Exhaled Pentane and Nitric Oxide Levels in Patients With Obstructive Sleep Apnea*

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Background: Upper airway inflammation is present in patients with obstructive sleep apnea (OSA).

Objective: To determine whether exhaled pentane and nitric oxide (NO) levels, two nonspecific markers of inflammation, are increased in patients with OSA.

Methods: Exhaled nasal and oral pentane and NO levels were determined before and after sleep in 20 patients with OSA (apnea-hypopnea index, 48±7; mean±SEM) and eight healthy control subjects.

Results: In patients with OSA, exhaled nasal and oral pentane levels after sleep were significantly higher than presleep values (6.1±1.2 nM vs 3.4±0.4 nM, and 7.0±1.3 nM vs 4.2±0.4 nM, respectively; p<0.05). Likewise, exhaled nasal and oral NO levels after sleep were significantly higher than presleep values in patients with OSA (39.7±3.8 ppb vs 28.4±2.9 ppb and 10.9±1.5 ppb vs 6.6±0.8 ppb, respectively; p<0.05). By contrast, there were no significant differences in exhaled nasal and oral pentane, and nasal NO levels before and after sleep in control subjects. Exhaled oral NO levels were significantly increased after sleep in comparison to presleep values in control subjects (p<0.05).

Conclusion: Exhaled nasal pentane and NO levels are increased after sleep in patients with moderate-severe OSA. These data suggest that upper airway inflammation is present in these patients after sleep.

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Key words: airway; inflammation; lipid peroxidation; nose; oxidants

Abbreviations: NO=nitric oxide; OSA=obstructive sleep apnea; ppb=parts per billion

It is estimated that 2% of women and 4% of men in the United States suffer from obstructive sleep apnea (OSA).1 This condition is characterized by repetitive episodes of upper airway obstruction during sleep leading to significant hypoxemia and frequent arousals from sleep.1 OSA is increasingly recognized as a major public health concern because it is associated with increased risk of developing cardiovascular complications, such as hypertension, myocardial infarction, and cerebrovascular accidents.1-4

Although the etiology of OSA is uncertain, mechanical obstruction of the upper airway during sleep characterizes this disorder.5-8 A large body of experimental evidence indicates that upper airway structure and function are altered in patients with OSA.8-12 Local pathophysiologic processes that could amplify these abnormalities may further compromise upper airway patency during sleep. One of these processes is upper airway inflammation, because it is associated with upper airway mucosal congestion.9,10

The presence of upper airway inflammation has been described recently in patients with OSA.9,10,12-16 Whether the presence of upper airway inflammation plays a role in the natural history of OSA is uncertain. We hypothesized that simple, noninvasive measures of upper airway inflammation could constitute the first step in addressing this issue.
To this end, determinations of exhaled pentane and nitric oxide (NO) levels have been suggested as simple, noninvasive, and reproducible markers of inflammation in human subjects.17–23

Hence, the purpose of this study was to determine exhaled pentane and NO levels in patients with OSA and healthy control subjects before and after sleep.

**MATERIALS AND METHODS**

**Study Population**

All subjects gave written informed consent for participation in the study as approved by the Committee for the Protection of Human Subjects from Research Risks at the University of Illinois at Chicago. All denied history of respiratory diseases, rhinitis, sinusitis, and none was receiving anti-inflammatory medications or smoked cigarettes within 3 months of enrollment into the study.

**Polysomnography**

Each subject underwent overnight polysomnography using standard techniques as previously described.26 Analysis and interpretation of the sleep studies were done using standard criteria.26

**Collection of Exhaled Air**

Four samples of exhaled air were collected from each subject: two immediately before sleep (about 10 PM) and two immediately after sleep (about 7 AM). Each subject, while sitting and wearing nose clips, was instructed to slowly inspire to total lung capacity and then slowly exhale to residual volume as previously described.25 The expired gas was collected through a mouthpiece into an impermeable 5-L collection bag (Tedlar; SKC; Eighty-Four, Pa). This procedure was repeated while the patient exhaled through the nose into the collection bag via a nasal continuous positive airway pressure mask (Respironies Inc; Murrysville, Pa). A sample of ambient air was collected simultaneously with the subject's samples and during sleep.

**Determination of Pentane Level**

A sample of the exhaled gas and ambient air was analyzed for pentane content using gas chromatography (model 3400; Varian; Sunnyvale, Calif) as previously described.17 The chromatogram was standardized with commercially prepared gases over a range of nil (pure air) to 100 nM pentane (Scott Specialty Gases Inc; Plumsteadville, Pa).

**Determination of NO Level**

The collected exhaled gas and ambient air were analyzed for NO levels using a chemiluminescence analyzer (model 425; Thermo Environmental Instruments Inc; Franklin, Mass) through a 40-cm narrow-bore Teflon-coated tubing as previously described.25 NO levels were recorded on a strip-chart recorder. The analyzer was calibrated daily using ultra-pure air and NO over a range of 0 to 150 parts per billion (ppb) (Scott Specialty Gases Inc).

**Statistical Analysis**

Data are expressed as mean±SEM. Statistical analysis was performed using the Wilcoxon signed-rank test. A p<0.05 was considered statistically significant.

**RESULTS**

**Anthropometric Data**

Anthropometric data are summarized in Table 1. Twenty patients had moderate-severe OSA (apnea-hypopnea index, 48±7) and eight healthy individuals served as control subjects (apnea-hypopnea index, 1±1). There were 12 men and eight women in the OSA group, and five men and three women in the control group. Although patients with OSA were older than control subjects, the difference was not statistically significant (42±2 years and 33±3 years, respectively; p>0.05). The weight of patients with OSA was significantly greater than that of control subjects (41±2 kg/m² vs 27±1 kg/m², respectively; p<0.05). Patients with OSA had significantly lower arterial oxygen saturation nadir during sleep in comparison to control subjects (79±3% vs 95±3%, respectively; p<0.05).

**Exhaled Pentane Level**

In patients with OSA, morning (AM) nasal pentane level was significantly higher than the presleep (PM) level (6.1±1.2 nM vs 3.4±0.4 nM, respectively; p<0.05; Fig 1). AM oral pentane level was also significantly higher than the PM level (7.0±1.3 nM vs 4.2±0.4 nM, respectively; p<0.05; Fig 1). By contrast, there was no significant difference between AM and PM nasal pentane levels (4.2±0.7 nM vs 3.1±0.9 nM, respectively; p>0.05) and in AM and PM oral pentane levels (4.7±1.1 nM vs 3.1±0.6 nM; p>0.05) in control subjects. No pentane was detected in ambient air either before or after sleep.

**Exhaled NO Level**

Exhaled NO level was not determined in four patients with OSA. In the remaining 16 patients, nasal NO level was significantly higher after sleep (AM) in comparison to the presleep (PM) values (39.7±3.8 ppb vs 28.4±2.9 ppb, respectively; p<0.05; Fig 2). Likewise, AM oral NO level was significantly higher than PM NO level (10.9±1.5 ppb vs 5.9±1.4 ppb, respectively; p<0.05). Table 1...

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<th>Table 1—Anthropometric Data*</th>
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<td><strong>Patients with OSA</strong></td>
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*BMI=body mass index; AHI=apnea-hypopnea index; Low SaO₂=lowest nocturnal transcutaneous hemoglobin saturation. *p<0.05 in comparison to control subjects.
vs 6.6±0.8 ppb, respectively; p<0.05; Fig 2). In control subjects, there was no significant difference between AM and PM nasal NO levels (32.1±5.2 ppb vs 22.8±5.5 ppb, respectively; p>0.05; Fig 2). However, there was a significant difference between AM and PM oral NO levels (16.9±6.2 ppb vs 6.8±1.3 ppb, respectively; p<0.05; Fig 2). Ambient NO levels before and after sleep were 16.1±3.5 ppb and 26.1±6.8 ppb, respectively (p<0.05).

**DISCUSSION**

The results of this study show that in patients with moderate-severe OSA, exhaled nasal and oral pentane and NO levels were significantly increased after sleep in comparison to presleep levels. By contrast, in control subjects, exhaled nasal and oral pentane levels were similar before and after sleep. Exhaled oral, but not nasal, NO levels in control subjects were increased after sleep in comparison to presleep values. Collectively, these data suggest the presence of upper airway inflammation after sleep in patients with moderate-severe OSA.

Current concepts suggest that reactive oxygen species induce tissue damage by attacking membrane lipids and proteins. Pentane is the most common product of polyunsaturated membrane fatty acid peroxidation by reactive oxygen species and is readily excreted through the lungs. Exhaled pentane has been suggested as a simple, objective, noninvasive marker of inflammation. For instance, exhaled pentane levels are increased in conditions associated with airway inflammation, such as cystic fibrosis and smoking. Moreover, Olopade et al showed recently that exhaled pentane levels are increased in patients with acute asthma and decrease significantly once acute asthma has subsided. The results of this study support and extend these observations by showing that exhaled nasal and oral pentane levels are increased after sleep in patients with OSA but not in control subjects. Overall, these data suggest that sleep is associated with airway inflammation in patients with moderate-severe OSA.

NO is produced by NO synthase in a reaction that converts L-arginine and oxygen to citrulline and NO. Although the precise cellular source of...
NO in the upper and lower airway is unknown, several cells have been implicated recently, including the lining epithelial cells. Exhaled NO levels are elevated in conditions associated with airway inflammation, such as asthma and bronchiectasis. We found higher nasal and oral NO levels after sleep in patients with moderate-severe OSA. These data corroborate the findings of elevated nasal and oral pentane levels in these patients and suggest the presence of airway inflammation. However, we observed a significant increase in oral NO after sleep compared to before sleep in control subjects while such an increase was not observed with pentane.

The level of exhaled oral NO in smokers is low in comparison to nonsmokers, and oral NO levels correlate with the severity of airflow obstruction. Damage to NO-producing bronchiolar epithelium has been suggested as a cause for the low levels of oral NO in smokers. Similar to the observation in smokers, we found lower oral NO levels in patients with OSA in comparison to control subjects before and after sleep. We postulate that in patients with OSA, increased levels of reactive oxygen species, as demonstrated by elevated oral pentane levels, may have damaged NO-producing airway epithelial cells leading to lower oral NO levels. We attribute the higher nasal NO levels in patients with OSA, despite the presence of reactive oxygen species, to local production of NO in the paranasal sinuses as previously described by other investigators. The higher nasal and oral NO levels in control subjects is in keeping with observation by Kanazawa et al. that in nonsmokers, exhaled NO levels tend to be higher. Collectively, these data suggest that exhaled nasal pentane and NO levels could be used as simple, noninvasive markers of upper airway inflammation after sleep in patients with moderate-severe OSA. The mechanisms underlying sleep-related increase in these parameters, such as recurrent hypoxemia and mechanical trauma to the upper airway, were not determined in this study and warrant further investigation.

There are some inherent limitations to our study. For instance, ambient pentane and NO levels could potentially affect the results. However, we found no detectable pentane in ambient air. Although NO was detected in ambient air of the sleep laboratory, Kharitonov et al. and Massaro et al. showed that ambient NO level had no significant effects on exhaled NO determinations in patients with asthma, a condition associated with airway inflammation. The contribution of extrapulmonary organs to exhaled pentane and NO levels in our subjects was minimized by excluding individuals with other known inflammatory conditions. We did not correlate exhaled pentane and NO levels with other indexes of inflammation in our patients. However, Rubinstein showed that nasal inflammation is present in patients with OSA and that this inflammation is amplified after sleep. In addition, Sekosan et al. showed that inflammation is present in the soft palate of patients with OSA. Collectively, these data suggest that the increase in nasal and oral pentane and NO levels observed after sleep in patients with moderate-severe OSA could be related, in part, to upper airway inflammation. Our findings are in keeping with the demonstration by Zakker et al. of decreased concentrations of neutral endopeptidase that cleave proinflammatory peptides in the uvula mucosa of patients with OSA in comparison to control subjects.

We did not determine whether obesity per se in the absence of OSA can lead to elevated levels of exhaled pentane and NO. Nevertheless, each subject served as his/her control subject and the changes in exhaled pentane and NO levels before and after sleep were still suggestive of an inflammatory process worsened after sleep. Clearly, additional studies are necessary to determine if hypoxemia and/or obesity, alone or in combination, can lead to increased levels of exhaled pentane and NO. It will also be essential to determine whether treatment of patients with OSA with nasal continuous positive airway pressure can lead to a reduction in the levels of exhaled pentane and NO.

In summary, we found that exhaled nasal and oral pentane and NO levels are significantly increased after sleep in patients with moderate-severe OSA. We conclude that these changes are suggestive of sleep-related upper airway inflammation and indicate that additional local factors contribute to upper airway obstruction in patients with OSA.

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REFERENCES

30 Morita S, Snider MT, Inada Y. Increased N-pentane excretion in humans: a consequence of pulmonary oxygen exposure. Anesthesiology 1986; 64:730-33