the ventilation circuit. Subjects were restricted to nasal breathing by placing tape over the mouth. Airflow was measured by a heated pneumotachometer (Model 3710,

Hans Rudolph Inc., Kansas City, MO) connected to the mask. Tidal volume ($V_T$) was obtained by integrating the pneumotachograph flow signal, (Model FV-156 Integrator, Validyne Inc., Northridge, CA). End-tidal carbon dioxide ($P_{ET}CO_2$) was measured with a gas analyzer (AEI Technologies, Pittsburgh, PA).

To confirm the central etiology of apnea and to ascertain upper airway mechanics, supraglottic pressure was measured with a solid-state catheter (Model MPC-500, Millar Instruments, Houston, TX). A 10% lidocaine spray was used prior to catheter insertion to provide topical anesthesia to one nostril and the pharynx. The catheter was threaded through a hole in the nasal mask, through the nose, and positioned in the hypopharynx just below the base of the tongue as determined by visual inspection of the tip. Airflow and supraglottic pressure were recorded using PowerLab data acquisition software (ADInstrument Pty Ltd, Castle Hill, NSW 2154, Australia) on a separate computer.

*Mechanical Ventilation Protocol*

Subjects were asked to restrict their sleep the night before the study to 4-6 hours, and the study was done under spontaneous sleep. Hyperventilation was achieved using a pressure support ventilator (Quantum PSV, Healthdyne Technologies, Marietta, GA) as previously described.[2;3] The nasal mask dead space was determined to be 110.5 ±1.5 ml. Accumulation of $CO_2$ in the circuit was prevented by the biased flow provided by the ventilator and from an expiratory mushroom valve in-line between the pneumotachometer and the ventilator tubing. No re-breathing of $CO_2$ took place as shown by the $P_{ET}CO_2$ at start of inspiration equivalent to room air values. For control subjects during the control and recovery periods, the ventilator was set at an expiratory positive airway pressure (EPAP) pressure of 2.0 $cmH_2O$. For OSA subjects during the control and recovery periods, the ventilator was set at an EPAP that eliminated apneas and hypopneas but at which flow limitation was present for 20-50% of the breaths (EPAP-FL). During periods of hyperventilation, the ventilator was set in spontaneous timed

mode with a back-up rate of 4 breaths per minute. Hyperventilation was achieved by increasing the inspiratory pressure of the ventilator, with adjustments made during expiration. For each successive trial, the inspiratory pressure was increased in 1 -2 cm $H_2O$ increments from the baseline EPAP (2 cm$H_2O$ for the control subjects, EPAP-FL for the OSA subjects), which resulted in increased tidal volume. Spontaneous respiratory effort remained in most trials as evidenced by persistence of an initial negative deflection of supraglottic pressure signal. Mechanical ventilation was continued for three minutes and was terminated during expiration to the baseline EPAP. Each trial was repeated twice with trials separated by a minimum of 5 minutes. The post-mechanical ventilation period, or recovery period, was observed for post-hyperventilatory inhibition. The ensuing hypocapnia resulted in either a hypopnea or central apnea depending on the magnitude of hypocapnia. Apnea was defined as a period of no airflow for at least 5.0 s. Stable NREM sleep in stage 2 or slow-wave was selected for each trial.

**Data Analysis and metrics of apnea susceptibility**

We analyzed only trials with stable sleep-state (Stages N2 and N3) as evidenced by the absence of arousal or ascent to a lighter sleep state. For each trial, $P_{ET}CO_2$ and minute ventilation ($V_E$) was measured breath by breath in the control and hyperventilation periods. The control period was represented by the average of five breaths immediately preceding the onset of mechanical ventilation. The hyperventilation data were the calculated average of the last five mechanically ventilated breaths prior to the ventilator being turned back to the baseline EPAP. The change in $P_{ET}CO_2$ ($\Delta P_{ET}CO_2$) was calculated as the difference between the control period and the last 5 mechanical ventilation breaths. $V_E$ was given a value of 0 during central apnea and the apnea length was set at a minimum of 5.0 seconds.

The ventilatory control during sleep operates as a negative feedback closed-loop cycle. A transient change in ventilation will result in a transient change in alveolar, then arterial blood gas tension. When the change reaches the chemoreceptors, ventilation will change in a direction opposite to the initial perturbation. Many authors have adopted the engineering concept of "loop gain" as an index to express the ventilatory change for a given perturbation, combining three types of gain: plant gain, mixing gain and controller gain.

Plant gain (Gp) is the relationship between $\Delta PaCO_2$ versus $\Delta V_E$. Contrary to popular belief, a low $PaCO_2$, for a given metabolic rate, promotes stability by requiring a larger increase in $V_E$ for a given reduction in arterial $PCO_2$. Therefore, a high $PaCO_2$ promotes instability. As the blood leaves the pulmonary capillaries, the change is attenuated by mixing of pulmonary capillary blood in the systemic circulation and delayed by the transit time; thus, $PaCO_2$ may not necessarily reflect the chemoreceptor $PCO_2$. Finally, the ventilatory response for a given change in chemical stimuli is referred to as the controller gain ($\Delta V_E$ versus $\Delta PaCO_2$). In our protocols, the controller gain represents the $CO_2$ chemoreflex sensitivity below eupnea in response to induced hypocapnia. An elevate chemoreflex sensitivity to $CO_2$ below eupnea results in reduced $CO_2$ reserve and increased propensity to central apnea.

Controller gain or chemoreflex sensitivity was calculated for each mechanical ventilation trial ending in a central apnea. Chemoreflex sensitivity was defined as the as the ratio of change in $V_E$ between control and apnea to the $\Delta P_{ET}CO_2$; for the apnea trials, we defined $V_E$=0 (see Figure E1).[4;5] The apnea threshold ($P_{ET}CO_2$-AT) was defined as the highest $P_{ET}CO_2$ that caused an apnea. The rationale for this definition is that, in some subjects, apneas resulted following multiple mechanical-ventilation trials; therefore, the

apnea closest to the last hypopnea most likely represented the trial that allowed for a determination of the apnea threshold. The $CO_2$ reserve ($\Delta P_{ET}CO_2$-AT) was defined as the change in $P_{ET}CO_2$ between eupneic (control) $P_{ET}CO_2$ and $P_{ET}CO_2$-AT.

Plant gain was determined as follows (see Figure E1). For each trial, we plotted the average $P_{ET}CO2$ for the last 5 breaths during control periods prior to the trials and the average of the last 5 mechanical ventilation breaths before the end of the trials. Using SigmaStat (v. 3.5), we fitted the data as a hyperbolic first order function ($V_I$= (A*$P_{ET}CO2$)/(B+$P_{ET}CO2$), where A and B are variables calculated by the curve fitted function, $P_{ET}CO2$ is the independent variable, and minute ventilation ($V_I$) is the dependent variable. After we determined the curve fitted function, we differentiated the function and took the derivative of the function at the operating point. The operating $P_{ET}CO2$ point was defined by the mean CO2 values recorded in the control periods for each subject. Mathematically, the derivative of the function is the slope of the curve. We defined plant gain as the inverse of the slope at the operating point.

**Statistical Analyses**

Statistical analysis was performed with Sigma Stat 3.0 (Jandel Scientific, San Rafael, CA). A $p<0.05$ was chosen as the accepted level of significance. All data are expressed as mean ±S.D.

For analysis #1, OSA and control subjects were paired for gender, age (±3 yrs) and BMI (±5 kg/m$^2$); matching occurred using a database in which data analysis is not reported to avoid bias in selection. The pairing resulted in 9 pairs of OSA and control

subjects for statistical analysis (Table E1). Paired t-tests were used to compare the NREM $P_{ET}CO_2$, $P_{ET}CO_2$-AT, $\Delta P_{ET}CO_2$-AT, and controller gain between the two groups.

For analysis #2, six OSA subjects were re-studied after the use of CPAP for an mean of 27.4±10.0 days (Table E2). In addition, five control subjects were studied after a median of 13 days (range 10-154 days), and 3 OSA subjects were re-studied without the use CPAP in the interval time (range 57-69 days; Table E3). Paired t-tests were used to compare the NREM $P_{ET}CO_2$, $P_{ET}CO_2$-AT, $\Delta P_{ET}CO_2$-AT, and controller gain before and after CPAP use for the OSA subjects and between studies for the control subjects.

*Examples of Raw Tracings*

Examples of raw tracings are provided in Figures E2-E4. In each of the figures, Panel A shows the last breaths of the control period, Panel B shows the beginning of the mechanical ventilation period and Panel C shows the final breaths of the last mechanical ventilation breaths and the ensuing apnea or hypopnea after the termination of mechanical ventilation. In Panel C of the figures note that there is no evidence of effort (negative deflection) in the supraglottic pressure ($P_{SG}$) tracing for the 2 trials ending in an apnea (Figure E1 and E2) while there is decreased effort in the trial ending in a hypopnea (Figure E3). Figure E2 is a tracing from a control subject. In this trial, the change in CO2 associated with this apnea was 3.91 mmHg. Figure E3 shows an tracing from a subject with obstructive sleep apnea. As can be seen, the change in CO2 associated with this apnea was smaller at 2.82 mmHg. Figure E4 shows the same subject after use of CPAP; in this trace, a smaller change in CO2 (2.36 mmHg) results in a hypopnea following the termination of mechanical ventilation, indicating a change in the CO2 reserve with CPAP use.

Table E1: Subject Demographics for Analysis #1

| | OSA Subjects | | | | | Control Subjects | | |
|---|---|---|---|---|---|---|---|---|
| Pair (Gender) | Age (yrs) | BMI ($kg/m^2$) | AHI (events/hr) | EPAP-FL ($cmH_2O$) | %IFL | Age (yrs) | BMI ($kg/m^2$) | %IFL* |
| 1 (M) | 22 | 26 | 58 | 12 | 15 | 25 | 23 | 11 |
| 2 (M) | 22 | 24 | 41 | 11 | 45 | 23 | 24 | 58 |
| 3 (M) | 38 | 29 | 17 | 10 | 95 | 34 | 28 | 33 |
| 4 (M) | 43 | 23 | 17 | 8 | 66 | 43 | 26 | 39 |
| 5 (M) | 46 | 33 | 53 | 12 | 12 | 44 | 30 | 42 |
| 6 (M) | 45 | 32 | 45 | 12 | 75 | 46 | 26 | 3 |
| 7 (F) | 19 | 23 | 30 | 15 | 35 | 24 | 25 | 23 |
| 8 (M) | 44 | 35 | 36 | 9 | 79 | 48 | 36 | 92 |
| 9 (M) | 41 | 31 | 43 | 8 | 15 | 40 | 26 | 80 |
| 10 (M) | 27 | 28 | 28 | 12 | 25 | 24 | 31 | 10 |
| 11 (M) | 26 | 27 | 47 | 7 | 50 | 24 | 24 | 10 |
| | 35.6±11.2 | 28.4±4.6 | 37.7±13.6 | 10.5±2.4 | 46.5±29.0 | 36.3±10.1 | 27.2±4.0 | 36.5±29.7 |

There was no statistical difference in age, BMI, or %IFL between the two groups.
*%IFL: percentage of breaths during eupneic breathing that demonstrate inspiratory flow limitation

Table E2: OSA Subject Demographics for Analysis #2

| Subject (Gender) | Age (yrs) | BMI (kg/m²) | AHI (events/hr) | CPAP Level ($cmH_2O$) | CPAP days used (%) | Ave use on days used (hrs) | Time between studies (days) |
|---|---|---|---|---|---|---|---|
| 1 (M) | 38 | 29 | 17 | 12 | 13 (33) | 3.9 | 60 |
| 2 (M) | 22 | 26 | 58 | 13 | 29 (60) | 1.3 | 65 |
| 3 (M) | 43 | 23 | 47 | 9 | 41 (98) | 5.2 | 43 |
| 4 (M) | 22 | 24 | 41 | 11 | 25 (50) | 4.4 | 51 |
| 5 (M) | 46 | 28 | 79 | 9 | 29 (60) | 1.4 | 63 |
| 6 (M) | 38 | 29 | 19 | 9 | * | * | 56 |
| 7 (M) | 27 | 28 | 28 | 12 | 35 (85) | 6.7 | 50 |
| | 33.7±10.4 | 26.7±2.6 | 36.9±24.0 | 11±3 | 28.7±9.5 d (64.4±23.7%) | 3.8±2.1 | 55±16 |

*objective compliance data could not be downloaded; subjective compliance was hrs/day.

Table E3: Control and OSA-noCPAP Subject Demographics for Analysis #2

| Subject (Gender) | Age (yrs) | BMI (kg/m²) | AHI (events/hr) | Time between studies (days) |
|---|---|---|---|---|
| 1 (F) | 23 | 21 | n/a | 127 |
| 2 (M) | 25 | 20 | n/a | 12 |
| 3 (F) | 24 | 25 | n/a | 10 |
| 4 (F) | 25 | 25 | n/a | 154 |
| 5 (M) | 28 | 22 | n/a | 13 |
| 6 (M) | 41 | 31 | 43 | 68 |
| 7 (M) | 46 | 33 | 53 | 57 |
| 8 (M) | 29 | 26 | 20 | 69 |
| | 30.1±8.6 | 25.4±4.6 | | 64±54 |

Reference List

E1. Rechtschaffen, A. and A. A. Kales 1968. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects National Institutes of Health, Washington, D.C.

E2. Rowley, J. A., X. S. Zhou, M. P. Diamond, and M. S. Badr. 2006. The determinants of the apnea threshold during NREM sleep in normal subjects. *Sleep* 29:95-103.

E3. Zhou, X. S., J. A. Rowley, F. Demirovic, M. P. Diamond, and M. S. Badr. 2003. Effect of testosterone on the apneic threshold in women during NREM sleep. *J.Appl.Physiol* 94:101-107.

E4. Dempsey, J. A. 2005. Crossing the apnoeic threshold: causes and consequences. *Exp.Physiol* 90:13-24.

E5. Dempsey, J. A., C. A. Smith, T. Przybylowski, B. Chenuel, A. Xie, H. Nakayama, and J. B. Skatrud. 2004. The ventilatory responsiveness to CO(2) below eupnoea as a determinant of ventilatory stability in sleep. *J Physiol* 560:1-11.

Figure E1

Determination of Plant Gain: For each trial, the average $P_{ET}CO_2$ for the last 5 breaths during control periods prior to the trials and the average of the last 5 mechanical ventilation breaths before the end of the trials were plotted. The data was fitted as a hyperbolic first order function. The operating $P_{ET}CO_2$ point was defined by the mean $CO_2$ values recorded in the control periods for each subject. Mathematically, the derivative of the function is the slope of the curve. Plant gain was defined as the inverse of the slope at the operating point.

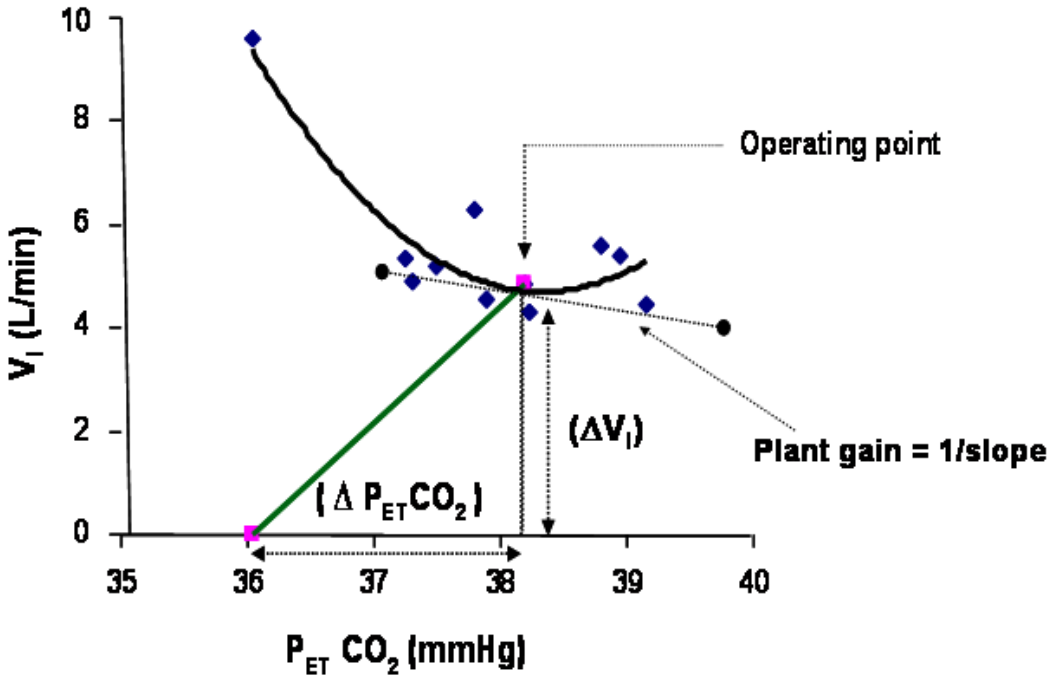


Figure E2

Raw tracing of a mechanical ventilation trial from a control subject.

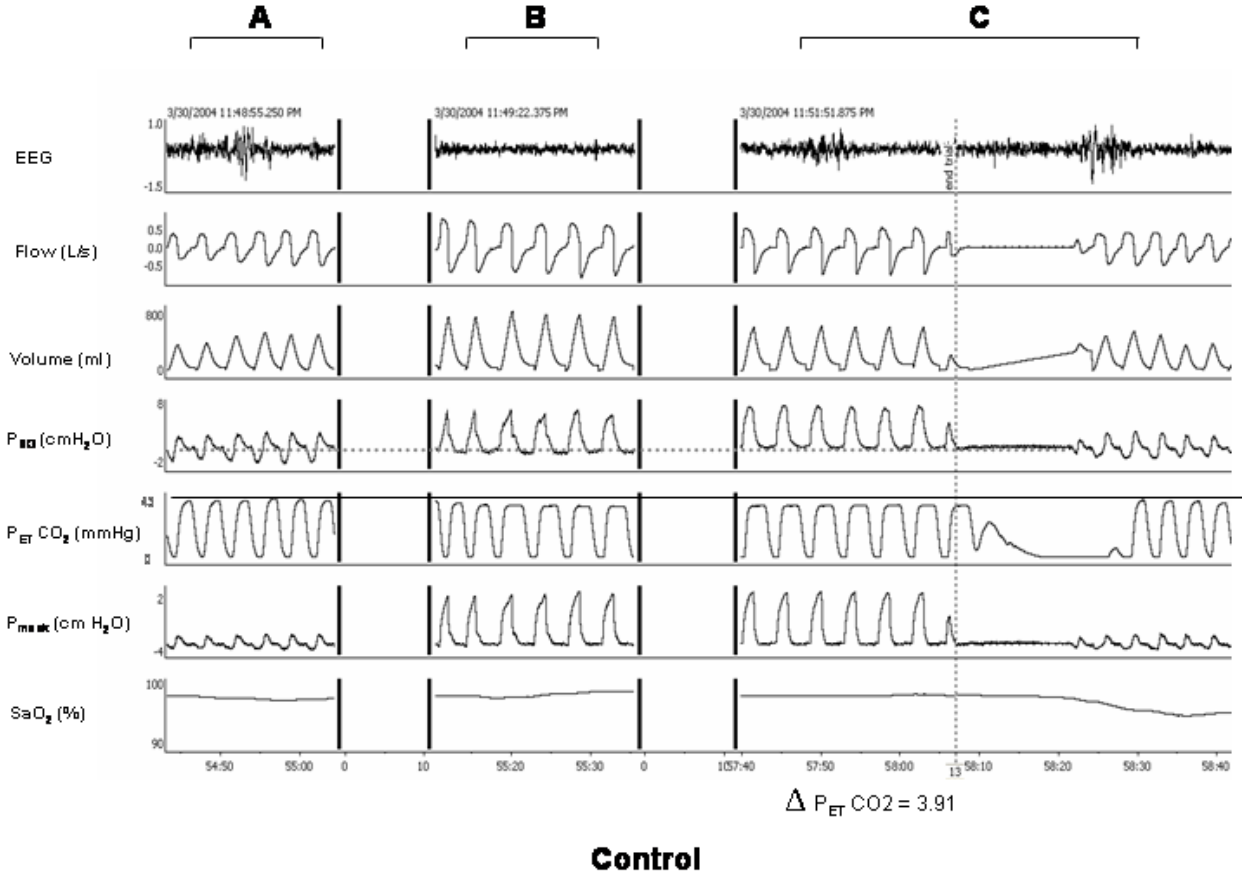


Figure E3

Raw tracing of a mechanical ventilation trial from a subject with OSA before use of CPAP.

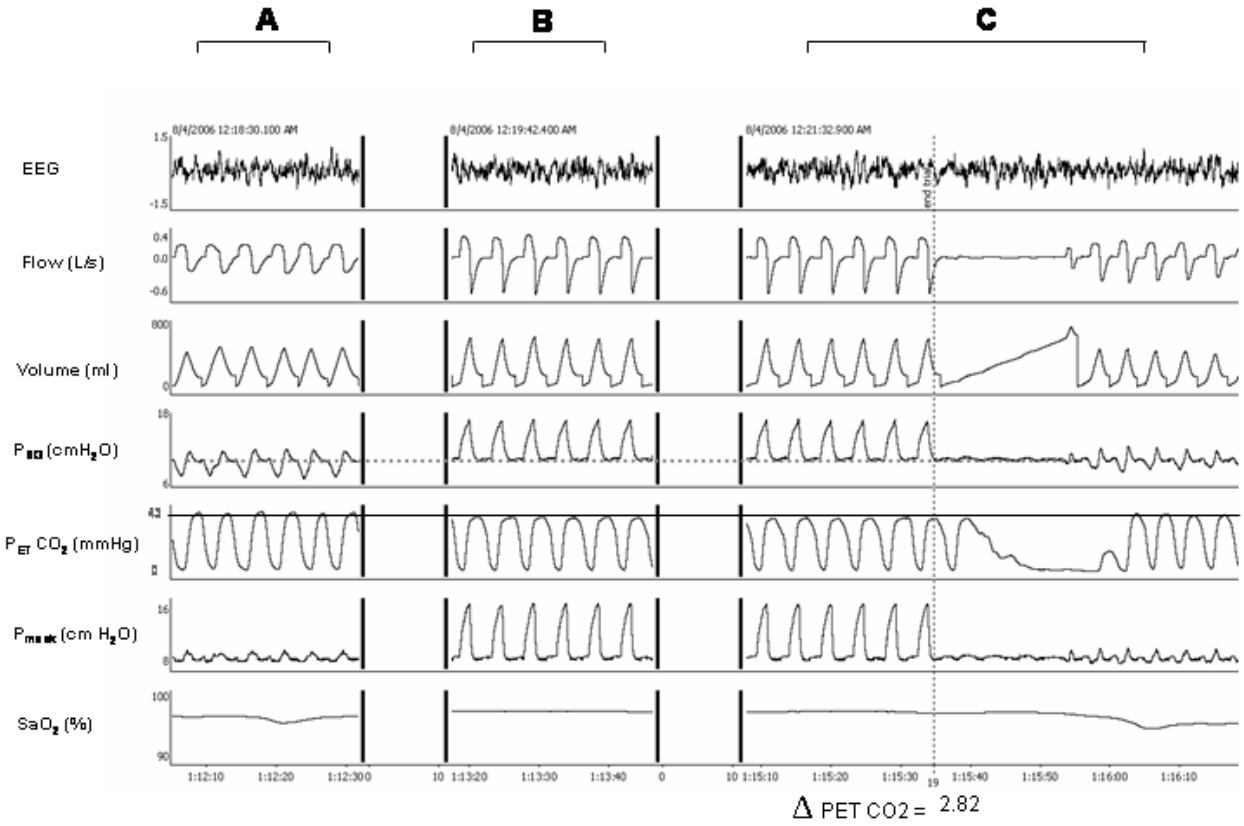
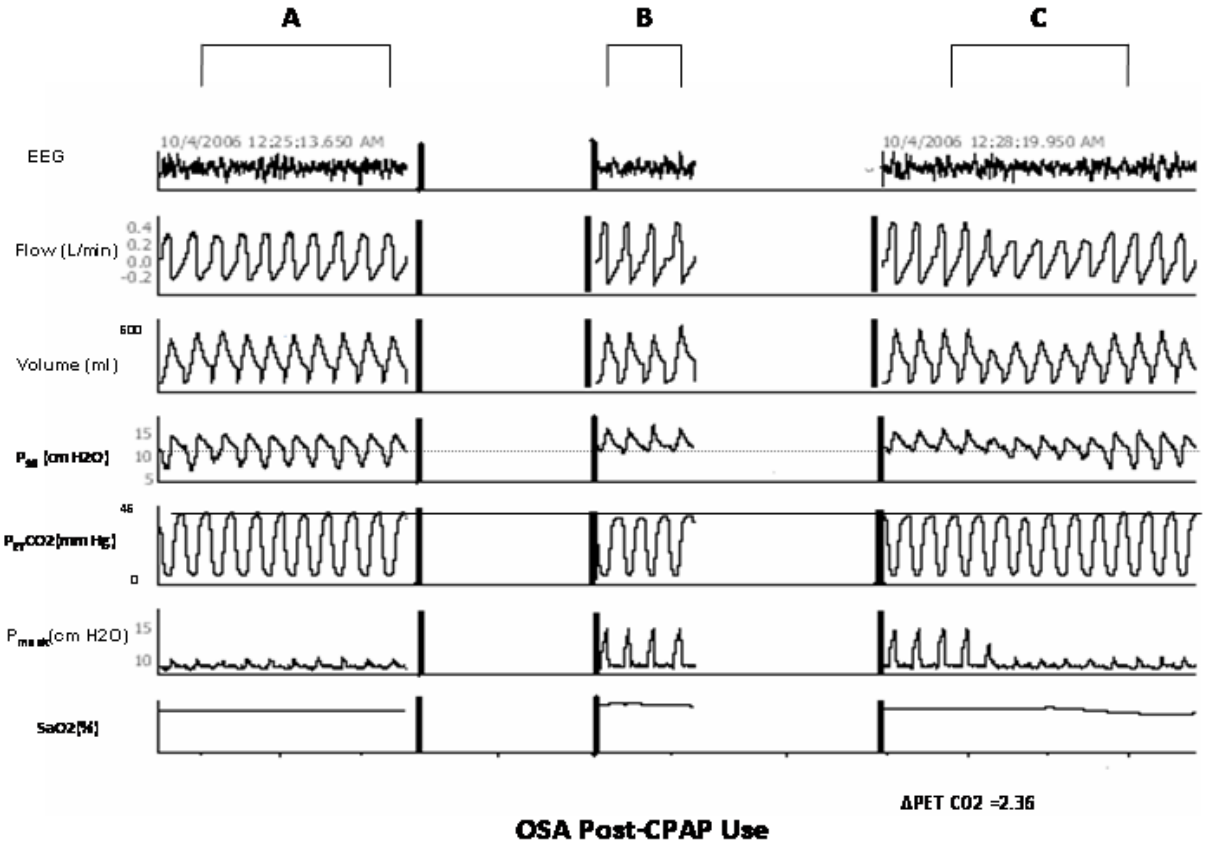


Figure E4

Raw tracing of a mechanical ventilation trial from a subject with OSA after 1 month of CPAP use.




OSA Post-CPAP Use