Increased oral nitric oxide in obstructive sleep apnoea

Increased oral nitric oxide in obstructive sleep apnoea

Beatrice Culla[a], Giuseppe Guida[a], Luisa Brussino[a], Antonella Tribolo[b], Alessandro Cicolin[b], Savino Sciascia[a], Iuliana Badiu[a], Sabrina Mietta[a], Caterina Bucca[a],

[a] Biomedical Science and Human Oncology, Internal Medicine V, University of Torino, Via Genova 3, 10128, Torino, Italy
[b] University Department of Neurology, University of Torino, via Cherasco 15, 10128, Torino, Italy

Background

Hypoxia and snoring-related mechanical trauma contribute to airway inflammation in obstructive sleep apnoea (OSA). Increased exhaled nitric oxide (FENO), an airway inflammation marker, has been reported in OSA patients. We propose the measure of NO in the oral cavity (oNO) as marker of oropharyngeal inflammation in OSA.

Methods

We compared oNO and FENO of 39 OSA patients with those of 26 mild asthmatics (ASTHMA), 15 patients with chronic rhinitis or rhinosinusitis (CRS) and 24 healthy subjects. A special device was used for oNO measurement. Apnoea/hypopnoea index (AHI), oxygen desaturation index, mean and nadir SaO2 were calculated from the polysomnography.

Results

oNO was significantly increased in OSA (104.2 95%CI 80.2–135.5 ppb) as compared to ASTHMA (71.9 95%CI 56.3–91.9 ppb; $p = 0.015$), CRS (54.4 95%CI 40.2–73.7 ppb;$p = 0.009$) and healthy subjects (63.6 95%CI 59–73 ppb; $p < 0.001$). oNO was directly related to AHI ($r = 0.466$, $p = 0.003$) and to minutes slept with SaO2 <90% ($r = 0.471$,$p = 0.011$) and it was inversely related to nadirSaO2 ($r = -0.393$, $p = 0.018$). FENO was highest in asthmatics (40.3 95%CI 32.5–50.1 ppb) and only slightly elevated in OSA (23.1 95%CI 19,8–28.3 ppb) and CRS (22.8 95%CI 16.8–32.5 ppb).

Conclusions

The finding that oral NO is increased in OSA and is related to upper airway obstructive episodes and to hypoxemia severity, strengthens the clinical and pathogenic role of oral inflammation in OSA.

Keywords

- Obstructive sleep apnoea;
- Exhaled nitric oxide;
- Oral nitric oxide;
- Oral inflammation

Introduction

Obstructive sleep apnoea (OSA) is a sleep disorder that leads to metabolic abnormalities and increased cardiovascular risks.[1] Airway and systemic inflammation has been proposed to have a central role in the pathophysiology of OSA.[2] Several studies reported increased inflammatory and oxidative stress markers in lower airway of OSA patients. Increased exhaled nitric oxide (FE_{NO}) and inflammatory metabolites were detected in the exhaled air of OSA compared to healthy subjects.[3, 4 and 5] All these markers were positively related to the severity of OSA (apnea / hypopnoea index). Airway inflammation in OSA is supposed to be the consequence of the release of inflammatory cytokines due to hypoxia and reperfusion cycles during episodes of apnoea or to snoring-related mechanical trauma. Inflammation has been described to involve the upper airways including the nose, the uvula, the soft palate and the pharyngolaryngeal tract. Local upper airway inflammation promotes oropharyngeal inspiratory muscle dysfunction and progressive local neurogenic lesion amplifying upper airway narrowing and collapsibility.[6] Localized inflammation with plasma cell infiltration and interstitial oedema has been documented in oropharynx, uvula and soft palate biopsies.[7] Nitric oxide (NO) is a molecule involved in oxidative stress and airway inflammation; reactive metabolites of nitric oxide may cause tissue damage through oxidation and nitration.[8] The role of nitric oxide in inflammatory airway diseases is well known and the usefulness of NO measurement both in the exhaled air and in the nasal cavity in asthma and nasal polyposis is widely recognized.[9 and 10]Increased oral nitric oxide production, evaluated by measuring salivary nitrites and nitrates, has been found in inflammatory oral diseases as periodontal disease.[11, 12 and 13]We previously found that oNO can be non invasively assessed using a special device which separates completely the oral cavity from the nose and the lower airway. oNO resulted to be significantly increased in healthy subjects during plaque deposition[14] and in edentulous patients[11] We wondered whether in OSA, oropharyngeal inflammation triggered by upper airway vibration, might lead to increased oral NO production.

The objective of the study was to determine whether oral nitric oxide production is increased in OSA patients and is related to disease severity. To this purpose we assessed oNO and FE_{NO} in patients with OSA as compared to patient with mild asthma and chronic rhinosinusitis (lower and upper airway inflammatory diseases respectively).

Methods

Patients

Thirty nine consecutive OSA patients diagnosed at the Sleep Medicine Center by polysomnography in the period June 2007–December 2008, were enrolled. Polysomnography was performed according to American Academy of Sleep Medicine Standards of Practice Committee of the American Sleep Disorders Association guidelines,[15] and OSA was diagnosed when the apnoea/hypopnoea index (AHI) was over 10 episodes per hour. Exclusion criteria were current smoking, past or present history of lower and upper airway disease (asthma, allergic rhinitis, chronic rhinosinusitis), respiratory infections during the last 8 weeks, systemic disease, use of corticosteroids in the last 2 months, dentures wearing, periodontal disease or coexisting oral disease. OSA patients were compared to 15 non smoking patients with chronic rhinitis or rhinosinusitis (CRS), and to 26 non smoking patients with mild asthma (ASTHMA). Asthma and CRS patients were selected among those followed-up in our clinic, who were not receiving oral or inhaled corticosteroids and had no oral or periodontal disease. Diagnosis and classification of rhinitis / rhinosinusitis and asthma

were performed according to ARIA[16 and 17] and GINA[18] guidelines respectively. Twenty four non smoking, non obese, healthy subjects (HS), with no oral disease, with a mean age of 41 years (range 18–65, 10 men and 14 women) served as control group for oNO measurement.

The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee. All the patients gave their informed consent for the use of their data in the study.

Study design

All subjects underwent history recording, clinical examination, lung function tests, and measurement of oNO and FE_{NO}.

Polysomnography

Overnight attended polysomnography was performed using an H_2O system for ambulatory recording (Grass-Telefactor; ASTRO-Med; West Warwick, RI) according to the guidelines of the American Academy of Sleep Medicine Standards of Practice Committee of the American Sleep Disorders Association,[15] using two EEG channels, two electrooculogram channels, surface mentalis and bilateral anterioris tibialis electromyography, electrocardiography, oronasal air flow (two-channel thermistor), respiratory movements (thoracic and abdominal belts), snoring and body position, and arterial oxygen saturation (SaO_2). Standardized criteria for staging sleep were used. EEG tracings were used to assess the total sleep time. Apnea was defined as complete cessation of air flow of at least 10 s in duration, and hypopnea as >50% amplitude reduction of air flow lasting 10 s, associated with 4% oxyhemoglobin desaturation during sleep. From the polysomnography tracing were calculated the following: AHI, oxygen desaturation index (ODI; number of oxyhemoglobin desaturations per hour of sleep), mean and nadir SaO_2 and minutes slept with a SaO_2 below 90% ($SaO_2 < 90\%$)

Lung function tests

Spirometry was performed using the computerized spirometer BAIRES (Biomedin, Padova, Italy). At least three maximal and repeatable flow-volume curves with vital capacity (VC) within 5% were recorded. From the curve with the greatest VC and maximal inspiratory and expiratory efforts, were calculated FEV_1, FEV_1/VC ratio, forced mid expiratory flow (FEF_{50}) and forced mid-inspiratory flow (FIF_{50}) and their ratio (FEF_{50}/FIF_{50}). VC, FEV_1 and FEF_{50} were expressed as percent of predicted.[19]

Exhaled nitric oxide measurement

Fractional Exhaled Nitric Oxide (FE_{NO}) was measured according to ATS/ERS recommendations[20] with a NO chemiluminescent analyzer (Sievers NOA 280, Sievers, Boulder, CO, USA) using a sampling flux of 50 mL/min. FE_{NO} values were calculated upon an end expiration plateau 3 s long at least. The patient performed three acceptable trials and the mean value was registered.

Oral nitric oxide measurement

Oral NO production was measured using a special device which consisted of a holed metal tube, welded at right angle to a shaped plate which served to press the tongue against the soft palate so as to isolate the mouth from upper and lower airway and from gastric cavity. Before the measurement, the subjects were asked to rinse accurately their mouth with tap water. A soft mouthpiece was fitted on the tube, to mantain the mouth closed off and to fasten the position of the device. A two-way valve connected the plate to a glass syringe, for delivery and withdrawal of air samples to and from the oral cavity, and, through a wide-bore Teflon tube (internal diameter 2 mm), to the NO meter (Fig. 1) Thirty ml NO-free air (NO below 5 ppb) were injected with a glass syringe through the device into the closed empty mouth and maintained for 30 s; the air was then withdrawn and shifted to the NO chemiluminescence analyzer (SIEVERS 280 NOA, Boulder, CO).[14] oNO measurement was repeated until three values having a variability within 5% were obtained. The mean of the three values was used in the analysis.

Statistics

Data were analysed with the statistical SPSS software package (version 13.0 for Windows, Chicago, IL, USA). Normal distribution of variables were assessed according to Kolmogorov-Smirnov's test of normality. In case of non normal distribution the variables were computed as logarithmic. Comparisons between continuous variables were estimated with unpaired and paired Student's t test or Mann-Whitney test, depending on the distribution of the variables. Categorical variables were compared with the Fisher's exact test. Correlations between parametric or non parametric variables were obtained using Pearson's regression analysis, Spearman's rank correlation tests, and logistic regression analysis. For the correlations of oNO with sleep disturbance, the following sleep parameters were used: AHI, oxygen desaturation index (ODI; number of oxyhemoglobin desaturations per hour of sleep), mean and nadir SaO2, and SaO2 < 90%.

A p value < 0.05 was considered to be significant.

Results

Demographical and clinical characteristics of patients are reported in Table 1. OSA patients, as compared to ASTHMA and CRS patients, had significantly older age, higher prevalence of males and higher BMI. These differences reflect the characteristics of the diseases, OSA being more frequent in obese men, adult asthma in women. There were no difference in lung function parameters apart from a significantly higher FEF50/ FIF50 ratio in OSA patients compared to asthmatics ($p = 0.05$).

Nitric oxide measurements

Mean values of oral NO in patients and healthy controls are reported in Fig. 2, panel A. OSA patients had significantly increased oNO values (104.2 95%CI 80.2–135.5 ppb) as compared either with healthy subjects (63.6 95%CI 59.0–73.0 ppb; $p < 0.001$) or with both ASTHMA (71.9 95%CI 56.3–91.9 ppb; $p = 0.015$) and CRS patients (54.4 95%CI 40.2–73.7 ppb; $p = 0.009$). In both ASTHMA and CRS groups oNO was similar to that of healthy controls.

Mean FENO values in patients and controls are reported in Fig. 2, panel B. In OSA patients FENO values (23.1 95%CI 19,8–28.3 ppb) were significantly higher than in healthy subjects (11.0 95%CI 8.7–14.8 ppb; $p < 0.001$), and were similar to those observed in CRS patients (22.8 95%CI 16.8–32.5 ppb). ASTHMA patients had the highest FENO values (40.3 95%CI 32.5–50.1 ppb).

No significant relationship between oNO and FENO was found either in OSA or in CRS, and in ASTHMA.

In OSA patients, oNO production was related to the severity of sleep disturbances. Actually, oNO was significantly positively related to AHI ($r = 0{,}466$, $p = 0.003$) (Fig. 3, A), to ODI ($r = 0.447$, $p = 0.017$), to SaO2 < 90% (available in 27 of the 39 patients) ($r = 0.471$,$p = 0.011$) (Fig. 3, B) and negatively related to nadir SaO2 ($r = -0.393$, $p = 0.018$). oNO was significantly related to BMI ($r = 0.505$, $p < 0.001$).

No correlation was found between oNO and spirometric parameters.

Discussion

The results of the present study show that patients with OSA have increased production of NO in oral cavity, which is proportional to the severity of the disease. Since NO is an inflammatory marker, this finding is in line with prior studies showing inflammation in the pharynx, uvula, soft palate and oral cavity of OSA patients.6 and 7 Oral inflammation in OSA is supposed to originate in the upper airway, where repetitive closing and opening during apnoeic episodes leads to increased production of inflammatory cytokines.21 Pharyngeal collapse is favoured by obesity, that is recognized as a major risk factor for OSAS. This may explain the correlation we found between BMI and oNO values. Oral inflammation in OSA may also be a consequence of intermittent hypoxia and reperfusion.22 Low oxygen tension is a known trigger of activation of polymorphonuclear neutrophils and macrophages, that interact with the endothelium causing the release of free oxygen radicals.23 Increased oNO, interacting with free oxygen radicals, may amplify the oxidative and nitrosative stress by generating reactive nitrogen oxide species.24 The effect of hypoxia on NO release is supported by the significant inverse correlation we found between oNO levels and nadir overnight desaturation.

Interestingly, oral NO in OSA patients was significantly higher than in patients with other types of upper and lower inflammatory diseases, such as rhinosinusitis and asthma. Actually, in both asthma and CRS the major sources of NO production are far from the mouth, paranasal sinuses in CRS,24 bronchi and alveoli in asthma.25 These differences in NO source are reflected by findings of exhaled nitric oxide measurements. In fact, FENO was highest in asthmatic patients and mildly increased in both patients with CRS and those with OSA. The mild increase of FENO levels in OSA patients is in agreement with several studies reporting increased inflammatory markers and metabolites in the exhaled air and condensate of OSA patients.3, 4 and 5 FENO levels measured in OSAS may reflect a systemic inflammatory response.26

In conclusion, the findings of this study indicate that oral nitric oxide measurement in OSA may be used as a marker of upper airway obstructive episodes due to mechanical trauma and of hypoxemia causing local oropharyngeal inflammation. Follow-up measurements of oNO after effective CPAP therapy would strengthen these findings. Oral NO, through its ability to amplify oxidative and nitrosative stress, may be suggested to have a pathogenetic role in OSA.

References

1. Bonsignore MR, Zito A. Metabolic effects of the obstructive sleep apnea syndrome and cardiovascular risk. Arch Physiol Biochem 2008;114(4):255e60.

2. Hatipoglu U, Rubinstein I. Inflammation and obstructive sleep apnea syndrome pathogenesis: a working hypothesis. Respiration 2003;70(6):665e71.

3. Olopade CO, Christon JA, Zakkar M, Hua C, Swedler WI, Scheff PA, et al. Exhaled pentane and nitric oxide levels in patients with obstructive sleep apnea. Chest 1997;111(6): 1500e4.

4. Carpagnano GE, Spanevello A, Sabato R, Depalo A, Turchiarelli V, Foschino Barbaro MP. Exhaled pH, exhaled nitric oxide, and induced sputum cellularity in obese patients with obstructive sleep apnea syndrome. Transl Res 2008;151(1): 45e50.

5. Petrosyan M, Perraki E, Simoes D, Koutsourelakis I, Vagiakis E, Roussos C, et al. Exhaled breath markers in patients with obstructive sleep apnoea. Sleep Breath 2008; 12(3):207e15.

6. Friberg D, Ansved T, Borg K, Carlsson-Nordlander B, Larsson H, Svanborg E. Histological indications of a progressive snorers disease in an upper airway muscle. Am J Respir Crit Care Med 1998;157(2):586e93.

7. Sekosan M, Zakkar M, Wenig BL, Olopade CO, Rubinstein I. Inflammation in the uvula mucosa of patients with obstructive sleep apnea. Laryngoscope 1996;106(8):1018e20.

8. Bove PF, van d V. Nitric oxide and reactive nitrogen species in airway epithelial signaling and inflammation. Free Radic Biol Med 2006;41(4):515e27.

9. Kharitonov SA, Yates D, Springall DR, Buttery L, Polak J, Robbins RA, et al. Exhaled nitric oxide is increased in asthma. Chest 1995;107(3 Suppl):156Se7S.

10. Ragab SM, Lund VJ, Saleh HA, Scadding G. Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy. Allergy 2006;61(6):717e24.

11. Bucca C, Cicolin A, Brussino L, Arienti A, Graziano A, Erovigni F, et al. Tooth loss and obstructive sleep apnoea. Respir Res 2006;7:8.

12. Ohashi M, Iwase M, Nagumo M. Elevated production of salivary nitric oxide in oral mucosal diseases. J Oral Pathol Med 1999; 28(8):355e9.

13. Paquette DW, Williams RC. Modulation of host inflammatory mediators as a treatment strategy for periodontal diseases. Periodontol 2000 2000;24:239e52.

14. Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, Brussino L, et al. Oral nitric oxide during plaque deposition. Eur J Clin Invest 2001;31(10):876e9.

15. Practice parameters for the indications for polysomnography and related procedures. Polysomnography task force, american sleep disorders association standards of practice committee. Sleep 1997;20(6):406e22.

16. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy 2008;63(Suppl. 86): 8e160.

17. Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. Rhinol Suppl 2007;20:1e136.

18. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; 31(1):143e78.
19. Gibson GJ. Standardised lung function testing. Eur Respir J 1993;6(2):155e7.
20. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171(8):912e30.
21. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997;82(5):1313e6.
22. Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during obstructive sleep apnea. Sleep 1993;16(8 Suppl):S15e21.
23. Schulz R, Mahmoudi S, Hattar K, Sibelius U, Olschewski H, Mayer K, et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. Am J Respir Crit Care Med 2000;162(2 Pt 1):566e70.
24. Maniscalco M, Sofia M, Pelaia G. Nitric oxide in upper airways inflammatory diseases. Inflamm Res 2007;56(2):58e69.
25. Brindicci C, Ito K, Barnes PJ, Kharitonov SA. Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity. Chest 2007;131(5):1353e62.
26. Depalo A, Carpagnano GE, Spanevello A, Sabato R, Cagnazzo MG, Gramiccioni C, et al. Exhaled NO and iNOS expression in sputum cells of healthy, obese and OSA subjects. J Intern Med 2008;263(1):70e8.

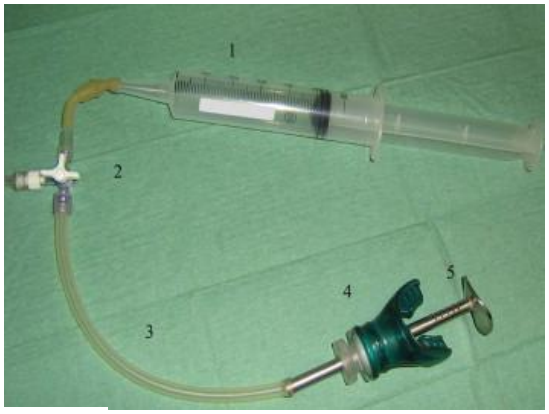

Figure 1.
Illustration of oral NO device. Siringe with NO-free air (1); two-way valve for delivery and withdrawal of air sample to and from the oral cavity and to the NO meter (2); wide-bore teflon tube (3); soft mouthpiece (4); Holed metal tube welded at a right angle to a shaped plate (5).

Table 1.
Demographical and clinical characteristics of OSA patients compared to ASTHMA and CRS groups.

	OSA ($N = 39$)	ASTHMA ($N = 26$)	CRS ($n = 15$)
Age, [range]	66 [38–81]a [and] b	45 [17–68]	50.4 [29–71]
Sex (M/F)	22/17a [and] c	8/18	6/9
BMI	30.7 (28.8–32.7)a [and] c	24.1 (22.6–25.5)	23.9 (21.9–25.9)
FVC	100.7 (92.9–108.5)	107.7 (99.9–115.5)	113.1(100.5–125.7)
FEV_1	99.4 (91.1–107.8)	92.6 (85.8–99.4)	99.7 (86.7–112.7)
FEV_1/VC	76.7 (73.5–79.9)	72.7 (67.9–77.3)	73.4 (67.3–79.5)
FEF_{50}/ FIF_{50}	112.7 (91.7–133.6)[d]	77.6 (52.6–102.7)	87 (62.7–111)

BMI,FVC, FEV_1, FEV_1/VC, FEF_{50}/ FIF_{50} are reported as means with 95% confidence intervals.

[a] $p < 0.001$ OSA vs ASTHMA
[b] $p < 0.05$ OSA VS CRS
[c] $p < 0.001$ OSA vs CRS
[d] $p < 0.05$ OSA VS ASTHMA

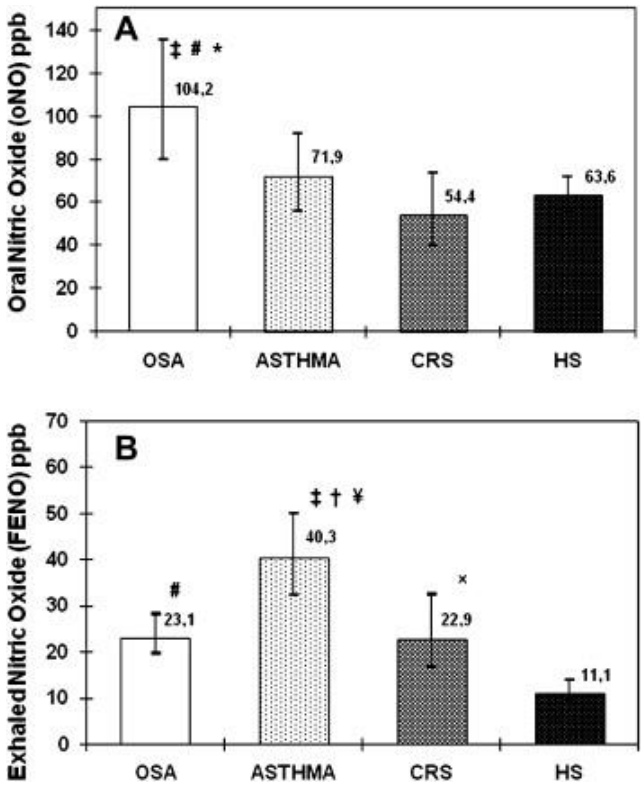

Figure 2.
Geometric means and SD (bars) of oNO (A) and FE_{NO} (B) in patients with OSA compared to asthmatics (ASTHMA), chronic rhinitis and rhinosinusitis (CRS) and healthy subjects (HS). [#]$p < 0.001$ OSA versus HS; [‡]$p < 0.05$ OSA versus ASTHMA; * $p < 0.05$ OSA vs CRS. [†]$p < 0.05$ ASTHMA versus CRS;[¥]$p < 0.001$ ASTHMA versus HS; [×]$p < 0.05$ CRS versus HS.

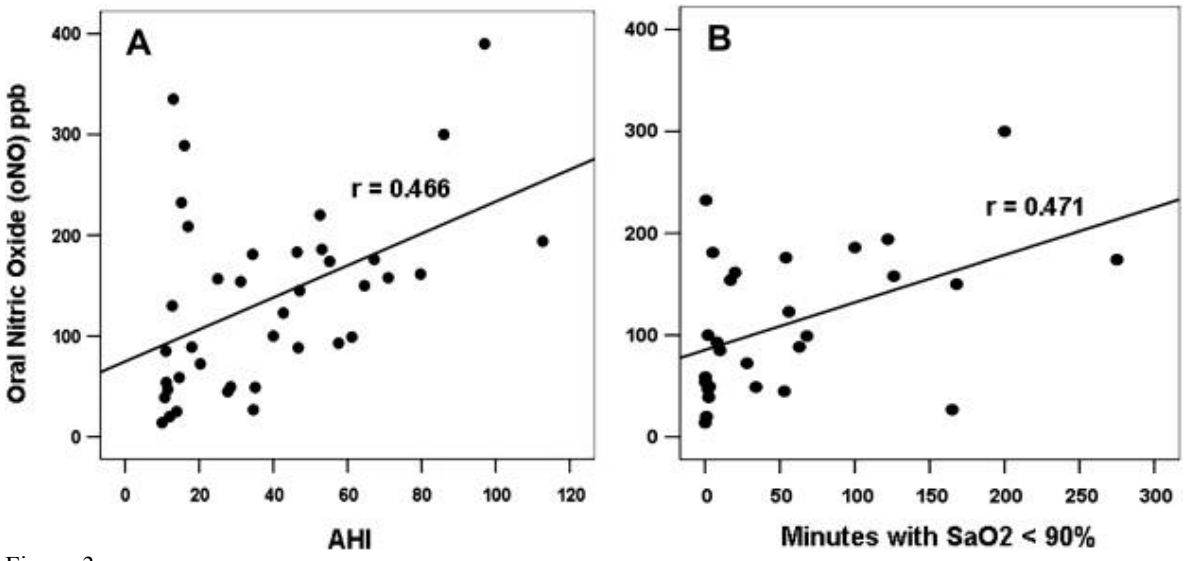

Figure 3.
Correlation of oNO with AHI (A) and with SaO2 < 90% (B) in OSA patients.