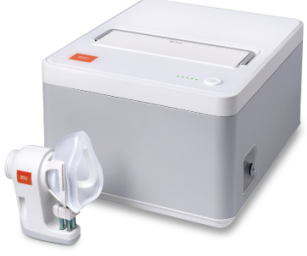
PAPER

Exhaled carbon monoxide levels in obstructive sleep apnoea



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Exhaled carbon monoxide levels in obstructive sleep apnoea

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Keywords: OSA, sleep, carbon monoxide, oxidative stress

Abstract

Background: Obstructive sleep apnoea (OSA) is characterised by chronic intermittent hypoxia, which enhances airway inflammation and oxidative stress. Exhaled carbon monoxide (eCO), a marker for oxidative stress, has been investigated in OSA. However, previous studies could be biased as they did not differentiate patients with OSA based on smoking history, a known factor influencing eCO levels. The aim of this study to investigate eCO levels in patients with OSA and non-OSA controls and compare evening to morning results. *Methods:* Exhaled carbon monoxide concentration was measured in the evening and in the morning following an in-hospital cardiorespiratory polygraphy in 60 never-smoker OSA patients, 14 ex-smoker OSA patients, 39 current-smoker OSA patients, 10 never-smoker asthmatic patients with OSA, 16 COPD patients with OSA and 20 never-smoker non-OSA controls. OSA was diagnosed based on the apnoea-hypopnoea index (AHI $>$ 5/h). *Results:* There was no difference between the never-smoker controls and never-smoker patients with OSA either in the evening (1.98 $\pm$ 1.00 ppm versus 1.95 $\pm$ 1.28 ppm, $p = 0.57$, OSA versus controls, respectively) or morning (1.95 $\pm$ 0.96 ppm versus 1.80 $\pm$ 0.95 ppm, $p = 0.42$), however there was a weak correlation between eCO and AHI in the evening ($r = 0.31$, $p = 0.01$). Accordingly, patients with severe OSA had higher eCO levels in the evening (2.43 $\pm$ 1.12 ppm) compared to mild OSA patients (1.57 $\pm$ 0.87 ppm, $p < 0.01$). Ex-smoker (3.07 $\pm$ 2.23 ppm), current-smoker (13.13 $\pm$ 11.35 ppm), asthmatic (2.70 $\pm$ 1.16 ppm) and COPD (18.25 $\pm$ 18.60 ppm) patients with OSA had higher levels of eCO compared to the non-smoker OSA group. *Conclusion:* Exhaled carbon monoxide is elevated only in severe never-smoker OSA suggesting accelerated oxidative stress. Previous smoking history is a major influencing factor which may explain differences between our findings and those of previous studies. Although our results show some impact of OSA on eCO measurements, the bias is small, and it does not significantly affect the clinical utility of eCO to monitor smoking cessation.

Background

Obstructive sleep apnoea (OSA) is a common disorder which is characterised by the repetitive episodes of complete or partial collapse of the upper airways during sleep. The resulting intermittent hypoxemic events lead to airway inflammation and oxidative stress [1] which may contribute to accelerated systemic inflammation [2] and subsequently to the development of cardiovascular and metabolic comorbidities [3]. Understanding early alterations in airway inflammation and oxidative stress may therefore facilitate the development of potential interventions to prevent end-organ dysfunction. The measurement of exhaled molecules is a non-invasive way to study inflammation and oxidative stress in the airways of patients with OSA [4–6].

The analysis of endogenous carbon monoxide could be of potential interest in OSA. Carbon monoxide is produced during heme degradation via the heme oxygenase enzyme. The inducible form of this enzyme is stimulated by oxidative and nitrosative

stress [7], which are enhanced in OSA [1]. In line with this, elevated levels of circulating CO have been reported in patients with sleep apnoea [8]. Carbon monoxide is not only a marker of cellular stress, but it also has an anti-inflammatory and anti-oxidative role [9].

Endogenous CO production can be studied in exhaled breath samples [7]. Although the laser-spectroscopic technique is more precise [10], commercial devices based on electrochemical detection are more widespread and are routinely used in clinical setting of smoking cessation [7, 11]. Exhaled carbon monoxide (eCO) is elevated in smokers [11], but can also be increased in inflammatory airway diseases, such as asthma [12], COPD [13] or cystic fibrosis [14] even in never- or ex-smokers. Interestingly, investigating nearly 15 000 volunteers in the Framingham Heart Study, higher eCO levels were associated with the development of metabolic syndrome and cardiovascular disease [15] which often associate with OSA. Only three studies have analysed eCO in OSA [16–18], and two of them reported higher levels [16, 17], but these studies did not differentiate ex-smoker and never-smoker volunteers. However, this could have been a bias, as smoking could have already led to the development of COPD [13], and the reliability of self-reported smoking abstinence in ex-smokers is low [19].

An overnight variation of exhaled biomarkers has been shown in OSA for exhaled nitric oxide [20, 21], volatile organic compounds [21, 22] and molecules in exhaled breath condensate [23]. These alterations may correspond to the acute effect of chronic intermittent hypoxia and fragmented sleep [1]. They also implicate methodological considerations to perform these measurements at the same time of day. However, no such guidance has been postulated in the latest European Respiratory Society technical standard document [24]. Overnight variations of exhaled carbon monoxide in OSA has been investigated in only one study, which reported no evening to morning difference [17]. Notably, the investigated population consisted of ex-smokers which may have confounded the results [13]. In addition, the control group included patients with mild OSA [17].

The primary aim of this study was to analyse evening and morning levels of exhaled carbon monoxide in never-smoker patients with OSA and controls. Secondly, as exploratory aims, we compared eCO levels between never-smoker patients with OSA to those with a previous smoking history, as well as to those with comorbid chronic airway disease.

Methods

Study subjects and design

In total 159 volunteers participated in this study who were referred for an overnight cardiorespiratory polygraphy at Sleep Laboratory, Department of Pulmonology, Semmelweis University. None of them had previously been diagnosed with OSA and they had not used anti-OSA treatment, including positive airway pressure therapy, mandibular advancement device or underwent upper airway surgery. Any malignancy or autoimmune disease in the past, and any infection in the 4 weeks prior to the study were exclusion criteria.

Medical and smoking history was taken, patients filled out the Epworth questionnaire and exhaled carbon monoxide was measured in the evening (between 7 and 9 pm). Patients then underwent an in-hospital polygraphy which was followed by a second exhaled carbon monoxide measurement in the morning (between 7 and 8 am). Chronic airway diseases such as asthma and COPD were diagnosed previously and treated according to guidelines. Study doctors, all specialists in respiratory medicine carefully reviewed the patients and differential diagnoses were made based on medical history and available lung function data. None of the asthmatic or COPD patients had an exacerbation within 4 weeks of the study.

The primary aim was to study differences in eCO between never-smoker OSA and control populations not suffering from any chronic airway disease at either time point. Our further, exploratory aims included investigating the effect of current and former-smoking on eCO in OSA, comparing OSA patients with and without chronic airway disease, and analysis of overnight changes in eCO.

The study was approved by the Semmelweis University Ethics Committee (TUKEB, 30/2014). Written informed consent was obtained from each patient included in the study.

Cardiorespiratory polygraphy

Patients attended an overnight cardiorespiratory polygraphy performed with Somnoscreen Plus Tele PSG (Somnomedics GmbH Germany). Thoracic and abdominal respiratory excursions, breath sounds, nasal pressure, electrocardiogram, and oxygen saturation were registered [25]. Cardiopulmonary events were scored manually according to the American Academy of Sleep Medicine (AASM) guidelines [26]. Apnoea-hypopnoea index (AHI) and oxygen desaturation index (ODI) were calculated to estimate OSA severity. OSA was defined as having an AHI $\geqslant$ 5/h.

Exhaled carbon monoxide measurement

Exhaled carbon monoxide was measured with a commercially available device (Pico Smokerlyzer, Bedfont Scientific Ltd, United Kingdom) according to the manufacturer's instructions. Briefly, after taking a deep breath in, subjects held their breath for 15 s and exhaled gently into the device. The instrument provided exhaled CO levels within seconds together with estimates of blood carboxyhaemoglobin. For our analysis, we used only the exhaled CO data. In the

current-smoker groups eCO levels were measured in the morning before smoking the cigarette; however, we did not control for the evening cigarette consumption. We did not ask to participants to fast prior to the evening samples, the morning breath measurements were performed before taking any food or medications. The coefficient variation of the test determined in triplicate measurements of 11 healthy smokers (own data) was 5%± 5% suggesting good accuracy.

Statistical analyses

Statistical analyses were carried out with GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). The normality of the data was assessed using the Kolmogorov–Smirnov test. Clinical characteristics were compared using the ANOVA and post-hoc Bonferroni tests, chi-square and Fisher tests among groups. Exhaled carbon monoxide levels were compared with Mann–Whitney and Wilcoxon (paired non-parametric test) as well as with Kruskal–Wallis and Dunn's post hoc tests among the groups. Spearmen's test was used to assess the relationship between exhaled carbon monoxide and clinical variables. The data are presented as mean ± standard deviation, a p value < 0.05 was considered significant.

The study was powered to investigate the primary outcome, namely, to find difference in eCO levels between never-smoker patients with OSA and controls at either time point. We hypothesised that this difference would be at least 1 ppm (the resolution of eCO levels measured by the Pico Smokerlyzer device). When computing the effect size, we used the distribution of eCO levels in OSA published before [17]. The sample size was calculated to reach a statistical power (1-β) of 0.80, an α error of 0.05 and effect size of 0.70 with respect to the asymptotic relative efficiency of non-parametric tests [27].

Results

Characteristics of the study groups

There were significant differences in age, gender distribution, BMI, the prevalence of comorbidities, AHI and ODI among the study groups. In particular, patients with OSA tended to be older, had a higher prevalence of comorbidities and had increased AHI and ODI compared to controls. Although the never-smoker OSA group had lower AHI and ODI than the other groups, this difference was not significant (as assessed with Kruskal–Wallis test and Dunn's test, $p > 0.05$). The smoker groups comprised of more males. Interestingly, there was no difference in ESS among the study groups. Clinical characteristics are summarised in table 1.

Comparison of eCO levels in never-smoker OSA and control participants

There was no difference in eCO levels between never-smoker OSA and control participants either in the evening (1.98 ± 1.00 ppm versus 1.95 ± 1.28 ppm, $p = 0.57$, OSA versus controls, respectively) or in the morning (1.95 ± 0.96 ppm versus 1.80 ± 0.95 ppm, $p = 0.42$, figure 1). In line with this, evening to morning changes were not significant in either group ($p = 0.85$ and $p = 0.41$ in OSA and controls, respectively). However, when participants were divided into AHI <15/h and $\geqslant 15$/h groups, eCO levels were significantly higher in the AHI $\geqslant 15$/h group both in the evening (2.21 ± 1.00 versus 1.76 ± 1.09 ppm, $p = 0.02$) and in the morning (2.13 ± 0.92 versus 1.71 ± 0.96 ppm, $p = 0.03$). When the OSA patients were divided into mild (AHI 5-14.9/h, $n = 21$), moderate (AHI 15-29.9/h, $n = 18$) and severe (AHI $\geqslant 30$/h, $n = 21$), there was a tendency for differences in the evening ($p = 0.05$) with a significant difference between the mild and severe groups ($p < 0.01$, figure 2).

Comparison of eCO levels in never-smoker, former-smoker and current-smoker OSA subjects

In the evening, eCO levels were significantly higher in former-smoker (3.07 ± 2.23 ppm, $p = 0.03$) and current-smoker (13.13 ± 11.35 ppm, $p < 0.01$) compared to never-smoker patients with OSA (1.98 ± 1.00 ppm). In contrast, there was no difference between former-smoker and never-smoker patients in the morning (2.29 ± 1.68 ppm versus 1.95 ± 0.96 ppm, $p = 0.81$), while the eCO levels of current-smokers were still significantly increased (7.33 ± 6.21 ppm, $p < 0.01$, figure 3).

There was a significant evening to morning drop in eCO levels in both groups ($p = 0.04$ and $p < 0.01$, in former- and current-smokers, respectively).

Comparison of OSA subjects with and without chronic airway disease

Patients with asthma and OSA tended to have elevated eCO levels compared to never-smoker OSA patients in the evening (2.70 ± 1.16 ppm versus 1.98 ± 1.00 ppm, $p = 0.05$); however, there was no significant difference in the morning (2.50 ± 1.27 ppm versus 1.95 ± 0.96 ppm, $p = 0.22$). Patients with COPD and OSA had higher eCO levels both in the evening (18.25 ± 18.60 ppm, $p < 0.01$) and in the morning (10.81 ± 10.48 ppm, $p < 0.01$, figure 4).

There was a significant evening to morning drop in the COPD group ($p < 0.01$), without a change in the asthmatic group ($p = 0.59$).

The relationship between eCO levels and clinical parameters in never-smoker OSA patients

There was a significant relationship between evening eCO levels and AHI ($r = 0.31$, $p = 0.01$) as well as

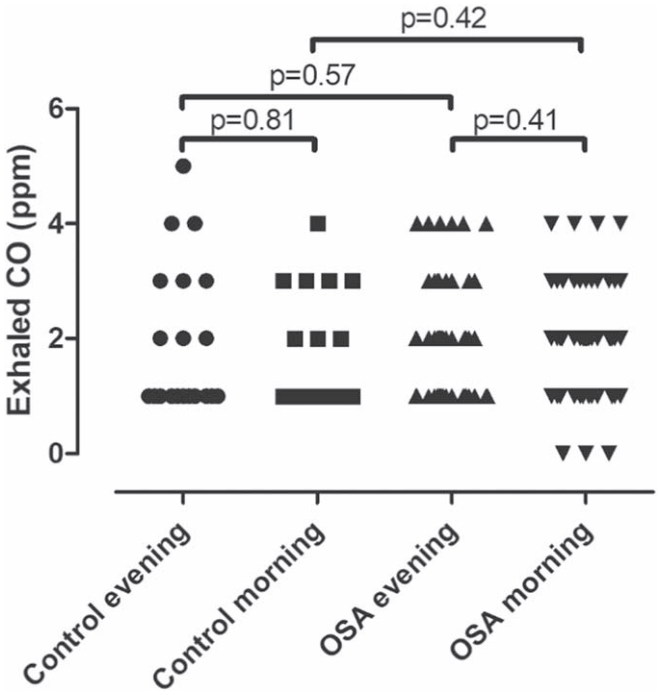

Figure 1. Exhaled carbon monoxide levels in never smoking patients with OSA and controls. There was no difference in exhaled carbon monoxide levels between groups, neither did it change from evening to morning in either group.

Table 1. Clinical characteristics of study groups.

	Never-smoker control ($n = 20$)	Never-smoker OSA ($n = 60$)	Former-smoker OSA ($n = 14$)	Current-smoker OSA ($n = 39$)	OSA with asthma ($n = 10$)	OSA with COPD ($n = 16$)	p
Age (years)	42 ± 16	57 ± 14	55 ± 15	53 ± 15	59 ± 9	58 ± 11	0.02
Gender (%males)	60	58	92	82	30	75	<0.01
	12/8	35/25	13/1	32/7	3/7	12/4	
BMI (kg m^{-2})	26.3 ± 4.7	36.0 ± 17.3	35.2 ± 10.9	33.1 ± 8.8	31.9 ± 10.7	38.1 ± 8.4	0.05
Cigarette pack years	NA	NA	14.9 ± 11.2	21.2 ± 17.6	NA	40.7 ± 28.2	<0.01
Hypertension (%)	20	82	71	67	70	94	<0.01
	4/16	49/11	10/4	26/13	7/3	15/1	
Diabetes (%)	0	17	21	23	20	38	0.06
	0/20	10/50	3/11	9/30	2/8	6/10	
Cardio/ cerebrovascular disease (%)	0	23	29	26	20	31	0.09
	0/20	14/46	4/10	10/29	2/8	5/11	
Arrhythmia (%)	10	33	7	26	50	37	0.06
	2/18	20/40	1/13	10/29	5/5	6/10	
ESS	6.0 ± 2.7	5.7 ± 4.2	8.0 ± 5.1	6.1 ± 3.8	7.9 ± 5.6	7.4 ± 3.9	0.31
AHI (1/h)	2.2 ± 1.5	28.4 ± 21.2	36.9 ± 23.7	33.2 ± 20.4	38.6 ± 23.8	38.3 ± 32.9	<0.01
ODI (1/h)	1.8 ± 1.6	25.7 ± 21.3	32.1 ± 24.6	27.7 ± 20.5	29.1 ± 23.6	35.9 ± 33.4	<0.01

AHI—apnoea-hypopnoea index, BMI—body mass index, COPD—chronic obstructive pulmonary disease, ESS—Epworth Sleepiness Scale, ODI—oxygen desaturation index, OSA—obstructive sleep apnoea

ODI ($r = 0.30$, $p = 0.02$), while these parameters did not correlate with the morning eCO levels (both $p > 0.05$). Interestingly, both evening ($p = 0.06$) and morning ($p = 0.07$) eCO levels tended to be higher in men. Morning eCO levels were lower in patients with cardiac arrhythmia ($p = 0.02$). There was no other correlation between demographics, comorbidities or ESS values and exhaled carbon monoxide levels (all $p > 0.10$). As univariate analyses showed negative results, no further multivariate analyses were performed to explore the relationship between eCO levels and clinical variables.

Discussion

This study investigated exhaled carbon monoxide levels in OSA. We found significant, but very small

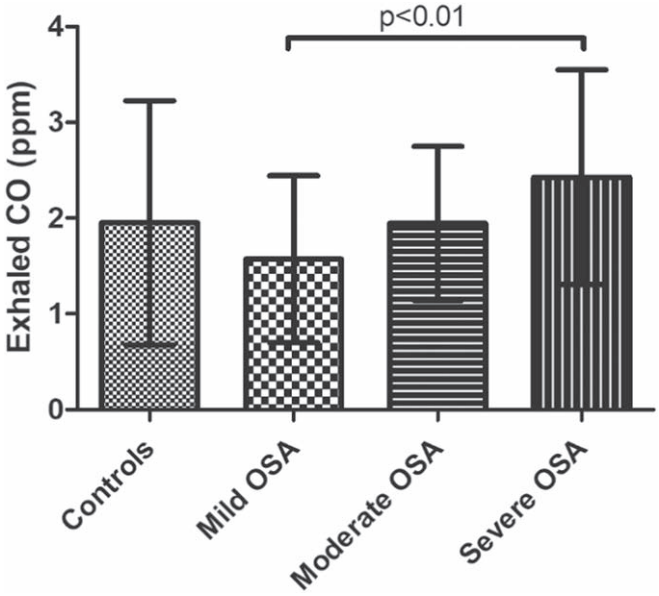

Figure 2. Exhaled carbon monoxide levels among different severities of OSA. There was a significant difference between the mild and severe OSA groups. Mean±SD of exhaled CO values in the evening are presented.

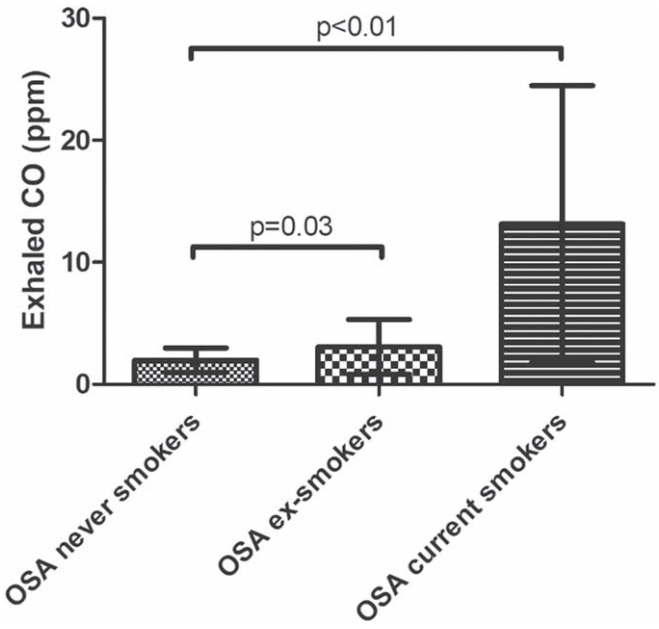

Figure 3. Exhaled carbon monoxide levels in never, ex- and current smokers with OSA. Exhaled CO levels were significantly higher in ex-smokers and current-smokers compared to the never-smoker participants with OSA. Mean±SD of exhaled CO values in the evening are presented.

increase in eCO only in severe disease. Evening to morning changes were relevant only in former and current smoking patients. Exhaled CO levels in OSA were increased in former smokers, therefore studies should avoid mixing never smokers and ex-smokers when evaluating exhaled carbon monoxide levels.

Chronic intermittent hypoxia is a hallmark of OSA which can induce airway inflammation in animal models and *in vitro* [28, 29]. In line with this, a significant association has been shown between various markers of airway inflammation and obstructive apnoeic events in patients with OSA [16, 30–34]. Only three studies have investigated exhaled carbon monoxide in OSA. Jafari and Mohsenin did not find any difference in OSA compared to controls [18]; however, that study focused on hypertension and not OSA. Furthermore, current smoker subjects were included and no subgroup analyses were performed in non-smokers [18]. On the contrary, exhaled CO levels were elevated in the two other studies which both compared OSA to controls [16, 17], however the latter study defined the control group by an AHI $<$ 15/h resulting

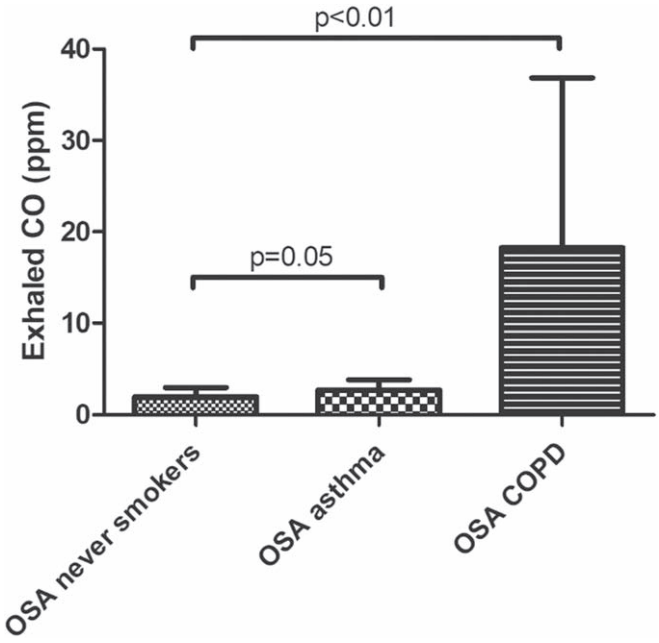

Figure 4. Exhaled carbon monoxide levels in OSA with and without chronic airway disease. Exhaled CO levels tended to be higher in asthma and were significantly higher in COPD. Mean±SD of exhaled CO values in the evening are presented.

in the inclusion of mild OSA patients [17]. Although both studies included patients who were abstinent from smoking over 12 months, the exhaled CO results in ex-smokers should be interpreted carefully, especially if COPD has not been excluded [13]. In addition, former smokers can inaccurately report on their smoking abstinence [19], which can reliably be detected only by the measurement of urine cotinine.

We did not find a significant difference between OSA and controls when the diagnostic criteria were set for AHI $\geqslant$ 5. We used this cut off as we hypothesised that chronic intermittent hypoxia even in mild disease can cause a significant increase in airway oxidative stress [22]. However, a significant elevation in eCO levels were present in more severe OSA participants which confirms the results of Azuma *et al* [17], but in never-smokers. In line with this, a significant, but weak direct relationship was observed between eCO levels and indices of OSA severity, however only in the evening. The exact reason for the lack of association in the morning levels is not known. The elevated eCO levels in severe OSA may suggest that eCO may have a potential to monitor oxidative stress in OSA following positive airway pressure therapy. Our study may serve a guide to design future interventional studies.

As obstructive hypoxemic events occur exclusively during sleep, one should hypothesise an overnight increase in the magnitude of airway inflammation in OSA. An overnight increase in exhaled nitric oxide [20, 21], pentane [21] in addition to alterations of the exhaled profile of VOCs [22] have been reported. Only one study has examined evening to morning variations in exhaled CO and reported no difference [17] which has been confirmed by the current study. As discussed above, we believe that the results of our study on never-smokers strengthen previous findings.

Cardiovascular and metabolic comorbidities are commonly associated with OSA [35]. Exhaled CO has previously been shown to be elevated in hypertensive patients with OSA [18]. Our study did not support these findings; however, the former study did not analyse smoking patients separately, which may have confounded the results [18]. We did not find any correlation with other comorbidities or obesity, either. Higher eCO levels have been reported in diabetes [36] and metabolic syndrome [37] before, but OSA has not been excluded in these patients. Our results suggest that the presence of severe OSA, which is highly prevalent in metabolic syndrome [35], might have at least partly biased the previous findings. Confirming the previous results [37], eCO was not related to body mass index. Common cardio-metabolic comorbidities and obesity may themselves induce systemic inflammation and oxidative stress which may have contributed to exhaled CO levels [38]. Unfortunately, we did not perform blood biomarker measurement in this study. These comorbidities may alter the composition of exhaled breath as well [39, 40]. The current study design does not allow further investigation on the influence of comorbidities on eCO levels in OSA.

Exhaled carbon monoxide was found to be elevated in bronchial asthma [12] and COPD [13]. Our study enabled us to investigate these differences within the OSA population. We found a tendency for increased eCO levels in asthma, interestingly only in the evening. The lack of difference in the morning may have resulted from taking inhaled medications which we did not control in the study. Exhaled CO levels

were increased in COPD patients with OSA as well. Apart from the effect of inflammation, all the patients with COPD had a significant smoking history which may have contributed to high eCO results. Of note, analysis of eCO levels in comorbid asthma and COPD in OSA was only exploratory and this study has not been powered to analyse the effect of airway diseases on eCO in OSA. Therefore, our results should be interpreted with caution.

Exhaled carbon monoxide is used to assess smoking habits and monitor smoking cessation in clinical practice [11]. Although overnight variations have not been investigated in smoker OSA patients before, the significant drop is most likely resulted from prolonged (>8 h) smoking cessation during sleep [11]. Of note, our study highlights the need for standardising the timing eCO measurements in clinical practice.

We used an electrochemical method to determine eCO levels. This is known to be less accurate than the laser-spectroscopic technique [10]. In addition, the commercially available device used in the current study has a lower limit of detection of 1 ppm. Therefore, the lack of differences observed in the current study may have been due methodological factors. Of note, the electrochemical method is much more widely used than the laser-spectroscopic method, therefore our data are more comparable to the previous studies.

In summary, OSA is not a major bias to interpret exhaled carbon monoxide levels in clinical practice. The mild, but significant rise in severe OSA possibly reflects airway oxidative stress, however more sensitive tests, such as exhaled volatile organic compound analysis should be carried out if someone aims to monitor this in OSA. Nevertheless, the results in smoker populations point out the need for standardising the timing of measurements when evaluating eCO levels in smokers.

Acknowledgments

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