Increased alveolar nitric oxide concentration is related to nocturnal oxygen desaturation in obstructive sleep apnoea

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ARTICLE INFO

Article history:
Received 25 July 2014
Revised 1 January 2015
Accepted 27 January 2015
Available online 30 January 2015

Keywords:
Exhaled nitric oxide
Alveolar inflammation
Obstructive sleep apnoea
Nocturnal oxygen desaturation

ABSTRACT

Purpose: To assess distal/alveolar inflammation in patients with suggestive symptoms of obstructive sleep apnoea (OSA) using exhaled nitric oxide (NO) measured by two-compartment model (2-CM) after correction for axial NO back-diffusion (trumpet model).

Methods: Ninety five patients suspected for OSA prospectively underwent pulmonary function test, over-night polysomnography (PSG), and exhaled NO measurement. Patients with apnoea–hypopnoea index (AHI) < 5/hour were included in non-OSA group. Exhaled NO was repeatedly measured after PSG in 21 OSA patients and 8 non-OSA subjects.

Results: Alveolar NO concentration (CANO) was significantly higher in OSA patients (n = 71; 4.07 ± 1.7 ppb) as compared with non-OSA subjects (n = 24; 2.24 ± 1.06 ppb; p < 0.0001) whilst maximal bronchial NO flux (J′awNO) and fractional exhaled NO (FENO) did not differ between the two groups. CANO was strongly associated to AHI (r = 0.701; p < 0.0001) and to recording time with SaO2 < 90% (ST-90%; r = 0.659; p < 0.0001) in OSA patients but not in non-OSA persons. The area under ROC curve for screening patients with OSA and significant nocturnal oxygen desaturation (ST-90% > 1%) was 0.865 ± 0.036 (95% IC, 0.793–0.937; p < 0.0001). CANO at 4.5 ppb could detect these patients with specificity of 94% and sensitivity of 46%. Increase of CANO measured after PSG was significantly related to oxygen desaturation index (ST-90%) in OSA patients.

Conclusions: Increased alveolar NO concentration was related to the severity of nocturnal oxygen desaturation in patients with OSA, linking the distal airway inflammation to intermittent hypoxia. (250 words)

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1. Introduction

Obstructive sleep apnoea (OSA) is an independent risk factor for cardiovascular morbidity and mortality [1], due to increased production of reactive oxygen species and pro-inflammatory cytokines, resulting from chronic intermittent hypoxia–reoxygenation [2]. Oxidative stress and inflammation cause endothelial dysfunction leading to cardiovascular diseases [3]. Thus, evaluation of pulmonary inflammation might be useful to screen for OSA and to predict its severity.

Nitric oxide (NO) plays an important role both as a physiological modulator of vascular tone and as a pathological pro-inflammatory biomarker implicated in many lung disorders [4]. NO can be easily measured in the exhaled air, and there are theoretical grounds to hypothesise that concentration of exhaled NO (FENO) may change in the two principal pathological processes observed in OSA: pulmonary inflammation and endothelial dysfunction. Increased exhaled NO reflects lung inflammation by over-expression of the inducible NO synthase (NOS) as observed in asthma and systemic sclerosis (SSc) [5,6], whilst reduced exhaled NO levels can be found in cardiovascular disorders associated with endothelial dysfunction such as pulmonary hypertension [7] and chronic heart failure [8], due to decreased endothelial NOS expression and activity.

In patients with OSA, lung inflammation and vascular injuries usually co-exist with, however, different degrees of severity. FENO, which reflects NO production from the large airways, has been found either
unchanged [9] or increased [10–17] in patients with OSA. $F_{ENO}$ is however a poor marker of NO production in the distal parts of the lungs, i.e. small airways and alveolar spaces. The two-compartment model (2-CM) allows quantification of maximal bronchial NO flux ($J_{awNO}$) and steady-state alveolar NO concentration ($C_{ANO}$) [18]. Using this simplified model, two studies reported a decrease of $C_{ANO}$ in patients with OSA [10,15] suggesting endothelial dysfunction that might be linked to systemic hypertension [10]. We hypothesised that in patients with advanced OSA and vascular diseases, distal/alveolar NO production might decrease [10,15] but in patients with moderate OSA and associated lung inflammation, $C_{ANO}$ might increase as observed in patients with systemic sclerosis [6].

It is recently suggested that taking into account NO axial back-diffusion, related to the trumpet shape of the cross-sectional area of the tracheal tree [18], can better characterise the proximal and distal exhaled NO origins in healthy subjects [19] and SSc patients [20].

In this prospective study, we aimed to assess the distal/alveolar inflammation in patients with suggestive symptoms of OSA, using this novel approach. We also studied the variation of exhaled NO after overnight PSG recording in OSA and non-OSA patients to see whether this variation was associated with sleep apnoea parameters.

2. Methods

2.1. Study population

All subjects (≥18 years-old) were recruited from our Sleep Research Unit, Department of Physiology, Cochin Hospital, Paris, France. They were consecutively referred for OSA diagnosis with suggestive symptoms (American Association of Sleep Medicine, AASM criteria) during a period of 2 years, from January 1, 2009 to December 31, 2010 [21]. The study was approved by the Ethics Committee of our institution and informed consents were obtained from all participants.

We excluded patients with advanced cardiovascular diseases or respiratory disorders, current smokers, patients with upper or lower respiratory infections, and those receiving oral corticosteroids within the last 4 weeks since these factors could modify exhaled NO.

All patients underwent thorough physical examination with medical history and pulmonary function tests before inclusion. On the examination day, exhaled NO was measured in the afternoon, then patients were submitted to overnight in-laboratory polysomnography (PSG). On the next morning, exhaled NO was re-evaluated in 31 patients with OSA (defined as $AHI \geq 5$/hour) and 8 patients without OSA. We included 30 healthy non-smokers (mainly from our medical staff and students) having exhaled NO measurement as controls.

2.2. Lung function measurement

Pulmonary function tests (PFT), assessing forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and total lung capacity (TLC) (MasterScreen® Body; VIASYS Healthcare GmbH, Hoechberg, Germany), were performed according to the international recommendations [22]. Blood gas analysis was done on the same day.

2.3. Exhaled NO measurement

Exhaled NO was measured using a chemiluminescent NO analyser (EndoNO 8000®; SERES, Aix-en-Provence, France), according to ATS/ERS recommendations [23]. NO analyser was calibrated daily with a standard NO source (100 ppb, Air Liquid, Paris, France). Exhaled NO measurement was strictly performed in all subjects during the medical visit preceding the PSG recordings, as previously described [20,24]. Patients who cannot perform a regular exhalation of more than 8 seconds to obtain a steady-state alveolar NO concentration during 3 seconds were excluded from the study [24].

Correction of $J_{awNO}$ and $C_{ANO}$ using the trumpet (TP) model to take into account axial NO back-diffusion was then applied for all measurements [19]. We used the linear relationship to express the variation of $V_{NO}$ (pl/s: picolitre/second) as a function of $V'$ ranging from 100 to 250 ml/s:

\[
V'_{NO} = (C_{ANO(TP)} + J_{awNO(TP)} \cdot 0.00078) \cdot V' + J_{awNO(TP)} / 1.7
\]

The slope of this linear relationship S and the intercept I was obtained by plotting the $V'_{NO}$ against $V'$, allowing the calculation of $C_{ANO(TP)}$ and $J_{awNO(TP)}$ from the $C_{ANO(2CM)}$ and $J_{awNO(2CM)}$ as previously described [18,20].

2.4. Polysomnography

All patients underwent overnight polysomnography (PSG) using a Medcare data-acquisition system (Rembrandt Analysis Manager, Buffalo, NY, USA) with standard electrodes and sensors according to AASM recommendations [25]. Briefly, electroencephalography electrodes applied at A2-C4, C4-C3, C3-A1, and C3-O1, two electrooculography, submental and anterior tibial electromyography channels were recorded. For respiratory parameters, thoracic and abdominal movements by inductance plethysmography, thermistors and nasal pressure cannulas were used simultaneously. Arterial oxygen saturation ($SaO_2$) was measured using pulse oximeter. To establish sleep stages, recorded nocturnal PSG were visually scored on the basis of 30-second epochs, using Rechtschaffen and Kales criteria, by two independent medical doctors with more than 10-year experience in sleep medicine [26]. Apnoea was defined as a complete cessation of oro-nasal airflow ≥10 seconds. Hypopnoea was defined as an important reduction in airflow (≥50%) for ≥10 seconds or moderate reduction (<50%) associated with EEG arousals and/or oxygen desaturation ($SaO_2$ decrease ≥3%). The apnoea–hypopnoea index (AHI) was defined as the addition of apnoea episodes and hypopnoea episodes per hour during sleep. Patients with OSA (AHI ≥5) were divided into mild-to-moderate group (5 ≤ AHI <30) and severe one (AHI ≥30) [26]. Subjects with AHI <5 constituted the non-OSA group. For the nocturnal oxygen desaturation (NOD), we recorded the mean $SaO_2$, nadir $SaO_2$ and the percentage of recording time with $SaO_2$ <90% on total sleep time (ST-90%). Patients with significant NOD were defined as ST-90% of more than 1% [27].

2.5. Statistical analysis

Data were analysed using SPSS 16.0 (Chicago, IL, USA). Values were expressed as mean ± standard derivation (SD) for continuous parameters, number and percentage for categorical variables. Normal distribution was determined using Kolmogorov–Smirnov test. Comparisons were made by Student’s t-test or Mann–Whitney test for quantitative variables and Chi-square test or Fisher’s exact test for qualitative variables, as appropriate. Exhaled NO levels before and after PSG were compared using Wilcoxon matched-pairs rank test. Linear correlations between $C_{ANO}$ with AHI and BMI were determined using Spearman’s correlation test. Multivariate linear regression was used to examine the association of AHI with $C_{ANO}$ and BMI. Statistical significance was two-sided at $p < 0.05$.

Diagnostic performance of $C_{ANO}$ for detecting patients with OSA (AHI ≥5/hour) and significant NOD (ST-90% > 1%) was done by the ROC curve. Overall discriminatory ability of ROC curve was determined by the area under ROC curve (mean ± SD; [95% CI]).
Exhaled nitric oxide (NO) was measured before polysomnography (PSG) by using the two-compartment model with correction for axial NO diffusion and PSG was recorded as described in Methods. CANO was significantly associated to AHI in OSA patients (p < 0.0001) but not in non-OSA persons (p = 0.18).

Table 1
Anthropometric, respiratory characteristics and exhaled nitric oxide parameters.

<table>
<thead>
<tr>
<th>OSA patients (n = 71)</th>
<th>Non-OSA patients (n = 24)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.2 ± 10.3</td>
<td>52.6 ± 15.3</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>46/25</td>
<td>11/13</td>
</tr>
<tr>
<td>Ex-smoking (n, %)</td>
<td>10; 14.1</td>
<td>2; 8.3</td>
</tr>
<tr>
<td>Alcohol user (n, %)</td>
<td>12; 16.9</td>
<td>3; 16.7</td>
</tr>
<tr>
<td>Epworth score</td>
<td>7.5 ± 5.2</td>
<td>7.3 ± 4.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.3 ± 6.5</td>
<td>27.4 ± 5.7</td>
</tr>
<tr>
<td>Obesity (n, %)</td>
<td>30; 42.3</td>
<td>9; 37.5</td>
</tr>
<tr>
<td>Systemic hypertension (n, %)</td>
<td>31; 43.7</td>
<td>6; 25</td>
</tr>
<tr>
<td>Arterial hypertension (n, %)</td>
<td>6; 8.4</td>
<td>0; 0</td>
</tr>
<tr>
<td>Coronary arterial disease (n, %)</td>
<td>1; 1.4</td>
<td>0; 0</td>
</tr>
<tr>
<td>Type 2 diabetes (n, %)</td>
<td>16; 22.5</td>
<td>2; 8.3</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>101.9 ± 14.2</td>
<td>101.1 ± 15.2</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>102.4 ± 15.4</td>
<td>106.9 ± 13.5</td>
</tr>
<tr>
<td>TLC (L)</td>
<td>106.9 ± 15.9</td>
<td>106.9 ± 11.8</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>88.6 ± 7.1</td>
<td>84.8 ± 6.5</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>37.8 ± 3.5</td>
<td>41 ± 4.1</td>
</tr>
<tr>
<td>AHI events/hour of sleep</td>
<td>26.4 ± 17.4</td>
<td>2.5 ± 1.5</td>
</tr>
<tr>
<td>Diurnal SaO₂ (%)</td>
<td>95.8 ± 1.6</td>
<td>96.5 ± 1.5</td>
</tr>
<tr>
<td>Nocturnal SaO₂ (%)</td>
<td>93.8 ± 1.5</td>
<td>94.8 ± 1</td>
</tr>
<tr>
<td>Nadir SaO₂ (%)</td>
<td>84.1 ± 8.2</td>
<td>92.3 ± 3.8</td>
</tr>
<tr>
<td>Recording time SaO₂ &lt; 90% (%)</td>
<td>7.3 ± 10.3</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>CANO (ppb)</td>
<td>4.07 ± 1.70</td>
<td>2.24 ± 1.06</td>
</tr>
<tr>
<td>FENO.50 (ppb)</td>
<td>1042 ± 948</td>
<td>1044 ± 563</td>
</tr>
<tr>
<td>R²</td>
<td>0.981 ± 0.021</td>
<td>0.975 ± 0.022</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD. OSA: obstructive sleep apnea; BMI: body mass index; FEV₁: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity; AHI: apnea–hypopnea index; CANO: alveolar nitric oxide concentration; J’awNO: maximal bronchial flux of nitric oxide; FENO.50: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values.

3. Results

We prospectively included 95 subjects (57 men) with mean age (56.8 ± 11.9 years) from January 2009 to December 2010. Characteristics of patients with and without OSA were presented in Table 1. There was no significant difference in term of age, sex, ex-smoking and alcohol status, percentage of obesity, and daytime sleepiness between the two groups. There was a tendency of increased overweight severity in the OSA group (p = 0.09). Percentages of type 2 diabetes and cardiac comorbidities including arterial hypertension, arrhythmias, and coronary arterial disease were not significantly different between OSA patients and non-OSA subjects (p > 0.05). All patients had normal results in pulmonary function test and arterial blood gas.

Regarding respiratory parameters, OSA patients had significantly higher AHI (26.4 ± 17.4 events/hour versus 2.5 ± 1.5 events/hour, p < 0.0001), lower mean nocturnal SaO₂ (93.1 ± 1.5% versus 94.6 ± 1.9%, p = 0.001), lower nadir SaO₂ (84.1 ± 8.2% versus 92.3 ± 3.8%, p < 0.0001), and longer recording time with SaO₂ < 90% (7.3 ± 10.3% versus 0.5 ± 0.8%, p < 0.0001) as compared with non-OSA subjects.

3.1. Exhaled nitric oxide parameters and OSA severity

Mean level of CANO from patients with OSA (n = 71; 4.07 ± 1.77 ppb) was significantly higher than that from non-OSA subjects (n = 24; 2.24 ± 1.06 ppb; p = 0.0001). However, there was no significant difference in J’awNO and FENO.50 between these two groups (p > 0.05) (Table 1). We also measured exhaled nitric oxide in 30 healthy, non-smoking, and non-obese subjects and found no significant difference in CANO (1.9 ± 1.16 ppb; p = 0.37), J’awNO (1149 ± 539 pl/s; p = 0.48), and FENO.50 (16.3 ± 6.6 ppb; p = 0.86) as compared with non-OSA subjects.

Regarding the severity of OSA, patients with severe OSA (AHI ≥ 30) had a significantly higher level of CANO (n = 21; 5.5 ± 1.56 ppb) as compared with those with mild-to-moderate OSA (n = 50; 3.46 ± 1.36 ppb; p = 0.0001). There was no difference in J’awNO and FENO.50 between these two groups (p > 0.05) (Table 2). Interestingly, we found a positive linear correlation between CANO and AHI (n = 71; r = 0.701; p < 0.0001) in patients with OSA while no correlation was found in the non-OSA group (p = 0.18) (Fig. 1).
3.2. Exhaled nitric oxide parameters and nocturnal oxygen desaturation (NOD)

Patients with OSA were then divided into a group with NOD (ST-90% > 1% TST), and the other without NOD. Mean level of CANO from patients with NOD (n = 41; 4.81 ± 1.6 ppb) was significantly higher than that from patients without NOD (n = 30; 3.05 ± 1.25 ppb; p < 0.0001). There was no difference in J′awNO and FENO.50 between these two groups (p > 0.05) (Table 3). We also showed a significant linear correlation between CANO and ST-90% in OSA patients (n = 71; r = 0.659; p < 0.0001) (Fig. 2).

### Table 3

<table>
<thead>
<tr>
<th>Patient group</th>
<th>CANO (ppb)</th>
<th>J′awNO (pl/s)</th>
<th>FENO.50 (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without NOD</td>
<td>3.05 ± 1.25</td>
<td>1077 ± 1064</td>
<td>16.6 ± 13.0</td>
</tr>
<tr>
<td>With NOD</td>
<td>4.81 ± 1.6</td>
<td>1017 ± 866</td>
<td>17.6 ± 10.4</td>
</tr>
</tbody>
</table>

N: nocturnal oxygen desaturation was defined as sleeping time with SaO2 < 90% more than 1% of total sleep time (# 5 minutes). Data were expressed as mean ± SD. CANO: alveolar nitric oxide concentration; J′awNO: maximal bronchial flux of nitric oxide; FENO.50: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values.

3.3. Alveolar exhaled nitric oxide for detecting OSA patients with NOD

Because the levels of CANO were highly correlated to the severity of OSA as measured by AHI and to the NOD degree as quantified by ST-90%, we used it for screening this group of patients at highest cardiovascular risk. The whole population was then divided into 2 groups: 41 patients with OSA (AHI ≥ 5) and NOD (ST-90% > 1%), and 54 patients without OSA (AHI < 5) and/or without NOD (ST-90% ≤ 1%). The area under ROC curve was statistically significant (p < 0.0001; AUROC: 0.865; [0.793–0.937]) (Fig. 3). The highest Youden index was at CANO level of 3.63 ppb with sensitivity of 78% and specificity of 77.8%. Another threshold of CANO at 4.5 ppb yielded higher specificity of 94.4% but lower sensitivity of 46.3%.

### Table 4

<table>
<thead>
<tr>
<th>Patient group</th>
<th>CANO (ppb)</th>
<th>J′awNO (pl/s)</th>
<th>FENO.50 (ppb)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PSG</td>
<td>4.06 (3.42–5.15)</td>
<td>885 (573–1356)</td>
<td>14.7 (10.6–22.4)</td>
<td>0.0001</td>
</tr>
<tr>
<td>After PSG</td>
<td>6.27 (4.11–8.3)</td>
<td>878 (577–1140)</td>
<td>16.8 (11.5–21.9)</td>
<td>0.15</td>
</tr>
<tr>
<td>Difference (p)</td>
<td>2.02 (1.43–2.86)</td>
<td>2.63 (2.06–3.73)</td>
<td>12.5 (8.7–16.9)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Data were expressed as median (IQR) and comparisons were made by Wilcoxon matched-pairs signed-rank test; OSA: Obstructive Sleep Apnea; PSG: overnight inpatient polysomnography; CANO: alveolar nitric oxide concentration; J′awNO: maximal bronchial flux of nitric oxide; FENO.50: fractional exhaled nitric oxide at 50 mL/s. Bold indicates statistically significant p-values (<0.05).
J′awNO and FENO.50 before and after PSG in OSA patients or in non-OSA subjects (p > 0.05) (Table 4).

3.5. Exhaled nitric oxide parameters with age, obesity, and systemic hypertension

There was no significant correlation between CANO, J′awNO or FENO.50 with age (p > 0.05) in patients with OSA and in non-OSA subjects (data not shown).

In OSA patients, there was a significant positive relation between CANO and BMI (n = 71; r = 0.247; p = 0.038). However, in linear regression analysis, AHI was significantly associated to CANO (β: 8.433 ± 0.865; p < 0.0001) but not to BMI (β: 0.288 ± 0.203; p = 0.16). There was no association between J′awNO or FENO.50 and BMI (Table 5).

CANO was not significantly different (p > 0.05) between OSA patients with and without hypertension. However, in the non-OSA group, CANO was significantly decreased in those with hypertension (n = 6; 1.29 ppb; [0.88–1.39]) as compared with non-hypertensive subjects (n = 18; 2.71 ppb; [1.85–3.17]; p = 0.002) (Table 6).

![Fig. 4. Increase of alveolar exhaled nitric oxide (CANO) after one night sleep with in-laboratory polysomnography (PSG) recording. Exhaled nitric oxide (NO) was measured after polysomnography (PSG) in 21 patients with OSA and in 8 non-OSA subjects. CANO levels post-sleep were significantly increased (p = 0.0001) to approximately 150% of pre-sleep levels only in OSA patients.](image)

![Fig. 5. Relation between increased levels of alveolar exhaled nitric oxide (Δ_CANO) after sleep and percentage of recording time spent at a SaO2 <90% (ST-90%) in 21 patients with OSA. Increase of CANO post-sleep was significantly associated (p = 0.004) to nocturnal oxygen desaturation index as evaluated by ST-90%.](image)
that was linked to over-expression of inducible NOS and 3-nitrotyrosine (3-NT) in palatine tonsils from patients with OSA as compared with healthy controls. High FENO levels were significantly correlated to the severity of OSA and to increased inducible NOS expression in induced sputum monocytes from OSA patients [12]. Thus, these inflammatory markers are tightly associated to the severity of OSA and, therefore, could be used in clinical monitoring of this syndrome. More recent studies [13,17] found a significant increase of FENO in OSA patients, which was partially reversible after 1-month CPAP treatment. These findings confirmed airway inflammation in patients with OSA (Table 7) that correlated to disease severity.

Several animal models that mimic OSA in humans have been used to study molecular and biological processes. In animals exposed to chronic intermittent hypoxia (CIH), increased expression of NF-kB correlates to severity and decreases after treatment of OSA [19]. These data support a critical role for iNOS in the CIH-induced oxidative stress and inflammatory response.

### Table 7

<table>
<thead>
<tr>
<th>n</th>
<th>NO parameter</th>
<th>Method</th>
<th>Expiratory flow rates (mL/s)</th>
<th>Main results</th>
<th>Interpretation</th>
<th>Ref.</th>
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<tr>
<td></td>
<td>Olopade CO et al., Chest1997</td>
<td>Nasal NO (nNO)</td>
<td>Offline NC</td>
<td>Increased nNO and oNO after sleep as compared with pre-sleep values in OSA patients but not in controls</td>
<td>Upper airway inflammation after sleep</td>
<td>[12]</td>
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<td></td>
<td>Agusti AC et al., Sleep 1999</td>
<td>FENO</td>
<td>Online NC</td>
<td>Unchanged FENO in OSA patients</td>
<td>(Upper and lower) Airway inflammation and oxidative stress in OSA patients</td>
<td>[10]</td>
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<td></td>
<td>Petrosyan M et al., Sleep Breath 2008</td>
<td>Nasal NO (nNO)</td>
<td>Online 250</td>
<td>Increased nNO and FENO in OSA patients, partially reversed after 1-month CPAP therapy</td>
<td>Lower airway inflammation in OSA patients</td>
<td>[17]</td>
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<td></td>
<td>Carpagnano GE et al., TransRes2008</td>
<td>FENO</td>
<td>Online 45</td>
<td>Decreased FENO in obese patients with or without OSA versus healthy controls</td>
<td>Airway inflammation in obese patients</td>
<td>[13]</td>
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<td></td>
<td>Depalo A et al., J Intern Med 2008</td>
<td>FENO (NIOX)</td>
<td>Online 45</td>
<td>Increased FENO in obese patients with or without OSA versus healthy controls</td>
<td>Airway inflammation in OSA and obese patients</td>
<td>[17]</td>
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<td>Verhulst SL et al., Chest 2008</td>
<td>FENO (NIOX)</td>
<td>Online 50</td>
<td>Increased FENO in obese patients with habitual snoring or OSA versus normal weight or obese healthy subjects</td>
<td>Airway inflammation in snoring and OSA patients but not in obese controls</td>
<td>[15]</td>
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<td>Chua AP et al., J Clin Sleep Med 2013</td>
<td>FENO (NIOX)</td>
<td>Online 50</td>
<td>Increased FENO in obese patients with habitual snoring or OSA versus normal weight or obese healthy subjects</td>
<td>Role of upper airway inflammation in the pathophysiology and treatment of OSA</td>
<td>[18]</td>
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<td></td>
<td>Foresi A et al., Chest 2007</td>
<td>FENO and CANO</td>
<td>2 CM model 50, 120, 190, 250, and 300</td>
<td>Decreased CANO in OSA patients, and in OSA patients with hypertension (HT) versus those without HT</td>
<td>Airway inflammation and endothelial dysfunction in severe OSA patients</td>
<td>[11]</td>
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<tr>
<td></td>
<td>Fortuna AM et al., Respir Med 2011</td>
<td>FENO and CANO</td>
<td>2 CM model 10, 30, 100, and 200</td>
<td>Increased FENO and decreased CANO in OSA patients, restored after 3-month CPAP treatment</td>
<td>Airway inflammation and endothelial dysfunction in severe OSA patients</td>
<td>[16]</td>
</tr>
</tbody>
</table>

NO: nitric oxide; OSA: obstructive sleep apnoea; AHt: apnoea–hypopnoea index; CANO: alveolar nitric oxide concentration; FENO: fractional exhaled nitric oxide; NOS: nitric oxide synthase; CPAP: continuous positive airway pressure.
inflammatory responses in OSA [2,3]. In another rat model, recurrent obstructive apnoeas alone or hypoxia/normoxia episodes alone could induce an inflammatory process by activating NF-κB and increasing TNF-α and IL-1β production in the lungs [32]. High levels of pro-inflammatory cytokines can stimulate the NF-κB pathway which, in turn, induced iNOS gene expression and NO overproduction [33]. These findings in animals were consistent with the main result of our study in humans that in OSA patients, the peripheral lung inflammation as assessed by CANO, was strongly related to the severity of OSA evaluated by apnoea–hypopnoea index (AHI) and nocturnal oxygen desaturation level (ST–90%).

Two studies using 2-CM model for exhaled NO measurement, however, found a decreased CANO in OSA patients [11,16]. Regarding demographic characteristics, their populations had more severe OSA and obesity as assessed by AHI and BMI, respectively, when compared with our patients. Percentage of OSA patients with systemic hypertension was also higher than that from our study [10]. These discrepancies might be linked to a peripheral/distal obstructive phenomenon, or to a decrease in NO synthesis and bio-availability related to endothelial dysfunction and advanced oxidative stress environment [2,4].

Our study showed that patients with OSA increased the levels of CANO after overnight PSCF. This augmentation was correlated to the oxygen desaturation severity, linking alveolar inflammation to acute intermittent hypoxia–reoxygenation. To the best of our knowledge, this phenomenon was not yet explored using the same model of exhaled NO measurement. In 1997, Olopade et al. [11] found an increased nasal NO levels after sleep in OSA patients as compared with control subjects, confirming an enhanced upper airway inflammation by intermittent sleep apnoeas. Recently, Chua et al. [17] showed that post-sleep FENO levels were significantly higher than pre-sleep values and the rising levels of FENO correlated to the severity of OSA.

Increased CANO in OSA patients reflected its severity, but how can we apply it in clinical practice? We performed the ROC curve on trumpet-model CANO for screening patients with OSA and nocturnal oxygen desaturation. The threshold of CANO at 4.5 ppb could identify specifically (94%) patients with high risk of OSA and nocturnal oxygen desaturation. These patients should get priority to polysomnography and potential CPAP treatment.

Our current study presented some limits. We decided to eliminate patients with advanced cardiovascular and/or respiratory disorders, especially those presenting daytime oxygen desaturation and those with heart failure in order to study specifically the effects of OSA on nocturnal hypoxemia and to exclude central sleep apnoea [34]. Patients that could not operate a satisfactory exhaled NO measurements with at least 8-second expiration and 3-second NO plateau levels were also eliminated [23]. As a result, only patients with less severity of OSA were included in this study.

Obesity can induce inflammation and oxidative stress observed in patients with metabolic syndrome and OSA [35]. We found a weak correlation between CANO and BMI in patients with OSA but in multiple linear regressions, the relationship between CANO and AHI was independent to BMI. Other studies using the 2-CM model did not find significant correlation between CANO and BMI, a result that might be accounted for their small populations [10,15].

In conclusion, our study using for the first time the trumpet model to characterise alveolar and bronchial exhaled NO production with correction for axial NO diffusion, demonstrated an increased CANO in patients with OSA, accentuated after sleep, linking the distal lung inflammation to intermittent hypoxia. We proposed exhaled NO measurement to screen for patients with oxygen desaturation OSA as they presented suggestive symptoms of OSA, especially snoring and daytime sleepiness. Exhaled NO should be further investigated in patients with morbid obesity and metabolic syndrome. Finally, CHI-exposed animal model investigations should be performed to directly study exhaled NO in the relation with NF-κB and inducible NOS pathways to demonstrate precisely what happens in human disease.

References

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