

8-Isoprostane, a Marker of Oxidative Stress, Is Increased in Exhaled Breath Condensate of Patients With Obstructive Sleep Apnea After Night and Is Reduced by Continuous Positive Airway Pressure Therapy*

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Study objectives: Obstructive sleep apnea (OSA) is characterized by recurrent apnea during sleep that may compromise oxidative balance. Oxidative stress is increased in the blood and in the airways of OSA patients.

Design: The aim of this study was to investigate whether oxidative stress is determined by nocturnal apneas and could be reduced by CPAP therapy, and whether there is a relation between local and systemic oxidative stress in these patients.

Patients and methods: Eighteen patients with OSA (13 men; mean [\pm SD] age, 48 ± 3 years) and 12 healthy age-matched and weight-matched subjects (8 men; mean age, 46 ± 7 years) were recruited. 8-Isoprostane was measured in exhaled breath condensate and blood by a specific enzyme immunoassay.

Measurements and results: Higher concentrations of 8-isoprostane were found in the morning exhaled condensate (9.5 ± 1.9 pg/mL) and plasma (9.7 ± 1.5 pg/mL) of OSA patients compared to healthy obese subjects (6.7 ± 0.2 and 7.1 ± 0.3 pg/mL, respectively; $p < 0.0001$). Elevated mean concentrations of exhaled 8-isoprostane were observed in the OSA patients at 8:00 AM (9.5 ± 1.9 pg/mL) but not at 8:00 PM (7.6 ± 0.8 pg/mL; $p < 0.0005$), and a significant reduction was seen after continuous positive airway pressure (CPAP) therapy (7.7 ± 0.9 pg/mL; before treatment, 9.6 ± 1.7 pg/mL; $p < 0.005$). A positive correlation was found between morning exhaled 8-isoprostane levels and the apnea-hypopnea index ($r = 0.8$; $p < 0.0001$), and 8-isoprostane levels and neck circumference ($r = 0.6$; $p < 0.0001$).

Conclusions: These findings suggest that systemic and local oxidative stress are increased in OSA patients, and that they are higher after nocturnal apnea and reduced by CPAP therapy.

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Key words: continuous positive airway pressure therapy; 8-isoprostane; obstructive sleep apnea; oxidative stress

Abbreviations: AHI = apnea-hypopnea index; CPAP = continuous positive airway pressure; ODI = oxygen desaturation index; OSA = obstructive sleep apnea; REM = rapid eye movement; ROS = reactive oxygen species; TST = total sleep time

Obstructive sleep apnea (OSA) is characterized by recurrent apnea due to occlusion of the upper airways during sleep leading to arterial oxygen de-

saturation and a decrease in tissue oxygenation, followed by a resumption of oxygen saturation during hyperventilation.^{1,2} This phenomenon has been described as hypoxia/reoxygenation and may alter the oxidative balance by inducing excessive oxygen free-

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radical production. Some studies have reported an increased oxidant burden, and a consequent increase in the markers of oxidative stress and inflammation in the blood of patients with OSA.^{3,4}

Although the etiology is uncertain, experimental evidence has shown that both the structure and function of the upper airways are altered in patients with OSA,^{5,6} and that mechanical obstruction during sleep is characteristic of this disorder.^{5,7} Moreover, the recurrent obstruction and reopening of the upper airways during sleep as many as 80 to 100 times per hour, may lead to mucosal congestion in the airways with a subsequent presence of local inflammation and oxidative stress.^{8,9}

The existence of upper airway inflammation in OSA patients has been confirmed.^{6,8-10} Direct study of the airways presents some difficulties due to the invasiveness of the methods currently available. Recently, we studied¹¹ airway inflammation and oxidative stress in OSA patients by finding two specific markers, interleukin-6 and 8-isoprostane, in exhaled breath condensate. This new technique produces samples that reflect the composition of airway lining fluid, and is simple and completely noninvasive. It already has been used to measure oxidative stress and inflammation in several respiratory diseases.^{12,13} The aim of this study was to investigate whether there is any relation between local and systemic oxidative stress in patients with moderate-to-severe OSA, to investigate whether the nocturnal apnea episodes could be responsible for oxidative stress, and whether continuous positive airway pressure (CPAP) therapy may have some beneficial effects on this marker of oxidative stress.

MATERIALS AND METHODS

Subjects

The study population consisted of 18 well-characterized OSA patients and 12 age-matched and weight-matched healthy control subjects. All subjects were white and recruited from the Sleep Laboratory of the Institute of Respiratory Diseases of the University of Bari, Italy. Written informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee. A complete physical examination was performed, including neurologic, cardiopulmonary, and ear, nose, and throat examinations. Inclusion criteria for this study were an apnea-hypopnea index (AHI) of >20 and symptoms of excessive daytime sleepiness, and an AHI of <5 for control subjects. The control group consisted of 12 obese subjects who were free of sleep disturbances and were in good health. The OSA patients and the control subjects did not have any endocrinologic diseases, psychiatric disorder, overt cardiopulmonary disease, airway obstruction, anatomic maxillo-mandibular skeletal abnormalities, or ear, nose, and throat disease. None of the subjects were heavy drinkers or used of any kind of drug. Patients with rhinitis, sinusitis, respiratory infections, and systemic infections also were

excluded. All subjects had stopped smoking at least 3 months before entering the study and had received no therapy for 4 weeks prior to study entry with inhaled, oral, or nasal steroids or other anti-inflammatory drugs. Exhaled breath condensate was collected in all OSA patients and healthy control subjects before sleeping (8:00 PM) and on waking (8:00 AM), and a venous blood sample was taken at the same time.

Ten subjects, who had a diagnosis of OSA based on polysomnography were rehospitalized for CPAP nasal treatment for two nights within 1 week of the diagnostic (baseline) polysomnography. Exhaled breath condensate was collected before and after receiving two nights of CPAP therapy.

Pulmonary Function Testing

Pulmonary function tests were performed within 1 day of the breath condensate measurements. FEV₁, FVC, and FEV₁/FVC ratio were measured using a spirometer (PK Morgan Ltd; Gillingham, UK). The best value of three maneuvers was expressed as a percentage of the predicted normal value.

Polysomnography

All subjects were evaluated in the sleep laboratory of the Institute of Respiratory Diseases of the University of Bari for one night, and they were monitored continuously for 8 h using a 19-channel polysomnograph (Compumedic; Sydney, Australia). Polysomnography was performed after one night of adaptation in the hospital. EEG, electrooculographic, and chin-electromyographic recordings were obtained with surface electrodes according to standard methods.¹⁴ Airflow was monitored by a thermistor placed at the nose and at the mouth. Abdominal and rib-cage movements were assessed by respiratory inductive plethysmography. Overnight continuous recordings of oxygen saturation were obtained by finger pulse oximetry. Snoring was recorded by a microphone placed at the neck, and note was taken of ECG findings and sleep position. *Apnea* was defined as the cessation of airflow lasting ≥ 10 s, *hypopnea* was defined as the discrete reduction (two thirds) of airflow and/or abdominal rib-cage movements lasting ≥ 10 s that are associated with a decrease of $>3\%$ in oxygen saturation or number of arousals. Since $>85\%$ of respiratory events were obstructive (characterized by an increasing ventilatory effort and paradoxical breathing), the specific pattern of apneic episodes was not taken into account in the statistical analysis. The number of events per hour was obtained by dividing the total number of events by the total sleep time (TST) and was defined as the AHI. We also measured the oxygen desaturation index (ODI). Hemoglobin desaturation was evaluated in terms of the percentage of TST with oxyhemoglobin saturation at $<90\%$. Sleep records were scored according to standardized criteria.¹⁴ Sleep was divided into that with non-rapid eye movement (REM) and that with REM. Then, the percentage of non-REM and REM sleep of the TST was calculated. Finally, the Epworth sleepiness scale was used to measure sleep propensity.^{15,16}

Exhaled Breath Condensate

Exhaled breath condensate was obtained by using a condenser (EcoScreen; Jaeger; Wurzburg, Germany) that noninvasively collected the nongaseous components of the expiratory air. Subjects breathed tidally through a mouthpiece and a two-way nonbreathing valve, which also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 10 min. If subjects felt saliva in their mouth, they were instructed to swallow it. The conden-

sate (at least 1 mL) was collected as ice at -20°C , was transferred to Eppendorf tubes, and was stored at -70°C immediately.

Measurement of 8-Isoprostane

A specific enzyme immunoassay kit (Cayman Chemical; Ann Arbor, MI) was used to measure 8-isoprostane concentrations in breath condensate and venous blood. The assay was validated directly by gas chromatography/mass spectrometry.¹⁷ The antiserum used in this assay has 100% cross-reactivity with 8-isoprostane, 0.2% with prostaglandin (PG) $\text{F}_{2\alpha}$, $\text{PGF}_{3\alpha}$, PGI_2 , and PGE_2 , and 0.1% with 6-keto $\text{PGF}_{1\alpha}$. The intra-assay and inter-assay variability were $\pm 5\%$ and 6% , respectively, and the detection limit of the assay was 4 pg/mL. The reproducibility of repeated 8-isoprostane measurements was assessed by the method of Bland and Altman¹⁸ and the coefficient of variation.

Statistical Analysis

Data were expressed as the mean \pm SD. A Mann-Whitney test was used to compare groups, and correlations between variables were performed using the Spearman rank correlation test. The Wilcoxon signed rank test was used to compare levels of 8-isoprostane within the same group of patients before sleeping and on waking, and between pretreatment and posttreatment. Significance was defined as a *p* value of < 0.05 .

RESULTS

Subject Characteristics

The anthropometric data are summarized in Table 1. Eighteen patients had moderate-to-severe OSA (mean AHI, 59.2 ± 5.4), and 12 healthy individuals served as control subjects (mean AHI, 3.0 ± 2.0). There were 13 men and 5 women in the OSA group, while the control group consisted of 8 men and 4 women. No subject presented with any diurnal hy-

poxemia. There were no statistically significant differences in age, BMI, and neck circumference between the OSA patients and control subjects.

8-Isoprostane Measurements

8-Isoprostane was measurable in the exhaled breath condensate of all subjects, and the mean morning concentrations were higher in the OSA patients (9.5 ± 1.9 pg/mL) than in the healthy subjects (6.7 ± 0.2 pg/mL; $p < 0.0001$). Higher morning levels of 8-isoprostane also were observed in the venous blood of the OSA patients (9.7 ± 1.5 pg/mL) compared to the control subjects (7.1 ± 0.3 pg/mL; $p < 0.0001$). No significant differences were found in the morning 8-isoprostane concentrations in the exhaled breath condensate and plasma of OSA patients and healthy controls ($p = 0.4$ and $p = 0.2$, respectively) [Fig 1, top, A]. A positive correlation was observed between morning levels of exhaled 8-isoprostane and plasma 8-isoprostane ($r = 0.8$; $p < 0.0001$) [Fig 1, bottom, B]. Greater concentrations of exhaled 8-isoprostane were found in OSA patients at 8:00 AM than at 8:00 PM (9.5 ± 1.9 and 7.6 ± 0.8 pg/mL, respectively; $p < 0.0005$) [Fig 2, top, A]. No differences were observed in evening levels of 8-isoprostane between OSA patients and control subjects (7.6 ± 0.8 and 6.8 ± 0.2 pg/mL, respectively; $p = 0.2$). A significant reduction in morning exhaled 8-isoprostane levels was found after CPAP therapy (before therapy, 9.6 ± 1.7 pg/mL; after therapy, 7.7 ± 0.9 pg/mL; $p < 0.005$) [Fig 2, bottom, B]. No differences were observed in the concentrations of exhaled 8-isoprostane at 8:00 AM and 8:00 PM in healthy subjects (6.7 ± 0.2 and 6.5 ± 0.9 pg/mL, respectively). A positive correlation was seen between morning levels of 8-isoprostane and AHI ($r = 0.8$; $p < 0.0001$) [Fig 3], and 8-isoprostane and neck circumference ($r = 0.6$; $p < 0.0001$), while no correlation was found between morning levels of 8-isoprostane and FEV_1 ($r = 0.1$; $p = 0.6$), FVC ($r = 0.2$; $p = 0.4$), PaO_2 ($r = 0.07$; $p = 0.7$), PaCO_2 ($r = -0.2$; $p = 0.3$), TST oxyhemoglobin saturation ($r = -0.2$; $p = 0.3$), or ODI ($r = 0.3$; $p = 0.5$). Furthermore, a negative correlation was observed between the percentage of REM sleep of the TST and exhaled 8-isoprostane concentrations ($r = -0.6$; $p < 0.0005$).

The reproducibility of exhaled 8-isoprostane measurements was assessed in 10 nonsmoking healthy subjects (6 men; mean age, 35 ± 7 years). In the majority of measurements, the differences between the two 8-isoprostane values laid within ± 2 SDs (mean difference, -0.14 ± 0.32 pg/mL) [Fig 4]. The coefficient of variation for 8-isoprostane measured was 4.4% [Fig 4].

Table 1—Subject Data*

Variables	Patients With OSA (n = 18)	Control Subjects (n = 12)	<i>p</i> Value†
Gender			
Male	13	8	
Female	5	4	
Age, yr	48 ± 3	46 ± 7	NS
BMI, kg/m^2	34 ± 0.9	32.6 ± 2	NS
Neck circumference, cm	42.6 ± 0.9	40.9 ± 0.3	NS
FVC, % predicted	86 ± 148	101 ± 4	< 0.005
FEV_1 , % predicted	9 ± 16	105 ± 8	< 0.005
FEV_1/FVC ratio	90 ± 7	103 ± 5	< 0.005
AHI	59.2 ± 5.4	3.6 ± 0.4	< 0.0001
ODI	66.4 ± 10.2	9.8 ± 1.1	< 0.0001
REM sleep, %	18.6 ± 2.7	23.6 ± 2.1	< 0.0001
TST $\text{Sao}_2 < 90\%$	32.2 ± 5.8	—	
Systolic BP, mm Hg	135 ± 15	127 ± 13	NS
Diastolic BP, mm Hg	83 ± 13	76 ± 11	NS

*Values given as the mean \pm SEM, unless otherwise indicated.

NS = not significant.

†Significance was defined as a *p* value of < 0.05 .

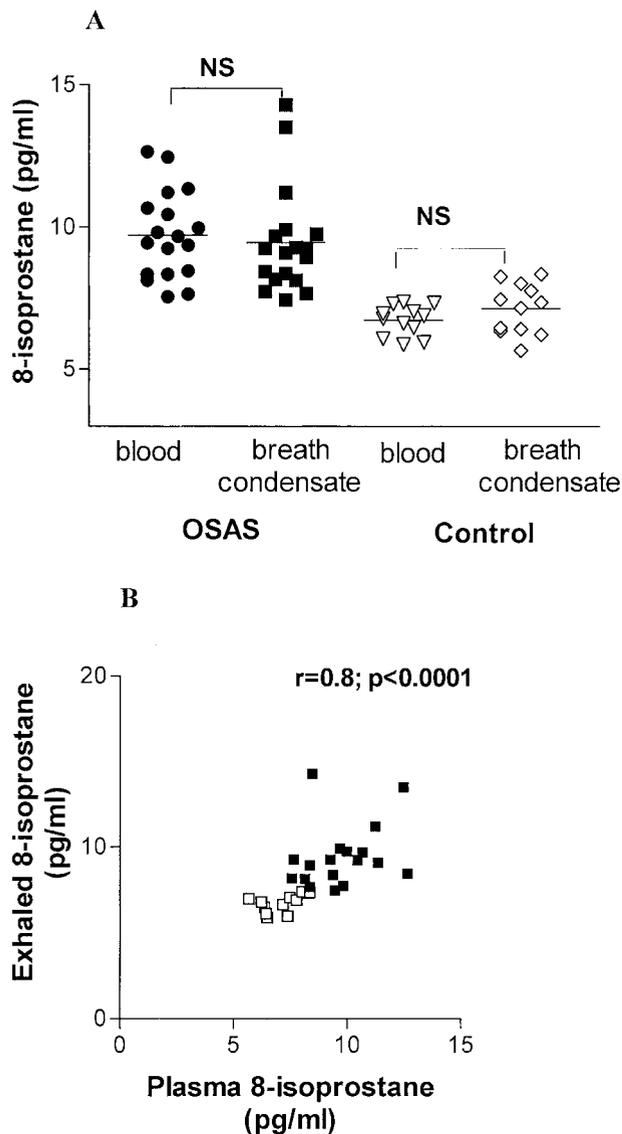


FIGURE 1. *Top, A:* morning 8-isoprostane concentrations in the exhaled breath condensate and blood of OSA patients and healthy control subjects. *Bottom, B:* correlation between 8-isoprostane levels in exhaled breath condensate and in plasma. NS = not significant; OSAS = OSA syndrome.

DISCUSSION

Our study confirms that 8-isoprostane is detectable in exhaled breath condensate and that its concentration is significantly higher in patients moderate-to-severe OSA, compared to healthy weight-matched subjects.¹¹ The concentrations of 8-isoprostane in the breath condensate of these patients were similar to the concentrations measured in plasma. There was a positive correlation between levels of exhaled 8-isoprostane and those of plasma 8-isoprostane in OSA patients and in control subjects. The concentrations of 8-isoprostane in breath condensate

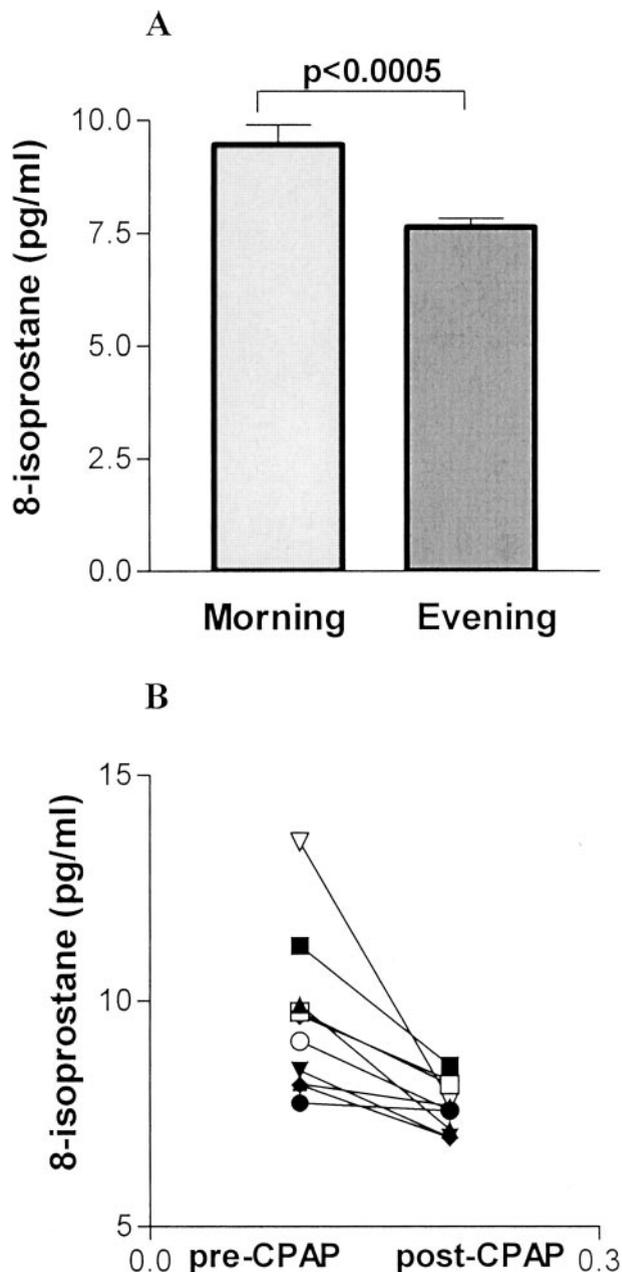


FIGURE 2. *Top, A:* exhaled 8-isoprostane concentrations in OSA patients at 8:00 PM and 8:00 AM. *Bottom, B:* morning exhaled 8-isoprostane levels before and after CPAP therapy.

were significantly higher in the morning (*ie*, 8:00 AM) than in the evening (8:00 PM), and this was reversible with CPAP therapy.

Isoprostanes have been used to quantify oxidative stress *in vivo*.¹⁹ Isoprostanes are formed by the effect of oxidative stress on arachidonic acid, which is generated from membrane phospholipids by phospholipase A₂.¹⁹ Due to their stability, specificity for lipid peroxidation, production *in vivo*, and relative abundance in biological fluids, isoprostanes are among the most reliable biomarkers of lipid peroxi-

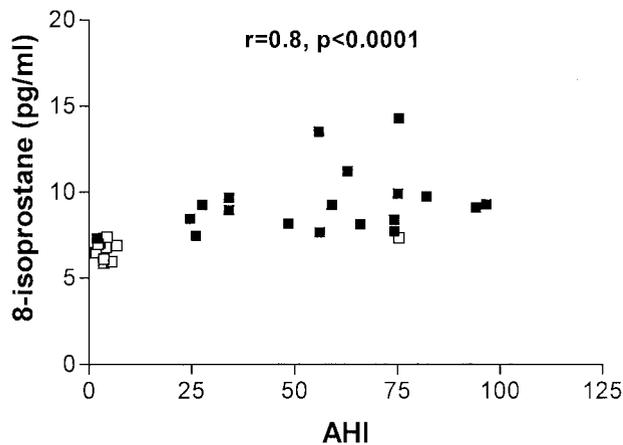


FIGURE 3. Correlations between exhaled 8-isoprostane levels and AHI.

dation and oxidative stress.¹⁷ For this reason, the measurement of 8-isoprostane levels in biological fluids has been considered to be the most promising method for quantifying oxidative stress under different pathophysiologic conditions. 8-Isoprostane, as a marker of oxidative stress, has now been widely investigated in pulmonary disease. For example, increased concentrations of 8-isoprostane have been reported in the BAL fluid of patients with interstitial lung disease,²⁰ in the plasma of patients with cystic fibrosis,²¹ in the urine of patients with COPD and in smokers,²² and in the exhaled breath condensates of patients with asthma,²³ COPD,²⁴ cystic fibrosis,¹⁷ ARDS²⁵ and OSA.¹¹ The measurement of plasma 8-isoprostane reflects systemic oxidative stress, whereas in exhaled breath condensate it is more likely to reflect local lipid peroxidation in the airways. A large body of experimental evidence indicates that upper airway structure and function are altered in OSA patients, although the results of their lung function tests may be normal.^{26,27} The measurement of 8-isoprostane in exhaled breath condensate therefore may be useful in monitoring these alterations, which result from increased oxidative stress in the airway mucosa.

Various authors have reported evidence for systemic oxidative stress in OSA patients. There is an increased release of superoxide from the neutrophils of OSA patients,⁴ and it has been suggested that the increased oxidative stress in OSA may be reduced by sleep.²⁸ By contrast, others have reported that hypoxic and nonhypoxic OSA patients show no difference from healthy control subjects in the susceptibility of low-density lipoproteins to oxidative stress.²⁹ For the first time, we also observed the existence of local oxidative stress in the airways of OSA patients that appears to be related to the severity of the

disease.¹¹ With the present study, we confirm the high concentrations of 8-isoprostane in the breath condensate of OSA patients and also demonstrate that there is a strong correlation between systemic and local oxidative stress in these patients.

The following several mechanisms have been proposed as being responsible for oxidant/antioxidant imbalance in OSA patients:

1. Sleep apnea patients undergo repetitive episodes of hypoxemia and reoxygenation during sleep, which result in the production of oxygen-derived free radicals, with subsequent tissue damage.³⁰ This phenomenon is called the *oxygen paradox*, and is dependent on the degree and duration of hypoxia.
2. An increase in sympathetic tone has been observed in OSA patients. Elevated catecholamine levels may undergo auto-oxidation, in which electrons are generated that in turn can produce reactive oxygen species (ROS). This is thought to be one of causes of catecholamine-induced cardiomyopathy, and it also may account for the cardiovascular complications in OSA patients.^{31,32}
3. OSA patients may have a marked reduction in REM sleep when their disorder is severe. This REM deprivation has been shown in an animal study³³ to cause both the activation of lipid peroxidation by free radicals and the inhibition of antioxidants. The negative correlation observed in our study between the percentage of REM sleep of the TST and exhaled 8-isoprostane concentrations provides some support for this hypothesis, which previously has been tested only in animals.

In addition, although the etiology of OSA is uncertain, mechanical obstruction of the upper airways during sleep is a characteristic of the disorder, and is associated with upper airway inflammation and mucosal congestion. This suggests that ROS formed during sleep in OSA patients may induce tissue damage. The presence of an inflammation of the upper airways induced by the repeated airflow turbulence following the mechanical obstruction already has been demonstrated in sleep apnea subjects, as evidenced by increased concentrations of the inflammatory cytokine interleukin-6 in exhaled condensate,¹¹ and could be responsible for the generation of increased 8-isoprostane concentrations at the luminal surface.³⁴

It is possible that the increased oxidative stress found systematically in OSA is a consequence of this local airway inflammation due to the mechanical injury. This could then account for the positive

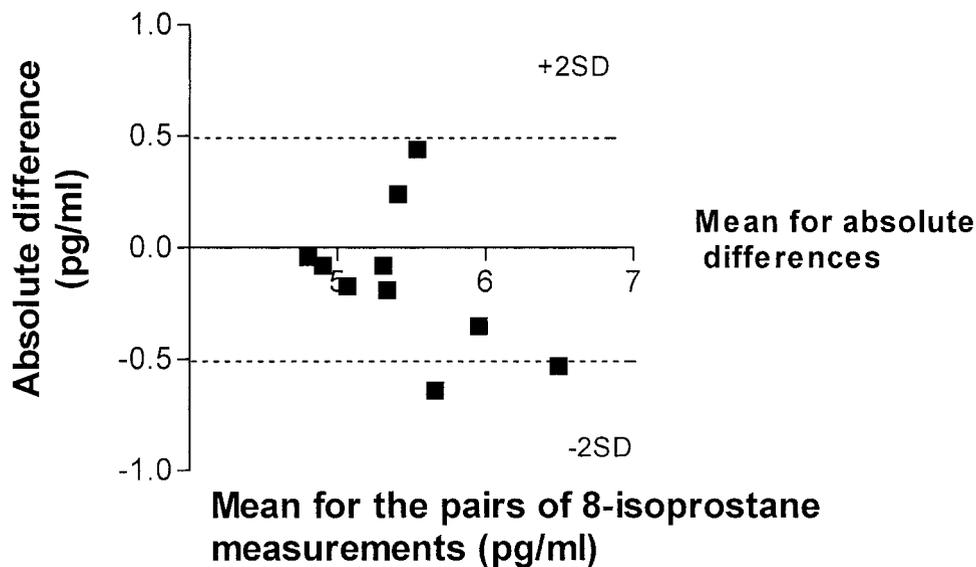


FIGURE 4. The degree of reproducibility between two successive measurements of 8-isoprostane concentrations in the breath condensate of healthy subjects using the Bland-Altman method.

correlation we found between 8-isoprostane concentrations in the exhaled breath condensate and in the plasma of OSA subjects.

Although the precise cellular source of ROS in the upper airway is unknown, Saul et al¹⁰ showed that inflammation is present in the soft palate of patients with OSA. Furthermore, Zakkar et al³⁵ demonstrated a decrease in the concentration of neutral endopeptidase (which cleaves proinflammatory peptides) in the uvula of patients with OSA compared to control subjects. It has been suggested that an imbalance between oxidants and antioxidants may lead to atherogenesis, and that this may account for the several chronic cardiovascular complications that frequently are related to OSA.^{36,37}

In confirmation of our previous results, we observed a positive correlation between morning exhaled 8-isoprostane concentrations and neck circumference, suggesting that the measurement of exhaled oxidative stress markers may be useful in screening obese subjects who are at high risk of developing sleep apnea and in monitoring the progression of this syndrome.

The lack of correlation of exhaled 8-isoprostane concentration with the percentage of TST oxyhemoglobin saturation at < 90% and ODI, in contrast to the positive correlation observed with AHI, argues against a role for hypoxia as a possible causative factor of the increase of the oxidative stress.⁴ However, it is possible that there could be a relationship between 8-isoprostane and the number of oxygen desaturation episodes, rather than their severity.

In the present study, we repeated measurements

of exhaled 8-isoprostane levels in OSA subjects before sleeping (*ie*, 8:00 PM) and at waking (8:00 AM), observing higher levels of this marker at 8:00 AM but observing their reduction after 12 h of being awake. These findings are consistent with previous observations, according to which the nocturnal apneas, through repeated hypoxia as a result of the mechanic obstruction, could be responsible for oxidative stress in patients with moderate-to-severe OSA.³

The rapid reduction of 8-isoprostane that we observed in OSA patients after CPAP therapy might be a further advantage of this therapy.³⁷ In this respect, our findings are consistent with those of Schulz et al,⁴ who also demonstrated a beneficial effect of CPAP on oxidative stress.

There are some potential limitations to our study that deserve comment. Due to the relatively small number of subjects included in the response to CPAP therapy, our conclusions require confirmation in a larger series of OSA patients. Nevertheless, in our study each subject served as his/her own control, and the changes in 8-isoprostane levels before and after sleeping or during CPAP treatment were still present after sleep.

In conclusion, oxidative stress, as measured by 8-isoprostane levels, was elevated in the airways and plasma of patients with OSA and was reduced by CPAP therapy. The concentration of 8-isoprostane increased after nocturnal apnea and was significantly reduced after CPAP therapy. Oxidative stress could have a key role in the link between OSA and the increased risk of cardiovascular diseases. Measurement of 8-isoprostane in the exhaled breath conden-

sate of these patients therefore may help to identify patients with a higher risk of developing cardiovascular diseases.

REFERENCES

- 1 Deegan PC, McNicholas WT. Predictive value of clinical features for the obstructive sleep apnoea syndrome. *Eur Respir J* 1996; 9:117–124
- 2 Marrone O, Bonsignore MR, Insalaco G, et al. What is the evidence that obstructive sleep apnoea is an important illness? *Monaldi Arch Chest Dis* 1998; 53:630–639
- 3 Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *Clin Endocrinol Metab* 1997; 82:1313–1316
- 4 Schulz R, Mahmoudi S, Hattar K, et al. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. *Am J Respir Crit Care Med* 2000; 162:566–570
- 5 Morrison DL, Launois SH, Isono S. Pharyngeal narrowing and closing pressures in patients with obstructive sleep apnea. *Am Rev Respir Dis* 1993; 148:606–611
- 6 Wynne JW. Obstruction of the nose and breathing during sleep. *Chest* 1982; 26:57–58
- 7 Rubinstein I, Slutsky AS, Zamel N, et al. Paradoxical glottic narrowing in patients with severe obstructive apnea. *J Clin Invest* 1988; 81:1051–1055
- 8 McNicholas WT, Tarlo S, Cole P, et al. Obstructive apneas during sleep in patients with seasonal allergic rhinitis. *Am Rev Respir Dis* 1982; 126:625–628
- 9 Leznoff A, Haight JS, Hoffstein V. Reversible obstructive sleep apnea caused by occupational exposure to guar gum dust. *Am Rev Respir Dis* 1986; 133:935–936
- 10 Saul S, Kimmelman CP, LiVolsi VA, et al. Histopathology of sleep apnea. *Trans Am Laryngol Assoc* 1988; 109:222–225
- 11 Carpagnano GE, Kharitonov SA, Resta O, et al. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnea patients. *Chest* 2002; 122:1162–1167
- 12 Kharitonov SA, Barnes PJ. Exhaled markers of inflammation. *Curr Opin Allergy Clin Immunol* 2001; 1:217–224
- 13 Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* 2001; 163:1693–1722
- 14 Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: US Government Printing Office, 1986; NIH Publication No. 204
- 15 Johns MW. Rethinking the assessment of sleepiness. *Sleep Med Rev* 1998; 2:3–15
- 16 Johns MW. Reliability and factor analysis of the Epworth Sleepiness Scale. *Sleep* 1992; 15:376–381
- 17 Montuschi P, Kharitonov SA, Ciabattini G, et al. Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. *Thorax* 2000; 55:205–209
- 18 Bland JM, Altman DG. Statistics notes: measurement error. *BMJ* 1996; 313:744
- 19 Janssen LJ. Isoprostanes: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol* 2001; 280:L1067–L1082
- 20 Montuschi P, Ciabattini G, Paredi P, et al. 8-isoprostane as a biomarker of oxidative stress in interstitial lung diseases. *Am J Respir Crit Care Med* 1998; 158:1524–1527
- 21 Brown RK, Kelly FJ. Evidence for increased oxidative damage in patients with cystic fibrosis. *Pediatr Res* 1994; 36:487–493
- 22 Pratico D, Basili S, Vieri M, et al. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F₂α-III, an index of oxidant stress. *Am J Respir Crit Care Med* 1998; 158:1709–1714
- 23 Montuschi P, Corradi M, Ciabattini G, et al. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999; 160:216–220
- 24 Montuschi P, Collins JV, Ciabattini G, et al. Exhaled 8-isoprostane as an *in vivo* biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000; 162:1175–1177
- 25 Carpenter CT, Price PV, Christman BW. Exhaled breath condensate isoprostanes are elevated in patients with acute lung injury or ARDS. *Chest* 1998; 114:1653–1659
- 26 Morrison DL, Launois SH, Isono S. Pharyngeal narrowing and closing pressure in patients with obstructive sleep apnea. *Am Rev Respir Dis* 1993; 148:606–611
- 27 Wynne JW. Obstruction of the nose and breathing during sleep. *Chest* 1982; 82:657–658
- 28 Reimund E. The free radical theory of sleep. *Med Hypotheses* 1994; 43:231–233
- 29 Wali SO, Bahammam AS, Massaeli H, et al. Susceptibility of LDL to oxidative stress in obstructive sleep apnea. *Sleep* 1999; 2:290–295
- 30 Khalid MA, Ashraf M. Direct detection of endogenous hydroxyl radical production in cultured adult cardiomyocyte during anoxia and reoxygenation: is the hydroxyl radical really the most damaging radical species? *Circ Res* 1993; 72:725–736
- 31 Kutryk MJB, Pierce GN. Effects of low density lipoprotein on calcium movements in cultured vascular smooth muscle cells. *Coron Artery Dis* 1991; 2:1093–1101
- 32 Singal PK, Kapur N, Dhilon KS, et al. Role of free radicals in catecholamine-induced cardiomyopathy. *Can J Physiol Pharmacol* 1982; 60:1390–1397
- 33 Kresinun VI, Rozhkovskij JV. Disruption of the structural organization of brain mitochondrial membranes during deprivation of the paradoxical sleep phase and correction for it using lithonite. *Ukr Biokhim Zh* 1991; 63:59–65
- 34 Ip MSM, Lam B, Chan LY, et al. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med* 2000; 162:2166–2171
- 35 Zakkar M, Sekosan M, Wenig B, et al. Decrease in immunoreactive neutral endopeptidase in uvula mucosa epithelium of patients with obstructive sleep apnea. *Ann Otol Rhinol Laryngol* 1997; 106:474–477
- 36 Barcelo A, Miralles C, Barbe F, et al. Abnormal lipid peroxidation in patients with sleep apnoea. *Eur Respir J* 2000; 16:644–647
- 37 He J, Kryger MH, Zorick FJ, et al. Mortality and apnea index in obstructive sleep apnea (experience in 385 male patients). *Chest* 1988; 94:9–14