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Control of breathing in children with mild sleep apnea: A six-year follow up study.

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Short title: Control of breathing in children with sleep apnea

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ABSTRACT.

We previously showed that children (average age 9 y) with mildly elevated obstructive apnea-hypopnea indices (OAHI) retained CO_2 at rest. Here, we report the results of a six-year follow up study on 14 children from that study.

Lung ventilation rate (V_E) and the partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) were measured during hypercapnic challenge.

OAHI fell from 7.5 ± 4.7 to 2.5 ± 1.8 from age 9 to age 15 ($P < 0.001$), despite an increase in BMI from 20 ± 4.6 to 26 ± 5.7 ($P < 0.0001$). Eupneic V_E increased from 4.1 ± 0.31 to 5.9 ± 0.4 L/min/m^2 ($P< 0.01$), while P_{ET} CO_2 fell from 44.1 ± 0.8 to 33 ± 1.0 mmHg ($P< 0.001$). The V_E - $P_{ET}CO_2$ obtained during hypercapnia was left-shifted, such that V_E at a $P_{ET}CO_2$ of 50 mmHg increased from 24 L/min at age 9, to 36 L/min at age 15. Central respiratory drive did not change.

We hypothesize that somatic growth of the pharynx coupled with a regression of tonsillar tissue mass with age lead to enlargement of the upper airway lumen, a reduction in airway resistance and increased respiratory airflow at a given level of ventilatory drive.

INTRODUCTION. We previously showed that resting $P_{ET}CO_2$ was higher in 6-12 year old children with relatively high obstructive apnea-hypoxia indices (OAHI) compared to age-matched controls with lower OAHI [1]. The initial studies were done in 1999 when the average age of our subjects was about 9 years. Here, we report the results of a six-year follow up study of 14 children from this original sample, now with an average age of 15 years. All of the children in our sample have intact tonsils and adenoids. The mechanism of the CO_2 retention is unknown, but could be due to increased upper airway resistance, to blunted central chemoreceptor sensing and/or to altered regulation of central ventilatory motor output.

We were able to re-study 14 children from this original sample in 2006-2007, and our results are reported herein. Preliminary analyses from the entire TuCASA cohort indicate that the OAHI improves with age in children, even in the absence of adenoidectomy and tonsillectomy ([2]; and see Table 1). Here, we test the hypothesis that these improvements in the OAHI are associated with improvements in ventilatory control. We were particularly interested in changes in the resting pulmonary ventilation rate (V_E), the resting $P_{ET}CO_2$, and changes in the sensitivity to inspired CO_2. Our unique longitudinal study shows that although the children increased their body-mass indices (BMIs) with age, they also had lower OAHIs, a marked diminution in resting $P_{ET}CO_2$, and a substantial leftward shift in the V_E- $P_{ET}CO_2$ response curve.

MATERIALS & METHODS.

Subjects. All methods used to recruit subjects and to collect the present data set were approved both by the University of Arizona Human Subjects Committee and the Tucson Unified School District Research Committee. In all cases, we obtained written informed consent from the parents, and assent from the children. Our initial sample (1999) was composed of subjects recruited through the Tucson Unified School District (TUSD), as described in detail previously [1]. For the present study we selected and attempted to contact all 50 subjects from our original cohort and asked them if they were interested in participating in a follow up study. Subjects that had adenoidectomy or tonsillectomy were excluded, and a total of 14 subjects that met our criteria agreed to participate (28% of the original cohort), with their anthropometric characteristics given in Table 1. The remaining children could not be located, had tonsillectomies or adenoidectomies (N= 19) and/or refused to participate. These latter children, in comparison to the 14 subjects who participated in the present study, did not differ with respect to mean age or BMI, gender or ethnicity distribution, or percent habitual snoring.

Polysomnography. Both in the 1999 study and the present one, children underwent unattended, nocturnal home polysomnography [3] using the Compumedics PS-2 system (Abbotsford, Victoria, Australia). The following signals were obtained: C_3/A_2 and C_4/A_1 electroencephalogram (EEG), right and left electrooculogram, a bipolar submental electromyogram, thoracic and abdominal displacement (inductive plethysmography bands), airflow (nasal/oral thermocouple), nasal pressure, electrocardiogram (single bipolar lead), snoring (microphone attached to a vest), body

position (Hg gauge sensor), pulse oximetry (Nonin, Plymouth, MN) and ambient light (sensor attached to the vest to record on/off). Using Compumedics W-Series Replay, v 2.0, release 22, sleep stages were scored according to standard criteria [4]. The respiratory disturbance index (RDI) was defined as the number of respiratory events (apneas and hypopneas) per hour of the total sleep time irrespective of any associated oxygen desaturation or arousal. Polysomnograms with less than 4 hours of scorable oximetry were classified as failed studies and were repeated if the participant consented. Central apneas were scored if both airflow and thoracoabdominal effort were absent. However, central events that occurred after movement were not included. Obstructive apneas were identified if the airflow signal decreased to below 25% of the "baseline amplitude". Hypopneas were scored if the magnitude of any ventilation signal decreased below approximately 70% of the "baseline" amplitude, as described previously [1]. Although more recent rules for scoring respiratory events have been published [5] we elected to score apneas and hypopneas using the same algorithm used in our 1999 study in order to make valid comparisons between the 2 time points, in each instance using the thermistor and/or the inductance plethysmography signal to score respiratory events.

The RDI that we routinely compute includes central apneas as well as obstructive apneas and hypopneas [3]. Based on the clinical and physiological uncertainty of central apneas in children [6, 7] we subtracted central events from the RDI to derive the OAHI. In our subjects this index represents primarily hypopneas. For example, in 1999 the number of frank obstructive events ranged from 0.1 – 0.8 per hour, except for one

subject that averaged 7 obstructions per hour in 1999. In 2006 there were no frank obstructions except in one subject who had 2 obstructions per hour.

Ventilatory Control Protocol. For this portion of the study, participants were studied between 9:00 AM and 4:00 PM, and were instructed to refrain from caffeinated beverages and food for one hour prior to the time of their scheduled experiment. Subjects were studied while seated and listened to music through headphones throughout the entire protocol. Analog waveforms from transducers monitoring expiratory airflow, mask pressure and the fractional concentrations of O_2 and CO_2 were passed through an analog-to-digital converter (Spike II, Cambridge Electronic Design), sampled at 2500 Hz per channel, and stored on the hard drive of an IBM-compatible computer (details of the measurements and equipment are given below). Estimated oxygen saturation of arterial blood was monitored and recorded manually in all studies with a pulse oximeter (Ohmeda).

The investigators were blinded to the OAHI status of the subject when conducting experiments and analyzing data. For hyperoxic hypercapnia the subjects breathed from humidified Douglas bags filled with 3, 5 or 7 % CO_2 in O_2. Subjects started by breathing room air for 3-5 minutes, and then breathed, in succession, each of the three CO_2-O_2 mixtures for 3 minutes each, and recovered by breathing room air. In all conditions, airway occlusions were applied twice per minute, to obtain measurements of $P_{0.1}$.

Measurement of pulmonary ventilation, inspired and expired gas concentrations and $P_{0.1}$. Subjects breathed through a tight fitting mask that covered the nose and mouth, and that allowed free breathing through either the oral or nasal airway (Hans-

Rudolph Pediatric rubber face mask). An additional rubber seal was created around the mask using Exaflex (GC America, Inc.) The mask was checked for leaks by instructing the subjects to hyperventilate while an investigator looked for leaks by sampling CO_2 around the mask seal. When leaks were noted, they were sealed with additional Exaflex. The end-tidal CO_2 on inspiration returned to zero under all conditions, indicating that the system dead space was sufficiently low to prevent rebreathing.

A low dead space non-rebreathing valve (Hans Rudolph 2600) was attached to the mask, and a short length of tubing and a pneumotachometer (Hans-Rudolph 4700) were placed on the expiratory side of the breathing valve for the measurement of expiratory airflow. The pressure drop across the pneumotachometer was measured with a differential pressure transducer with a ± 2.5 cmH_20 diaphragm (Validyne MP 45). The pneumotachometer was calibrated with a precision Matheson Rotameter before each experiment. The respiratory period was measured from the flow signal, and used to compute breathing frequency (f). Expired flow was integrated by the computer off-line to derive expired tidal volume (V_T), which was converted from ambient temperature, pressure and humidity conditions (ATPS) to body temperature, pressure and humidity conditions (BTPS), with the assumption that body temperature was 37 ^oC. Expired ventilation (V_E) was computed offline as the product of V_T and f. Breath-by-breath values for CO_2 and O_2 were measured with a rapidly responding analyzer (Raytech Instruments) that was connected to the non-rebreathing valve by small-bore tubing. The output of the analyzer was digitized and used to compute the end-tidal levels of CO_2 and O_2. Mask pressure was measured by connecting a short

length of PE 200 tubing to the center of the non-rebreathing valve, and attaching the opposite end to a differential pressure transducer with a ± 56 cm H_20 diaphragm (Validyne MP 45).

For the measurement of $P_{0.1}$, a Hans-Rudolph automated balloon valve was attached to the inspiratory side of the non-rebreathing valve. The balloon was connected to a compressed air source, and was inflated or deflated with a solenoid valve and vacuum pump, respectively. The computer controlled the activation and deactivation of the solenoid valve and pump. The inspiratory port was occluded during expiration, and the occlusion was maintained for about 200 ms into the ensuing inspiration, allowing sufficient time to obtain measurements of $P_{0.1}$ [8]. The computer measured the drop in mask pressure exactly 0.1 seconds after the onset of the occluded inspiratory effort, and denoted this value as $P_{0.1}$.

Data processing and statistical analysis. Six-to-ten breaths obtained over the last 30-40 seconds under control conditions and at each level of hyperoxic hypercapnia, were used to calculate average values of V_T, f, V_E and the partial pressures of end-tidal O_2 ($P_{ET}O_2$) and CO_2 ($P_{ET}CO_2$). All $P_{0.1}$ values obtained in each condition were averaged, so that each subject had a single $P_{0.1}$ measurement for each of the experimental conditions. All $P_{0.1}$ values were expressed in units of cmH_2O.

The V_E values for each subject were divided by the subject's body surface area (BSA, m^2) to account for the large differences in size (and hence tidal volume) over the 6-year age range of our pediatric subject population. This analysis allowed us to compare the hypercapnic ventilatory responses of our subjects with previously published results in children of various ages.

To determine if the severity of SDB correlates with ventilatory drive, we first plotted both V_E and $P_{0.1}$ against $P_{ET}CO_2$ for each subject, and computed the slope of the relation as an index of hypercapnic ventilatory drive using linear regression analysis. We then plotted each subject's hypercapnic response slope against their OAHI, and subjected the data to a linear regression analysis. To determine if resting CO_2 retention was correlated with the OAHI, we plotted the resting level of $P_{ET}CO_2$ for each subject against his or her OAHI. We used a simple linear regression model followed by analysis of variance (ANOVA, Sigma Stat 3.0) to determine if the relation was statistically significant, with significance defined as a P value < 0.05.

RESULTS. As shown in Table 1, the subject's average RDI and OAHI values were significantly lower in 2006 compared to 1999. Figures 1 A and 1B show the RDI and OAHI values for each subject, and it is clear that these values fell with age in all but three subjects, in whom the values were very low to begin with. Weight, height, BMI and BSA all were significantly higher in 2006 compared to 1999 (Table 1). The BMI percentile adjusted for age, gender and ethnicity (calculated from the US Centers for Disease Control and Prevention growth charts, http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/index.htm) was higher in 2006 than in 1999 for 11 of the 14 subjects (Fig. 1C). Total sleep time and SaO_2 nadir measured in the 1999 and 2006 nocturnal sleep studies did not differ significantly (Table 1).

The absolute pulmonary ventilation rate measured under resting conditions increased with age (Fig. 2A), but was the same when it was expressed as a function of

body surface area (P=0.26, Fig. 2B). The rise in resting ventilatory output was associated with a much lower $P_{ET}CO_2$, from 44.1 ± 0.8 mmHg in 1999 to 33 ± 1.0 in 2006 (P<0.001; Fig. 2C). In contrast to the increase in lung ventilation with age, the resting $P_{0.1}$ showed marked variability at both ages, and the mean values were not significantly different (Fig. 2D). It is important to point out here that our baseline $P_{0.1}$ values are within the range reported for children of similar age by Marcus and colleagues [9].

We estimated central CO_2 sensitivity by conducting steady-state CO_2 response tests at 3, 5 and 7% inspired CO_2. The individual data points that were used to define the slope of the V_E-$P_{ET}CO_2$ response for all subjects are shown in Fig. 3A. It is clear from this graph that although the slopes in 1999 and 2006 are not different (1999, y = 1.28 (x) - 35.6; 2006, y = 1.31 (x) - 28.6, t= 0.08, P = 0.94), the curve is markedly left-shifted, such that V_E was much higher at any given $P_{ET}CO_2$ in 2006 compared to 1999 (Fig. 3A). For example, at a $P_{ET}CO_2$ of 50 mmHg the V_E estimated from the data shown in Fig. 3A would be 28.4 L/min in 1999 and 36.9 in 2006. Similarly, the average x-intercept calculated from the slopes, the so-called “apnea point” [10], was significantly different (1999, 33.1 ± 1.1; 2006, 26.4 ± 2.7, t = 2.41, P = 0.03). Figure 3B shows that the left-shift in the V_E-$P_{ET}CO_2$ response curves is due to a corresponding left-shift in the V_T-$P_{ET}CO_2$ response (1999, y = 0.05 (x) – 1.08; 2006, y = 0.07 (x) - 0.34, t = 1.43, P = 0.16), coupled with a frequency response that was identical in 1999 and 2006 (data not shown). To correct for changes in V_E secondary to growth, we also examined the V_E-$P_{ET}CO_2$ response curves with V_E expressed as a function of the body surface area.

Again, there were no significant differences in the slope of the relationship (1999, y = 0.65 (x) - 19.6; 2006, y = 0.72 (x) - 15.3, t=0.36, P=0.72).

Interestingly, the $P_{0.1}$-$P_{ET}CO_2$ slopes in 1999 and 2006 (Fig. 4) were statistically identical (1999, y = 0.23 (x) – 5.7; 2006, y = 0.22 (x) - 5, t=0.16, P=0.87), and were only slightly left-shifted, such that the apnea point was the same in 1999 and 2006 (1999, 26.7 ± 2.2; 2006, 23.7 ± 1.8, t = 1.24, P = 0.24). Individual V_E-$P_{ET}CO_2$ and $P_{0.1}$-$P_{ET}CO_2$ slopes for each subject are shown in Fig. 5, A and C. With a few exceptions, the slopes did not change with age, and the group average slopes were the same in 1999 and 2006 (for V_E-$P_{ET}CO_2$, 1999 = 1.7 ± 0.24; 2006 = 1.65 ± 0.22, t = 0.14, P=0.89; for $P_{0.1}$-$P_{ET}CO_2$, 1999 = 0.28 ± 0.04; 2006 = 0.32 ± 0.05, t=0.6969, P=0.49). The individual apnea points for each subject are shown in Fig. 5 B and D. Statistical analysis of the individual apnea points revealed significant differences for the V_E-$P_{ET}CO_2$ curves (1999, 35.5 $\pm$ 1.3; 2006, 24.6 ± 3.2, t = 3.16, P = 0.0082), but not for the $P_{0.1}$-$P_{ET}CO_2$ curves (1999, 29.9 ± 2.5; 2006, 23.7 ± 2.0, t = 1.4, P = 0.19).

DISCUSSION. This is the first longitudinal study of developmental alterations in the ventilatory control of children with sleep-disordered breathing in early-childhood. Our subjects were, on average, 9 years of age on initial study, and 15 years when the current study was completed. Our main finding is that subjects now have much lower OAHIs and retain much less CO_2 than they did in 1999, despite similar mass-specific resting ventilation rates and a substantial increase in BMI. We also showed that, on average, the slope of either the V_E-$P_{ET}CO_2$ curve or the $P_{0.1}$- $P_{ET}CO_2$ curve were not significantly different in 1999 and 2006, indicating that CO_2 sensitivity during steady

state hypercapnic challenge was unchanged. However, the V_E-$P_{ET}CO_2$ response was markedly left-shifted in 2006 compared to 1999, indicating greater V_E at a given $P_{ET}CO_2$, despite no change in CO_2 sensitivity. This left-shift also resulted in much lower apnea points [10]. Interestingly, there was no left-shift in the $P_{0.1}$- $P_{ET}CO_2$ curve. As discussed below, our observations are most likely explained by changes in mechanical factors, e.g., a decrease in airflow resistance, rather than changes in the central control of breathing. That the left-shift in the V_E-$P_{ET}CO_2$ response curve was the result of changes in tidal volume rather than breathing frequency also supports the idea that the effects were mechanical and not central.

In theory, the increased lung ventilation rate at a given $P_{ET}CO_2$ with no change in the sensitivity to CO_2 could be explained by a reduction in airway resistance. This is due in large part to somatic growth of the airway as children grow taller [11]. Using the regression equations derived by Zapletal and Chalupova [11], we estimate that nasopharyngeal resistance in our subjects would have fallen by 23% on the basis of the change in height alone (136 cm tall in 1999 vs. 163 inches tall in 2006, Table 1). In addition, it is well known that tonsil size declines with age after reaching a peak size between 4 and 8 years of age [12]. There have been anecdotal suggestions that enlarged tonsils, and thus a narrow pharyngeal airway, predispose children to nasal breathing, which in turn leads to hypoventilation through the high-resistance nasal pathway. When our subjects were studied initially, in 1999, we found that their resting $P_{ET}CO_2$ during wakefulness was significantly elevated, and that it was correlated with the OAHI [1]. Our previous MRI studies showed that a sub-sample of the group studied in 1999 had large adenoids, tonsils and soft palates [13]. We analyzed those data by

computing the sum of the cross-sectional areas of these soft tissue structures and expressed the sum as a percentage of the nasopharyngeal cross sectional area. We found that the children with sleep-disordered breathing had a narrow nasopharynx as a result of increased soft tissue mass. The present data suggest that this ratio is now smaller. In other words, if their pharyngeal airway grew at a faster rate than the surrounding soft tissue structures, the lumen of the nasopharynx would be enlarged, leading to lower airway resistance. Although we do not have MRI data as part of this study, the increased ventilation and lack of CO_2 retention in the absence of a change in sensitivity to $P_{ET}CO_2$ is consistent with a larger upper airway lumen. The results of this study and our earlier one are consistent with other data showing hypoventilation and CO_2 retention in young children with enlarged tonsils and sleep disordered breathing [14, 15].

We observed that both the RDI and OAHI decreased over the approximate 6-year interval between PSGs in these subjects. Preliminary examination of data from the entire TuCASA cohort confirms this finding [2]. In contrast to our findings, a previous study in Thai children showed that 5 of 7 children with obstructive sleep apnea had a higher OAHI over a 3-year interval [16]. However, PSG was performed in these children because they had symptoms of OSA, and thus there may have been some selection bias. As discussed above, we suspect that in our study the observed decrease in RDI and OAHI is related to somatic growth of the pharynx, coupled with regression of tonsillar tissue with age. At the initial TuCASA examination, children were studied between the ages of 6 and 11 years. This is the age range where some children will have large tonsils resulting in an elevated RDI and OAHI. With normal regression in

tonsil size as they become adolescents, there should be a decrease in RDI and OAHI as we have found.

Although our observations are consistent with changes in airway resistance, the exact mechanism of the CO_2 retention during quiet breathing at an average age of 9 years, but not approximately six years later remains unknown. One possibility is that the young children "chose" to hypoventilate rather than fight the increased flow resistance and thus higher work of breathing that would have been required to drop their $P_{ET}CO_2$. This is consistent with the strategy employed by highly trained athletes during peak exercise, wherein they allow themselves to become hypoxic and relatively hypercapnic rather than consume the extra energy that would be required to elevate alveolar ventilation sufficiently to fully correct the blood gas and acid base derangements [17].

The left-shifted ventilatory response to $P_{ET}CO_2$ is often considered to be due to an "extra" stimulus to breathe. If this were the case we would have expected a left-shift in the relation between $P_{0.1}$ and $P_{ET}CO_2$, which we did not observe (Fig. 4). Complicating the relation between $P_{0.1}$ and $P_{ET}CO_2$ is that the former can be influenced by both respiratory muscle strength and end-expiratory lung volume. Weak inspiratory muscles lead to lower $P_{0.1}$ values during hypercapnic challenges, although the effects are small until the $P_{ET}CO_2$ exceeds 60 mmHg [18]. This would have little or no impact on our data as the $P_{ET}CO_2$ values were less than 60 mmHg in every case (Figs. 3 and 4). Inspiratory muscle strength increases by about 20% from age 9 to 15 in boys [19], suggesting that changes in strength alone as the subjects grew would result in slightly higher $P_{0.1}$ values in 2006 compared to 1999 (Figure 4), but this was not seen.

The $P_{0.1}$ can also be influenced by changes in end-expiratory lung volume, with lower volumes associated with a greater $P_{0.1}$, due to improved muscle length-tension properties and thus improved mechanical advantage [20, 21]. However, the pertinent issue is the end-expiratory lung volume as a percentage of an individuals total lung capacity, as this dictates the length-tension relationship of the respiratory muscles for that particular system [22]. End-expiratory lung volume as a percentage of total lung capacity increases from approximately 46% in 9 year olds (the average age of our subjects in 1999), to 53% in 15 year olds (average age in 2006), corresponding to a volume increment of about 400 ml [23]. It has been shown that an increase in FRC of 500 ml reduces respiratory muscle pressure development by about 10% [22]. In our subjects this would translate into at most an 8% decrease in the $P_{0.1}$ (400/500 x 10%), which would result in only a negligible shift in the $P_{0.1}$-$P_{ET}CO_2$ curve (Figure 4). Obesity can reduce end-expiratory lung volume independently of age and height, although the effects are small and variable [24, 25] except in severe obesity [26]. Most of our subjects were in a higher BMI percentile in 2006 than they were in 1999 (Fig. 1C), with some of them exhibiting severe obesity (i.e., BMI values at or above the 95th percentile). This could also contribute to a slight leftward shift in the $P_{0.1}$-$P_{ET}CO_2$ relation, but again, this was not observed. Finally, although it is possible that hypercapnia could increase airway resistance to a variable extent across the subject population, the $P_{0.1}$ is uninfluenced by airway resistance and behavioral adjustments in ventilatory output [8, 22]. Taken together, our longitudinal data support the contention that the elevated resting $P_{ET}CO_2$ and the left-shifted V_E-$P_{ET}CO_2$ curve in younger children is the result of

reduced flow resistance, and likely not to the addition of an “extra” excitatory stimulus to breathe.

The functional consequences of the elevated eupneic $P_{ET}CO_2$ when the children were younger are unknown. Given that the apnea point was significantly higher in 1999 than in 2006, one might surmise that the tendency for apnea was greater in the young children. However, the difference between the apnea point and the eupneic $P_{ET}CO_2$ was the same in 1999 and 2006 (1999, 7.7 $\pm$ 1; 2006, 6.9 $\pm$ 3, P=NS). This difference has been called the CO_2 reserve, and it has been suggested that a smaller reserve increases the propensity for apnea in adult human subjects [27]. Our subjects had higher RDI values in 1999 than in 2006 despite a similar CO_2 reserve, suggesting that the CO_2 reserve may not predict a predisposition to apnea in children.

In conclusion, we have examined changes in the control of breathing from childhood to adolescence in a group of subjects that had mild sleep-disordered breathing as young children. The main finding is that the rate of pulmonary ventilation at a given $P_{ET}CO_2$ was much higher, and the eupneic $P_{ET}CO_2$ much lower at an average age of 15 compared to an average age of 9. This occurred in the absence of changes in sensitivity to inspired CO_2, suggesting that upper airway resistance dropped as the children grew, leading to improved alveolar ventilation in the absence of significant changes in central ventilatory drive.

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Table 1. Anthropometric and sleep data in 1999 and 2006.

	1999	2006	P value
N	14	14	
Gender	8M, 6F	8M, 6F	
Age (y)	8.7 ± 0.91 (7-10)	15 ± 1.3 (12-17)	< 0.0001
Weight (kg)	38 ± 12 (24-67)	71 ± 18 (49.6-101)	< 0.0001
Height (cm)	136 ± 8.3 (120-149)	163 ± 7.1 (149-174)	< 0.0001
BMI (Kg/m^2)	20 ± 4.6 (15-30)	26.8 ± 5.7 (19-37)	< 0.0001
BSA (m^2)	1.18 ± 0.21 (0.90-1.61)	1.75 ± 0.22 (1.4-2.1)	<0.0001
RDI (events/h)	8.6 ± 5.3 (2.7-19)	2.7 ± 1.8 (0.5-7.8)	0.0018
OAHI (events/h)	7.5 ± 4.7 (1.8-17)	2.5 ± 1.8 (0.3-7.6)	0.001
Total Sleep time (min)	487 ± 56 (314-530)	446 ± 51 (367-528)	NS
SaO_2 nadir (%)	89 ± 13 (87-91)	91 ± 2.9 (86-94)	NS
SBP (mmHg)	102 ± 1.3 (72-122)	107 ± 9.9 (88-122)	NS
DBP (mmHg)	65 ± 10 (50-88)	61 ± 7.6 (48-74)	NS

Values are mean ± SD, with range in parentheses. N, number of subjects; M, male, F, female; BMI, body mass index; BSA, body surface area; RDI, respiratory disturbance index; OAHI, obstructive apnea-hypopnea index; SaO_2, oxygen saturation of arterial blood; SBP systolic blood pressure; DBP, diastolic blood pressure (see text for definitions).

FIGURE LEGENDS.

Figure 1. The respiratory disturbance index (RDI, *panel A*) and the obstructive apnea-hypopnea index (OAHI, *panel B*) recorded in 1999 and in 2006, for all 14 subjects. The OAHI declined in every subject. See Table 1 for average values, and text for description of how the RDI and OAHI values were calculated. **, Different than 1999 at P<0.01; ***, Different than 1999 at P<0.001. Panel C shows the BMI percentile values for each subject, in 1999 and 2006. The line of identity is shown, and it is clear that most subjects were in a much higher percentile in 2006 compared to 1999 (see text for details).

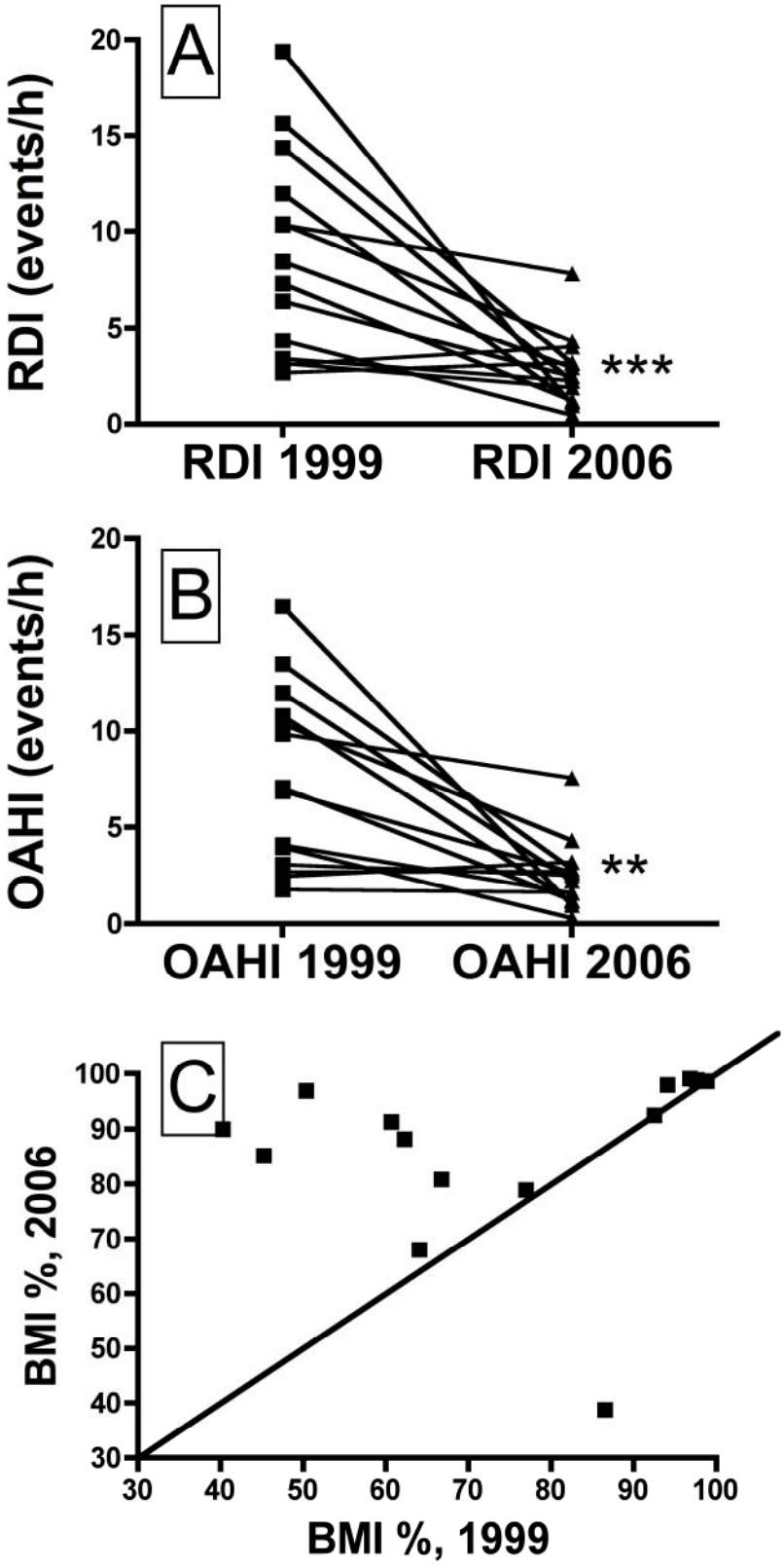

A
RDI (events/h)
20
15
10
5
0
***
RDI 1999
RDI 2006
B
OAHI (events/h)
20
15
10
5
0
**
OAHI 1999
OAHI 2006
C
BMI %, 2006
100
90
80
70
60
50
40
30
30
40
50
60
70
80
90
100
BMI %, 1999

Figure 2. Resting values for absolute expired minute ventilation (V_E, *panel A*), V_E corrected for body surface area (BSA, *panel B*), the partial pressure of end tidal CO_2 ($P_{ET}CO_2$, *panel C*) and the mouth pressure measured 100 ms after the onset of inspiratory effort ($P_{0.1}$, *panel D*). V_E was higher in every subject in 2006 compared to 1999, but V_E corrected for BSA (*panel B*) was the same in 1999 and 2006. $P_{ET}CO_2$, declined in every subject (*panel C)*, while $P_{0.1}$ did not change (*panel D*). **, Different than 1999 at P<0.01; ***, different than 1999 at P<0.001.

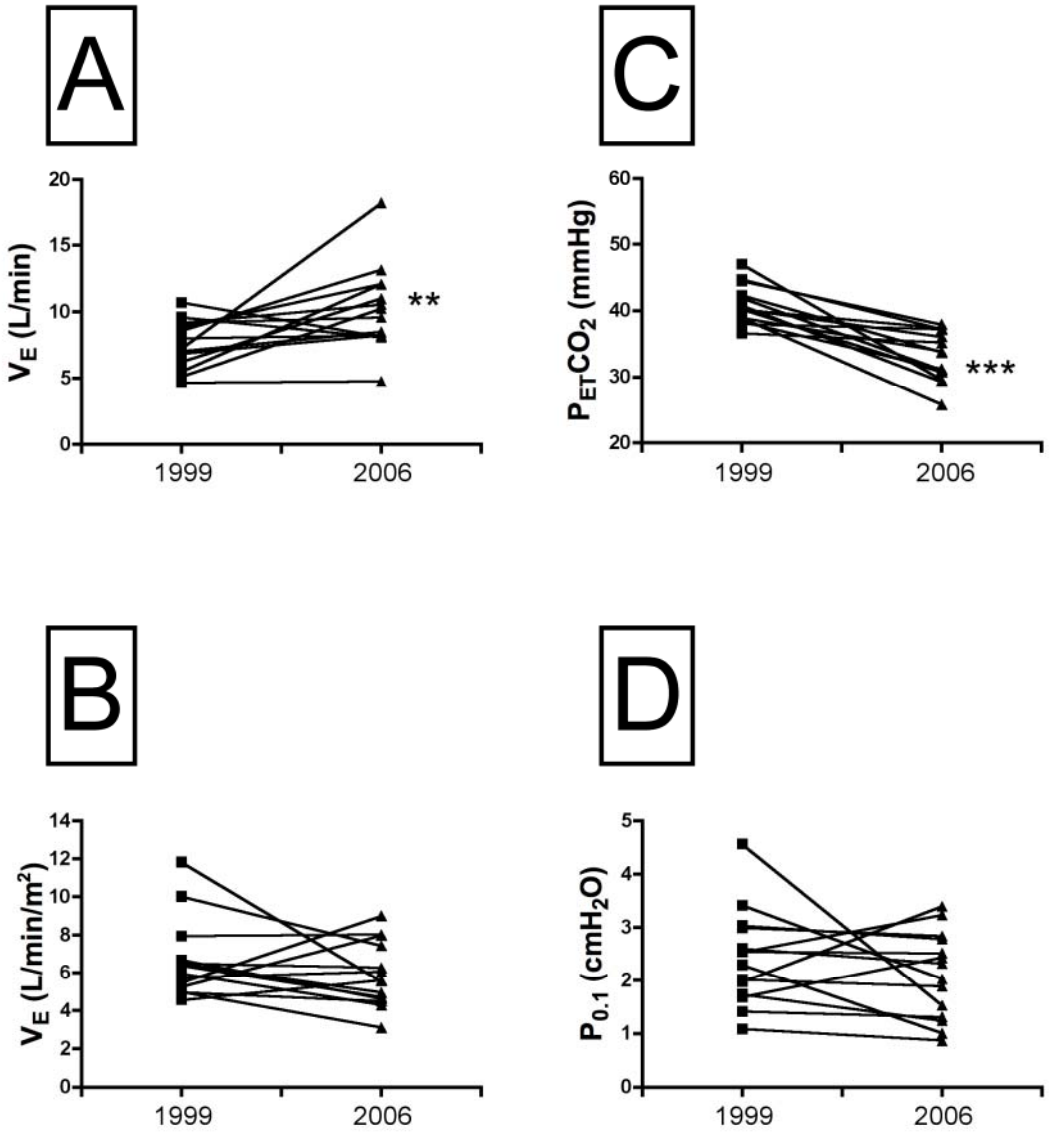
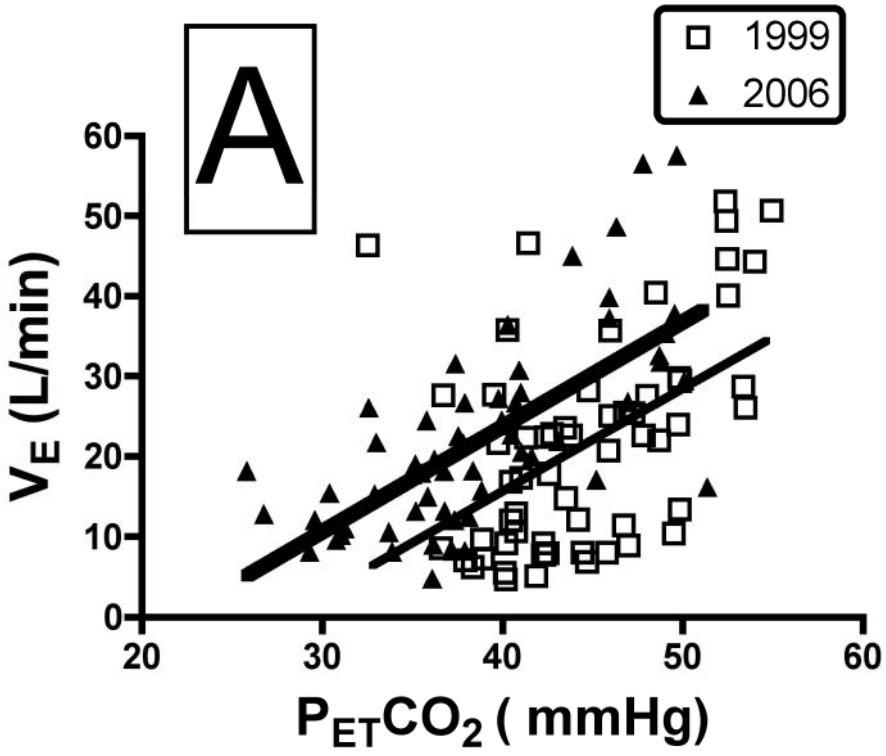


Figure 3. *Panel A* shows the V_E- $P_{ET}CO_2$ relation in 1999 (open squares) and 2006 (filled triangles). Each point represents data points obtained at rest, and in the steady state of breathing gas mixtures with 3, 5 and 7% inspired CO_2 in all 14 subjects, as described in Methods. The slope of the relation was the same in 1999 and 2006 (1999, y=1.28 (x) - 35.6; 2006, y=1.31 (x) - 28.6, t= 0.08, P = 0.94), but the regression

line obtained in 2006 was markedly left-shifted, as discussed in text. *Panel B* shows the relationship between tidal volume (V_T) and $P_{ET}CO_2$ in all subjects. As with V_E, the slope of the relation was the same in 1999 and 2006 (1999, y=0.045 (x) – 1.1; 2006, y= 0.066 (x) – 1.45, t= 1.43, P = 0.16), but the curve in 2006 was markedly left-shifted. These data indicate that the left-shift in the ventilatory response to CO_2 (*panel A*) was due to differences in the V_T response, as the frequency response was unaltered (data not shown).


A
1999
2006
60
50
40
30
20
10
0
V_E (L/min)
20
30
40
50
60
$P_{ET}CO_2$ (mmHg)

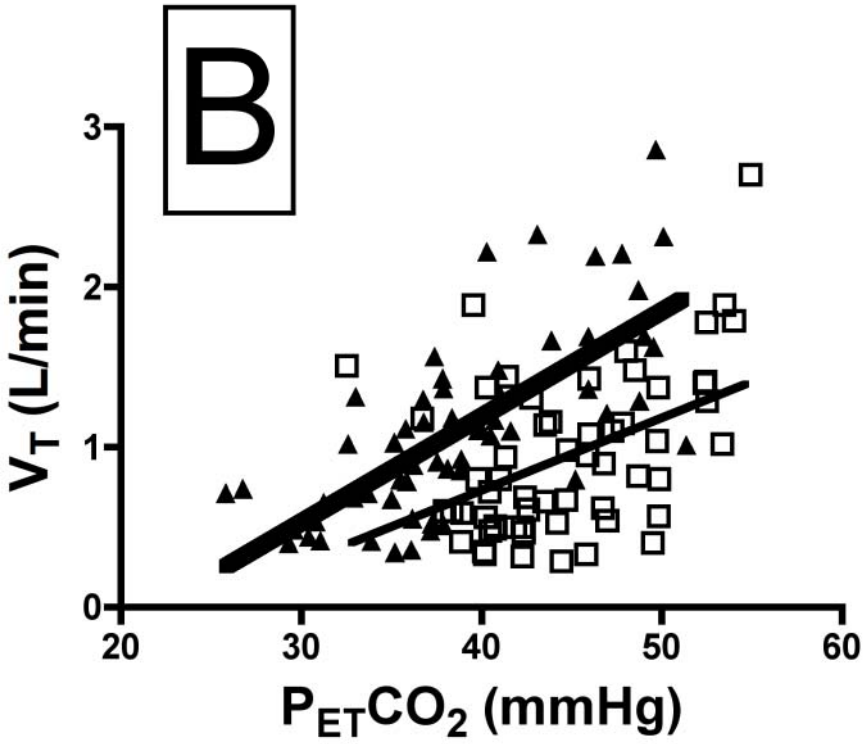

B
3
2
1
0
V_T (L/min)
20
30
40
50
60
$P_{ET}CO_2$ (mmHg)

Figure 4. Relationship between mouth occlusion pressures measured 100 ms after onset of inspiratory effort ($P_{0.1}$) and $P_{ET}CO_2$ in all subjects. The slopes calculated in 1999 and 2006 were the same (1999, y=0.23 (x) - 5.7; 2006, y=0.22 (x) – 5.0, t=0.16, P=0.87), suggesting that the central ventilatory response to CO_2 did not change (see Discussion).

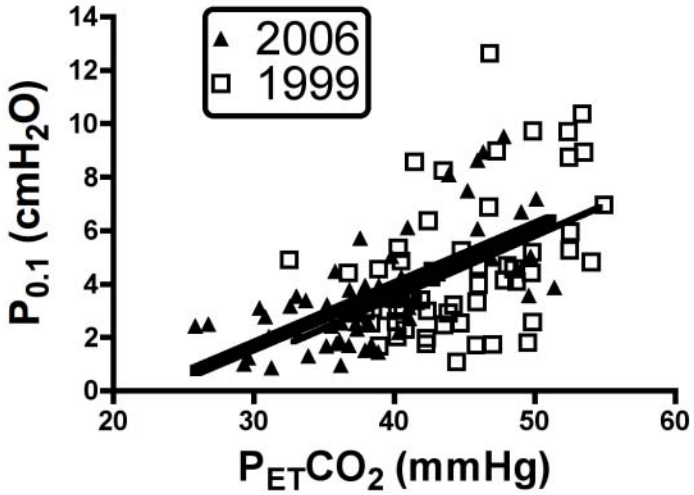


Figure 5. Individual V_E-$P_{ET}CO_2$ (panel A) and $P_{0.1}$-$P_{ET}CO_2$ (panel C) slopes in 1999 and 2006, for each of the 14 subjects. These data demonstrate the variability between subjects with respect to the V_E-$P_{ET}CO_2$, and the $P_{0.1}$-$P_{ET}CO_2$ relation, and that on average there was no age-dependent change in either of these relationships. Panels B and D show the apnea points computed from V_E-$P_{ET}CO_2$ and $P_{0.1}$-$P_{ET}CO_2$, curves, respectively. The apnea point derived from the V_E-$P_{ET}CO_2$ curve was significantly lower in 2006 compared to 1999 (panel B), consistent with the left shift in the V_E-$P_{ET}CO_2$ calculated from the composite data (Fig. 3A). The apnea points derived from the $P_{0.1}$-$P_{ET}CO_2$ curve were the same in 1999 and 2006, consistent with the lack of

change in central ventilatory drive, as shown for the composite data in Fig. 4. *, Different than 1999 at P<0.05.