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Effects of aging on peripheral chemoreceptor CO_2 response during sleep and wakefulness in healthy men

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ABSTRACT

Sleep-disordered breathing (SDB) prevalence multiplies with age and the mechanisms underlying this disorder are complex. Fifteen young and 13 elderly healthy male volunteers, ages ranging from 25 to 38 and from 55 to 76 years, were studied on three consecutive nights at the sleep laboratory. The peripheral chemoreceptor CO_2 response was estimated by the single breath CO_2 test ($SBCO_2T$). The apnea-hypopnea index (AHI) was non-significantly higher in elderly men than in young men. During rapid eye movement (REM) sleep and non-REM sleep $SBCO_2T$ was similar in young and elderly subjects. During wakefulness, young had a tendency to higher $SBCO_2T$ than elderly (0.25 ± 0.08 L/min/mm Hg vs. 0.19 ± 0.07 L/min/mm Hg, respectively; $p = 0.054$), but the five elderly subjects with AHI > 5 had significantly higher $SBCO_2T$ than the remaining elderly subjects (0.24 ± 0.07 L/min/mm Hg vs. 0.16 ± 0.04 L/min/mm Hg, respectively; $p = 0.024$). Aging seems to spare the $SBCO_2T$ during sleep. Investigation of $SBCO_2T$ during transition from wakefulness to sleep, as a factor in the pathogenesis of disordered breathing in elderly men, is justified.

1. Introduction

Sleep apnea is a medical condition with grave physical, mental, and social consequences (Flemons, 2002; Caples et al., 2005; Durmer and Dinges, 2005; AlGhanim et al., 2008). The prevalence of sleep-disordered breathing (SDB) increases with age and peaks during the sixth decade in men (Bixler et al., 1998) and during the seventh decade in women (Bixler et al., 2001). Depending on the definition of SDB and sleep apnea syndrome, their prevalences can vary from 27 to 75% of the elderly population (Feinsilver, 2003). The reason for the observed increase in the prevalence of SDB with aging has been clearly linked to menopause in women, but remains to be elucidated in men (Jordan and McEvoy, 2003).

The association of SDB with maturity may be a consequence of several factors, including an age-related increase in body weight, loss of upper airway muscle tone, and non-morbid processes germane to aging that operate on the ventilatory control system. Among the complex interplay of ventilatory control system components, the peripheral chemoreceptors are actors of particular interest in the SDB scenario, since their fast response characteristics place their activity within the time envelope in which SDB takes place (Shaw et al., 1982; Smith et al., 2003; Dempsey et al., 2004; Gauda et al., 2007). Although not all disordered breathing events are due to control system instability, it is interesting that both reduced and increased peripheral chemoreflex can be implicated in the pathogenesis of sleep apnea. Carotid body denervated (Nakayama et al., 2003) or dopamine-treated dogs show smaller CO_2 reserves and increased propensities for apnea in response to even smaller transient reductions in $PaCO_2$ (Chenuel et al., 2005). On the other hand, increases in system gain can destabilize the ventilatory control system leading to SDB (Cherniack and Longobardo, 2006).

Carotid bodies respond within seconds to changes in arterial levels of O2, CO_2, and H^+, being these responses mediated by different transduction mechanisms and interconnected in a complex fashion (Phillipson et al., 1981; Ursino and Magosso, 2002). The single breath CO_2 test ($SBCO_2T$) constitutes a valid appraisal of the CO_2 peripheral chemoreflex. In awake subjects, the test proved to be risk-free, reproducible, and independent of the central chemoreflex (McClean et al., 1988). Although it seems ideally suited for investigations of the peripheral chemoreceptor CO_2 response during sleep, at the present time, this test has not been employed in sleeping subjects.

To determine whether there is any underlying age-related increase or decrease in healthy male subjects' peripheral chemoreceptor CO_2 responsiveness that might explain the propensity for breathing instability observed in elderly individuals during sleep, the response to a single breath of CO_2 was studied in healthy men of two age groups during wakefulness and sleep.

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2. Methods

2.1. Subjects

Male volunteers from the hospital staff and the community, with ages ranging from 25 to 38 years and 55–76 years, were screened based on the following criteria: no history of snoring, restless sleep, or morning headaches; no past history of stroke or seizures; no present history of allergic rhinitis, hay fever, or other cause of nasal obstruction; no history of pulmonary or cardiac disease. The subjects could not be taking medications and had to be non-smokers, light users only of alcohol, and not regular users of any caffeinated beverage at night. Individuals with life-long obesity or with body mass index greater than 30 kg/m^2 were also excluded.

Fifteen healthy young men and 13 healthy elderly men (Table 1) were included in the study. They all lived in the Toronto area for at least 1 year before the study. Twenty-six were of European ancestry, one young subject was Japanese, and one elderly subject was East Indian. The subjects were screened in an interview, in person or by telephone. They were instructed to arrive at the sleep laboratory 1 h before their usual bedtime without changing their routines, but to abstain from alcohol entirely and caffeinated beverages after noon during the 3 days of the study. The research project was approved by the University of Toronto review board. Subjects gave informed consent and were paid an honorarium.

2.2. Polysomnography

The polysomnogram was recorded using conventional techniques described previously (Bradley et al., 1986) and was analyzed using standard criteria (Rechtschaffen and Kales, 1968). Ventilation was monitored every night using a respiratory inductive plethysmograph (RIP; Respitrace, Ardsley, NY, USA) and, on the night of the chemoreflex testing, was monitored with a pneumotachygraph (PNT; Godart Stathan, USA). The RIP was calibrated against a Spirotel water spirometer (Computer Instrument, Hampstead, NY, USA) by the two-position method with the calibration factor calculated by the least squares method (Chadha et al., 1982). Apneas were defined as periods of breathing cessation for at least 10 s. Hypopneas were defined as periods 10 s or longer with tidal volumes smaller than 50% of the previous stable baseline tidal volumes. Periodic breathing was characterized by the presence of at least three consecutive cycles of oscillation of the tidal volume, showing a crescendo–decrescendo pattern, without a phase of stable ventilation, and without paradoxical movement of the rib cage and abdomen. Ventilation was analyzed from the sum channel of the RIP. Tidal volume (V_T), inspiratory time (T_i), total duration of the ventilatory cycle (T_{tot}), ventilatory frequency (f), V_T/T_i, and T_i/T_{tot} were represented by the mean and standard deviation of 30 breaths taken from epochs of electrographically stable sleep/wakefulness stages.

Table 1
Anthropometric and pulmonary function characteristics of the study subjects

	Young men (n = 15)	Elderly men (n = 13)	p
Age (years)	29 ± 4	63 ± 5	
Height (cm)	176 ± 7	176 ± 6	0.98
Weight (kg)	71 ± 10	80 ± 10	0.025
BMI (kg/m^2)	22.7 ± 2.3	25.7 ± 2.6	0.003
VC (% predicted)	116 ± 15	120 ± 14	0.48
F_EV1 (% predicted)	122 ± 14	124 ± 12	0.71
V50 (% predicted)	112 ± 20	85 ± 23	0.002
V25 (% predicted)	103 ± 31	47 ± 13	0.000

BMI, body mass index; VC, vital capacity; F_EV1, forced expiratory volume. V50, expiratory flow at 50% of VC; V25, expiratory flow at 25% of VC.

On the first night, for adaptation, only the sleep variables were recorded during undisturbed sleep; on the second night, the ventilation variables were measured as well as the sleep variables during undisturbed sleep; on the third night, ventilatory responses to the single breaths of CO_2 were measured. The last six subjects were studied without the adaptation night, following the protocol of the second and third nights. On the third night, the subjects were initially asked to stay awake in bed while the $SBCO_2T$ was performed 6–10 times.

2.3. $SBCO_2$ test

The response to $SBCO_2$ was measured as described previously (McClean et al., 1988). The CO_2 concentration was sampled from inside a light-weight, transparent, tight fitting mask with a 60 mL dead space and built-in inspiratory and expiratory valves (Downs CPAP mask, Vital Signs, East Rutherford, NJ, USA) and measured with a LB2 analyzer (Beckman, Schiller Park, IL, USA) with sampling flow rate of 0.5 L/min and response time to detect 95% of a square-wave change in CO_2 fraction of 100 ms.

The inspiratory port of the mask was connected to two lines by means of a Y tube (Fig. 1). In each branch of this Y tube, a small balloon could be inflated or deflated to block or allow air flow without noise. One of the lines was connected to the box and the other to the bag of a bag-in-box system. The PNT system placed at the input to the box was linear from 0 to .346 L/s. The bag contained a mixture of 13% CO_2, 21% oxygen, and balance nitrogen, obtaining an inspiratory CO_2 fraction ($FICO_2$) of around 10%. The recorders were located in a room adjacent to the bedroom, from where the polysomnogram was monitored and the tests performed. For the measurement, ventilation during four breaths was recorded simultaneously with the inspired and expired CO_2 fractions ($FECO_2$).

If ventilation was stable, during the fifth inspiration, the inspiratory line from the bag containing the CO_2 mixture was opened and the line from room air was closed by the balloons. After the subjects inspired the CO_2 mixture, the balloons were immediately switched back to the original situation. The ventilation and F_ECO_2 for the test

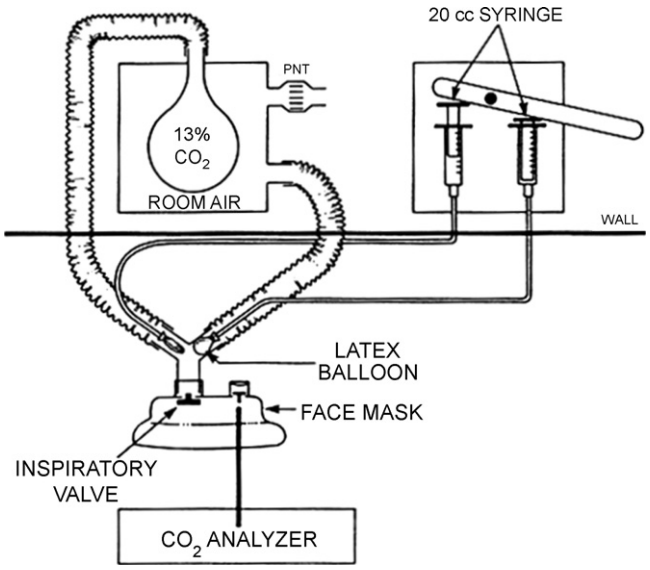

Fig. 1. Diagram of the circuit used to deliver a breath of 13% CO_2. The subject wears the mask all night and inspires room air from the line connected to the box through the pneumotachygraph (PNT). CO_2 in the mask is sampled continuously. To perform the test, the lever controlling the syringes is lowered to the position shown in the figure, occluding the line connected to room air and opening the line from the bag with CO_2. The system is noiseless.

breath and all subsequent breaths for 30 s were recorded and the breath with the largest ventilation was considered the response breath. Tests were repeated with 2- to 3-min intervals between exposures. The delay between the beginning of the CO_2 breath and the beginning of the response breath was measured to certify that the response was occurring in a time frame compatible with the peripheral chemoreflex. The response was calculated as the difference in ventilation from baseline to the response breath divided by the difference in $PETCO_2$ from baseline to the stimulus breath and expressed in L/min/mm Hg. When an arousal, awakening, or stage change occurred during the procedure, the test was discarded.

To validate the $SBCO_2T$ procedure, six young and five elderly men repeated the test during the day in the pulmonary function laboratory in the sitting position. In five subjects, just before bedtime, $SBCO_2T$ was performed seated on the bed to allow comparison between seated and supine responses during wakefulness.

2.4. Statistical analysis

The SAS program (SAS, Cary, NC, USA) was used to calculate statistics. Student's *t*-test was used to compare two means and repeated measures ANOVA was used for comparisons between multiple sleep/wake states. Multivariate regression analysis was utilized to adjust for the effects of confounding variables. Correlations with apnea-hypopnea index (AHI), due to its non-normal distribution, were measured employing a non-parametric test. The specific statistical test employed and the value of p will be mentioned for each result in the next section.

3. Results

3.1. Sleep architecture

To assess the sleep architecture across age groups, sleep variables on the second night were compared by means of Student's *t*-test. There was only a tendency for sleep efficiency to be lower in the elderly group ($p = 0.051$). Results are presented in Table 2. The first 22 subjects who underwent an adaptation night did not differ from the last 6 subjects with no adaptation (4 from young, 2 from elderly group) in all tested sleep architecture and sleep stages variables; for example, sleep efficiency was $87 \pm 10\%$ vs. $88 \pm 4\%$, respectively ($p = 0.26$) and sleep latency was 8 ± 5 min vs. 10 ± 4 min, respectively ($p = 0.3$).

3.2. Pattern of breathing

Descriptors of the pattern of breathing are shown in Fig. 2. The pattern of breathing was analyzed from ventilation measured by RIP, using repeated measures ANOVA, with the five stages (wakefulness, S1, S2, slow wave sleep (SWS), and rapid eye movement (REM) sleep) as within-subjects variables and with age group as between-subjects factor. In two elderly men, it was impossible to measure ventilation in S1 because of the short duration of this stage; in one elderly man, ventilation was not measured during REM sleep due to numerous apneas. In the ANOVA, V_T ($p < 0.01$), (dot over) V_I ($p < 0.005$) and V_T/T_i ($p < 0.005$) were significantly higher in elderly men, demonstrating an age effect. V_T and f did not reveal an effect of sleep stage in ANOVA, while V_T/T_i ($p < 0.005$) and T_i/T_{tot} ($p < 0.0001$) did. Wakefulness and REM sleep represent extremes and the three stages of NREM sleep represent intermediate states, as indicated by Duncan's multiple range test in ANOVA. In wakefulness, V_T/T_i of young and elderly men combined is at its highest (0.29 L/s) and T_i/T_{tot} is at its lowest (0.39). Coefficients of variation of the 30 breaths sampled in each stage indicate that ventilation in REM, wakefulness, and S1, in that order, was more unstable in these stages and, without exception, was the most stable in SWS.

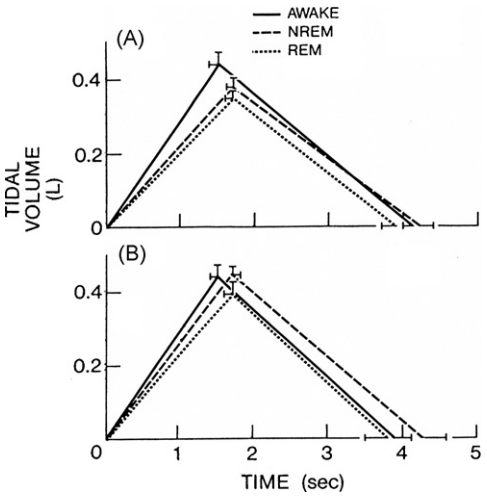

Fig. 2. Diagram representing the effect of sleep on the breathing pattern measured with RIP in young (A) and elderly men (B). In both groups, there is a significant reduction in V_T/T_i (the mean slope of the inspiratory phase) from wakefulness to NREM sleep and from NREM to REM sleep.

Table 2
Means and standard deviations of the sleep variables

	Young men ($n = 15$)	Elderly men ($n = 13$)	p
Total time in bed (min)	400 ± 34	395 ± 34	0.71
Sleep efficiency (%)	92 ± 7	85 ± 11	0.051
Sleep latency (min)	8 ± 5	9 ± 5	0.6
Stage changes (n)	63 ± 14	67 ± 15	0.48
Movement arousals (n)	68 ± 26	76 ± 37	0.52
WASO (%)	6 ± 7	12 ± 11	0.09
Stage 1 (%)	4 ± 2	5 ± 2	0.22
Stage 2 (%)	51 ± 7	51 ± 11	0.97
Slow wave sleep (%)	20 ± 7	17 ± 6	0.24
REM sleep (%)	18 ± 6	15 ± 7	0.23

WASO, wakefulness after sleep onset; REM, rapid eye movement sleep. Data obtained after one adaptation night for 11 young and 11 elderly subjects.

3.3. Sleep-disordered breathing

The AHI was higher, but non-significantly different, in elderly men (8 ± 13 AH/h) than in young men (3 ± 4 AH/h; *t*-test; $p = 0.18$). The SDBs per hour of each sleep stage were calculated (Table 3). ANOVA of SDB per stage shows significant effect of age group ($p < 0.01$) and a clear clustering during stages 1 and REM in both age groups ($p < 0.005$). Comparing the number of episodes of PB between age groups and sleep stages, the elderly men had more episodes in stage 1 ($p < 0.05$). The mean desaturations during episodes of disordered breathing did not show effect of sleep stage or age group in ANOVA.

Two young subjects (with 12 and 13 AH/h) and five elderly subjects (with 7, 12, 16, 19, and 47 AH/h) had AHI values greater than 5 AH/h. Four of these five elderly subjects had predominantly obstructive apneas (3, 7, 9, and 45% central apneas and hypopneas). Only one of these subjects (16 AH/h) had predominantly central events (76 central AH and 41 obstructive AH; 65% central).

Comparison between elderly men with AHI > 5 and the remaining elderly men in terms of age, BMI, and pulmonary function tests showed trivial differences that were not statistically or biologically significant. For instance, the five subjects with AHI > 5 were 4 years younger and 0.6 kg/m^2 heavier. Comparing the young men with and without AHI > 5, the two subjects with SDB were 1 year older

Table 3
Means and standard deviations of breathing dysrhythmias in each sleep stage and in total sleep time in young and elderly men

		Central apneas		Obstructive apneas		Hypopneas		Periodic breathing		Total breathing dysrhythmias	
		#	Index	#	Index	#	Index	#	Index	#	Index
Young	Stage 1	1 ± 1	4.8 ± 7.1	0 ± 0	0.4 ± 1.1	1 ± 1	4.0 ± 5.0	0 ± 1	1.1 ± 3.1	2 ± 1	7 ± 4.2
	Stage 2	4 ± 5	1.1 ± 1.5	1 ± 2	0.3 ± 0.7	13 ± 20	3.6 ± 5.0	1 ± 4	0.5 ± 1.2	18 ± 9	5.4 ± 2.4
	SWS	0 ± 0	0.0 ± 0.1	0	0.0	1 ± 2	1.3 ± 3.4	0 ± 0	0.1 ± 0.3	1 ± 1	0.8 ± 0.8
	REM	2 ± 3	1.8 ± 2.4	1 ± 1	0.7 ± 1.2	9 ± 12	8.1 ± 9.4	0	0.0	12 ± 5	10.3 ± 5.3
	TST	7 ± 2	1.1 ± 1.1	2 ± 1	0.3 ± 0.5	24 ± 25	4.1 ± 4.1	2 ± 4	0.3 ± 0.8	17 ± 21	3 ± 4
Elderly	Stage 1	7 ± 13	18.9 ± 31	1 ± 3	3.8 ± 7.8	8 ± 18	20 ± 41	4 ± 1	9.3 ± 18	16 ± 8	53.3 ± 33.3
	Stage 2	4 ± 7	1.1 ± 2.0	9 ± 25	2.6 ± 6.4	11 ± 13	3.2 ± 3.4	3 ± 5	1.0 ± 1.8	24 ± 16	10.8 ± 3.9
	SWS	0	0	0	0	1 ± 1	0.8 ± 1.5	0 ± 1	0.4 ± 1.6	1 ± 1	0.9 ± 0.3
	REM	2 ± 3	4.3 ± 12	7 ± 16	6.5 ± 12	10 ± 16	16 ± 27	0 ± 1	0.7 ± 2.5	19 ± 12	20 ± 15.7
	TST	13 ± 6	2.6 ± 4.4	17 ± 11	3.1 ± 6.9	29 ± 37	5.9 ± 8.1	8 ± 12	1.5 ± 2.4	46 ± 80	8.4 ± 13.2

SWS, slow wave sleep; REM, rapid eye movement sleep; TST, total sleep time.

and 1.2 kg/m^2 lighter than the others. Even combining the young and elderly groups to obtain a larger number of subjects and comparing the seven cases with SDB to the remaining 21 without SDB, no significance emerged in assessment of average BMI, which was 24.8 kg/m^2 vs. 23.9 kg/m^2 (t-test; $p = 0.5$), respectively.

3.4. Chemoreflex

On average, 30 acceptable $SBCO_2T$ per subject were obtained and the results were calculated for each subject in wakefulness, S2, SWS, and REM sleep, as the mean of at least three tests (range 3–22 tests). For one subject, in wakefulness, two tests were accepted since they produced similar values: 0.20 and 0.25 L/min/mm Hg. When less than three tests were obtained in a given sleep stage, the subjects were not included in the analysis. This happened because either they did not have enough time in a stage (usually in SWS) or breathing was too unstable to establish baseline ventilation (usually in REM sleep). For the repeated measures ANOVA, the missing values in REM and SWS were replaced with group means.

In the awake stage, the young men had a tendency to have a higher response (0.25 ± 0.08 L/min/mm Hg) than elderly men (0.19 ± 0.07 L/min/mm Hg; t-test; $p = 0.054$; Fig. 3a). When one outlier in the elderly group was removed from the analysis on the basis that he was the youngest in the group (56 years old; response = 0.36 L/min/mm Hg), then $p = 0.01$. In S1, most data were unusable due to short duration and breathing instability. In young men, the $SBCO_2T$ during wakefulness (0.25 ± 0.08 L/min/mm Hg) differed from $SBCO_2T$ measured during S2 (0.18 ± 0.06 L/min/mm Hg) and SWS (0.19 ± 0.11 L/min/mm Hg; ANOVA, $p < 0.01$ for both differences), but not from $SBCO_2T$ measured during REM sleep (0.26 ± 0.09 L/min/mm Hg). In elderly men, no significant differences were seen between the stages.

The five elderly subjects with AHI ≥ 5 had $SBCO_2T$ in wakefulness higher than the remaining older subjects with AHI < 5 (0.24 ± 0.07 L/min/mm Hg vs. 0.16 ± 0.04 L/min/mm Hg, respectively; $p = 0.024$), but showed no difference in any single sleep stage (Fig. 3b). Combining two young and five elderly subjects with AHI ≥ 5 and comparing them with the remaining 21 without SDB, no difference between the two SDB groups was found in $SBCO_2T$ during wakefulness (0.24 L/min/mm Hg vs. 0.22 L/min/mm Hg, respectively) or any sleep stage (ANOVA; $p = 0.3$).

The delay from the beginning of the CO_2 breath to the beginning of the response breath ranged from 6 to 22 s, with a mean 15 ± 3 s for all subjects and stages, attesting that the responses were mediated by the peripheral chemoreceptors. Comparison of the delay in wakefulness with the delay in all sleep stages, by paired t-test, revealed that in sleep, the delay was significantly longer (1.2 s longer for young men and 0.8 s longer for elderly men; $p < 0.05$).

The validation of the $SBCO_2T$ procedure, in six young and five elderly men, during the day in the pulmonary function laboratory, in the sitting position, obtained responses that were 0.18 ± 0.13 L/min/mm Hg higher then the ones measured at night, when recumbent. In the five subjects in whom $SBCO_2T$ was performed seated on the bed, just before bed time, the response was,

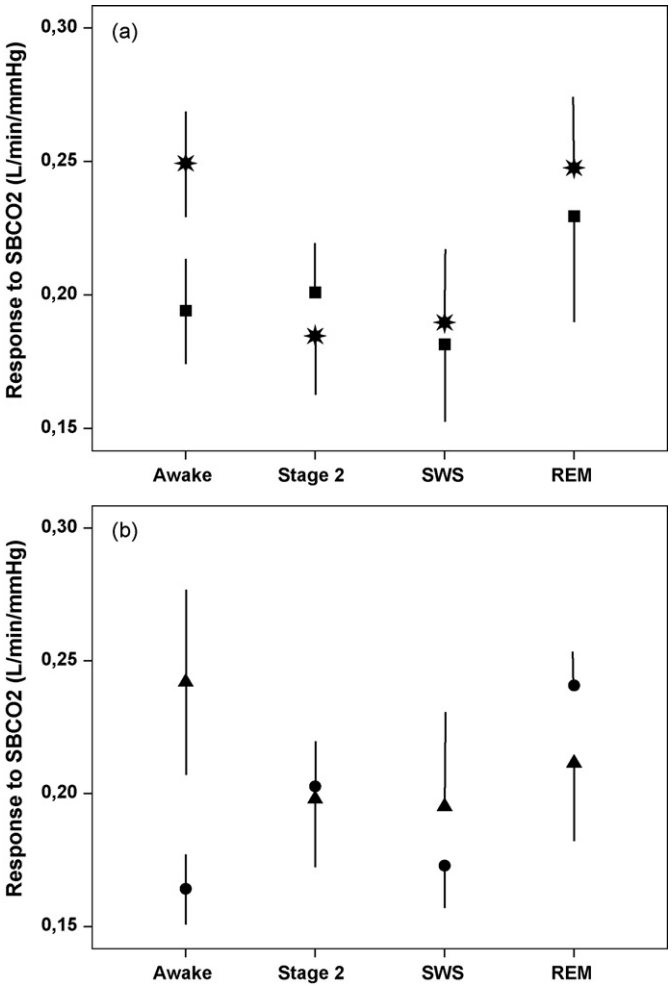

Fig. 3. Plot of the mean $SBCO_2$ responses during wakefulness, stage 2, slow wave sleep (SWS), and REM sleep. Panel a, data on young (star) and elderly men (square); the differences are non-significant. Panel b, data on elderly men with (triangle) and without (circle) apnea-hypopnea index ≥ 5; in awake stage the difference is statistically significant ($p = 0.024$). Bars represent one standard error of the mean.

on average, 0.09 L/min/mm Hg higher than in the supine position (range 0.04–0.26 L/min/mm Hg).

4. Discussion

This is the first report of data comparing peripheral chemoreceptor CO_2 response during sleep in healthy young and elderly men. During S2 and REM sleep, typically the stages in which most SDB occurs, as well as in SWS, when breathing is more stable, the $SBCO_2T$ was similar in elderly and young men as well as in individuals with or without SDB (Figs. 3a and b). The pattern of breathing is similar during wakefulness and during REM and NREM sleep when comparing the elderly and the young groups (Fig. 2). These findings point toward the integrity of the ventilatory control system during sleep in the age range studied and are in agreement with previous works that analyzed the central chemoreflex (Naifeh et al., 1989; Browne et al., 2003) and peripheral chemoreceptor response to hypoxia (Vovk et al., 2004).

During wakefulness there is a tendency for lower peripheral chemoreceptor CO_2 response in the whole elderly group. Significance, however, is reached only when one outlier is excluded. This uncertainty could be clarified with larger sample size. During wakefulness elderly men with AHI > 5 maintain elevated $SBCO_2T$ responses, at the level of young subjects. This finding may implicate the peripheral chemoreflex in the pathogenesis of SDB, since, after an arousal, increased CO_2 response may be the source of ventilatory instability in the transition from wake to sleep. Unfortunately, the above hypothesis was not addressed in the present study designed specifically to measure the $SBCO_2$ in young and elderly men and not to compare elderly men with and without SDB. Future research should assess the timing of SDB in relation to arousals to confirm whether the interval is compatible with a role of the peripheral chemoreceptors. Furthermore, before concluding on the role of the peripheral chemoreflex in aging-related SDB, it will be necessary to quantify the response to O_2 changes, both hyperoxia and hypoxia, in a larger number of cases.

Because the volunteers recruited in this study are not representative of the general population it is difficult to establish the significance of a higher proportion of cases with AHI > 5 among elderly subjects. Although this is similar to prevalence estimates reported in population studies, the nature of this sample, selected for absence of snoring and sleepiness, prohibit any comparison with the literature on SDB epidemiology. Also, Bixler et al. have reported a decrease in SDB severity with increasing age. The same phenomenon was seen within the elderly group but the same objection to comparison holds.

The difference in CO_2 response from wakefulness to sleep is one possible speculative link between SDB and aging since arousal and sleep–wake transitions have been reported as particularly prone to generate SDB (Xie et al., 1994). It must be noted that the higher $SBCO_2T$ in elderly subjects with AHI > 5 was encountered in an a posteriori analysis. The higher peripheral chemoreceptor CO_2 response shown by these five elderly individuals during wakefulness may induce more intense hyperventilation at the moment of an arousal. Greater ventilatory response to arousal from sleep, with a consequent decrease in ventilation thereafter, has been suggested as a possible explanation for why males have more SDB than females by Jordan et al. (2003). This, however, cannot be the sole explanation for the SDB in elderly men, since young men display similar levels of $SBCO_2T$ during wakefulness without obvious SDB. The fact that for the young group, higher $SBCO_2T$ during wakefulness does not cause SDB may be due to a combination of anatomical and functional aging-related factors (fat deposits around the upper airways, muscle fiber loss, longer circulation time, etc.). Two of the elderly subjects with AHI > 5 had a significant proportion of central events (45 and 65% central AH) what may indicate a different mechanism of SDB in these two cases. Exploration of these factors was beyond the scope of the present study. A study with the primary objective of comparing elderly men with and without SDB is necessary to resolve this topic.

The tendency observed in young men only to have a higher $SBCO_2T$ during wakefulness may indicate an enhanced "waking neural drive" in younger individuals (Bradley and Phillipson, 1992), rather than a sleep-related loss of ventilatory drive. Accordingly, previous studies have shown that differences in chemoreflex between young and elderly subjects may be due to exceptionally elevated results in the young groups rather than below average results in the elderly groups (Naifeh et al., 1989; Ahmed et al., 1991; AlGhanim et al., 2008).

A large number of tests during REM sleep had to be rejected due to the variability of ventilation typical of this stage. During REM sleep only a trend was seen towards an increase of the $SBCO_2$ response measured in eight young and five elderly subjects. The higher $SBCO_2$ response in REM sleep is consistent with the existence of an endogenous REM-related drive to breathe described in cats (Orem et al., 2000, 2005). Lack of statistical significance is probably a consequence of the small number of subjects with adequate tests. Future experiments which repeat the $SBCO_2$ tests more frequently may be able to obtain more numerous data during REM sleep and overcome the high rejection rate imposed by this stage.

By design, only male subjects were studied, which could limit the generalization of the present results. Although the phase of the menstrual cycle seems not to affect the peripheral chemoreflex (White et al., 1982), the main reason for not including women is, obviously, the insurmountable problem of menopause. With aging, the prevalence of obstructive sleep apnea-hypopnea syndrome becomes similar in men and women; if a higher CO_2 peripheral chemoreflex during wakefulness is involved in the pathogenesis of SDB, it is likely to be detected also in women.

Subjects who snored were excluded since the upper airway size of snorers is in between that of normal individuals and of patients with obstructive sleep apnea (Hoffstein et al., 1986). Anatomical and functional upper airway abnormalities are known causes of SDB (Svanborg, 2005) and would be unacceptable confounding factors in this study. There is, however, one important issue. Although efforts were made to keep the groups matched, the elderly male subjects were heavier. This could confound age-related changes in ventilatory control mechanisms and their relationship to SDB, since an increase in body mass could have anatomically predisposed the subjects in the elderly group to increased upper airway resistance and more SDB. Also, the increased body mass could have altered the ventilatory load in the elderly group, potentially dampening the $SBCO_2$ response. This confounder could have been a problem if SDB had shown a significant correlation with BMI ($r = 0.16$; $p = 0.6$); this is not the case, however, because in this selected population the elderly group with AHI > 5 is neither heavier than the remaining subjects (26.0 kg/m^2 vs. 25.4 kg/m^2; $p = 0.7$) nor has a different $SBCO_2T$ during sleep (S2 $p = 0.86$; SWS $p = 0.43$; REM $p = 0.39$) to indicate ventilatory load. The role of excess weight in the elderly group as a possible cause of SDB cannot be elucidated in the present study. Exclusively age-related lung function and muscle performance losses in the elderly, however, have been shown to be insufficient to explain the reduction in CO_2 response (Peterson et al., 1981).

The $SBCO_2T$ measured in the sleep laboratory, with the subjects lying down before lights out in awake state, is 42% lower (0.224 L/min/mm Hg for combined young and elderly men) than that measured in the pulmonary function laboratory during wakefulness in the sitting position in a different population

(0.385 L/min/mm Hg; McClean et al., 1988). Reasons for a lower response include: (1) possible leaks in the mask, which would not happen with a mouthpiece and nose clip; (2) the different state of arousal dependent on the environment, time of the day, etc.; and (3) an increase in the ventilatory load in recumbence (Milic-Emili and Zin, 1986). The reduction in ventilatory response observed in five subjects in this study between sitting and supine positions was 40%. Interestingly, Xie et al. (1993) have shown a 43% reduction, almost exactly the same decrease, in peripheral chemoreceptor-mediated ventilatory response to isocapnic progressive hypoxia from upright to supine positions.

The variability of the $SBCO_2T$ observed in this study during wakefulness (36%) is equal to that measured in the pulmonary function laboratory (McClean et al., 1988). Tests in which a stable ventilation plateau could not be identified were excluded. Excluding tests in which a movement arousal or a sleep stage change occurred helped to reduce variability, but these were not recurring technical problems. Ventilatory instability happened notably in REM sleep.

In summary, this is the first study of the peripheral chemoreceptor CO_2 response during nocturnal sleep in healthy elderly men aged up to 76 years, showing $SBCO_2T$ during all sleep stages similar to the young group and providing additional evidence that aging spares peripheral chemoreceptor CO_2 response during sleep. The higher CO_2 peripheral chemoreflex during wakefulness in the minority of elderly men with infraclinical SDB, as compared to elderly men without SDB, suggests a possible role for this reflex in the pathogenesis of SDB.

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