Evidence for Lipid Peroxidation in Obstructive Sleep Apnea

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INTRODUCTION

OBSTRUCTIVE SLEEP APNEA SYNDROME (OSA) THAT IS ASSOCIATED WITH CARDIOVASCULAR MORBIDITY HAS ALSO BEEN SHOWN TO BE ASSOCIATED WITH INCREASED OXIDATIVE STRESS AND A STATE OF INFLAMMATORY CELL ACTIVATION.1-3 It has been suggested that the apnea-induced hypoxic stress in OSA initiates atherogenesis, which eventually leads to cardiovascular disorders.4 Oxidative modification of low-density lipoproteins (LDL) in the arterial wall is a key feature of atherogenesis and is widely believed to cause and/or accelerate lesion development.5 Quantification of thiobarbituric reactive substances (TBARS) and peroxides (PD) in plasma is one of the most common methods used to assess lipid peroxidation.6,7 It has been reported that plasma lipid peroxidation is significantly higher in patients with atherosclerosis in comparison with controls8,9 and after acute ischemic stroke.10 Furthermore, increased TBARS concentrations in plasma have predicted restenosis after coronary balloon angioplasty.11 However, this information in OSA is very limited and inconsistent. While Wali et al12 failed to identify abnormal lipid peroxidation in a small group of OSA patients, Barcelo et al13 showed increased levels of TBARS, which were reduced by 9 months of treatment with nasal-continuous positive airway pressure (nCPAP). Identification of increased concentrations of autoantibodies against oxidized LDL in OSA patients further supports the notion of increased lipid peroxidation in these patients.14

Protective mechanisms that prevent LDL oxidation, such as paraoxonase-1 (PON1) activity also play a role in atherogenesis and, thus, are relevant to OSA as well. Paraoxonase-1, a calcium-dependent esterase, physically associated with high-density lipoproteins (HDL), has been shown to protect both LDL and HDL against lipid peroxidation15 and, thereby, is postulated to at least partially protect against atherosclerosis. Serum PON1 activity has been shown to be reduced in coronary artery disease, in patients with familial hypercholesterolemia, and in diabetes mellitus.16,17 Moreover, PON1 activity has been shown to selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions.18 After myocardial infarction (MI), PON1 activity decreased dramatically, suggesting that PON1 may be a predictive risk factor for MI.20 Thus, we hypothesized that while TBARS and PD would be increased in patients with OSA, the levels of serum PON1 would be decreased in comparison with those in nonapneic controls; we also hypothesized that nCPAP treatment would decrease lipid peroxidation.

METHODS

Experiment 1

Participants

We investigated fasting levels of TBARS, PD, and PON1 in a total of 144 participants. These included 114 patients with untreated OSA, of whom 59 had cardiovascular disease (CVD) (hypertension, ischemic heart disease [IHD], or a history of myocardial infarction or stroke), 55 had untreated OSA but were without CVD, and 30 were controls without OSA. Patients with either hypertension, IHD, or a history of MI or stroke were designated as +OSA/+CVD, whereas patients without cardiovascular comorbidity were designated +OSA/-CVD. Normal controls were recruited from the population referred to the sleep laboratory with suspected sleep apnea and were found to have a respiratory disturbance...
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Thiobarbituric Reactive Substances

The TBARS assay was based on the method by Buege and Aust.\textsuperscript{21} Malonaldehyde (MDA) was dissolved in tetramethoxy propane, which decomposes under assay conditions to give MDA and is used as the standard in the range of 0.0-7.50 nmol of MDA in 500 µL. One milliliter of TBARS working solution (0.26M TBA) was added to samples or standard tubes, then heated for 20 minutes at 100°C, and centrifuged at 1000 x g for 10 minutes. The absorbance of the supernatant was read spectrophotometrically at 532 nm against the blank. The data are expressed as nmol MDA/mL plasma.

Lipid Peroxides

The PD assay was based on the method by El-Saadani et al.\textsuperscript{22} One milliliter of color reagent (CHOD-iodide-Merk cat. no 14106) was added to 100 µL diluted plasma samples, vortexed, and let stand for 30 minutes in the dark. Absorbance was read at 365 nm against the color reagent as the blank. Data are expressed as nmol PD/mL plasma.

Paraoxonase 1-arylesterase Activity

Serum arylesterase activity was measured spectrophotometrically at 270 nm with phenyl acetate as the substrate. The assay mixture consisted of 1 mmol/L of phenyl acetate and 0.9 mmol/L CaCl\textsubscript{2} in 20 mmol/L Tris HCl, pH of 8.0, at 25°C. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The data are presented as Units per minute per milliliter of serum. One unit of arylesterase activity is equal to 1 µmol of phenyl acetate hydrolyzed per minute per milliliter of serum.\textsuperscript{23}

Blood Chemistry

The lipid profile (total cholesterol, LDL and HDL cholesterol, and triglycerides), glucose, and creatinine in serum were determined by routine laboratory techniques using a Vitros 250 Chemistry System (Johnson & Johnson Clinical Diagnostics; Rochester, NY) by the American Medical Laboratories, Israel.

Statistical Analysis

In Experiment 1, univariate analysis was used to compare the demographic and clinical data of the 3 groups: +OSA/-CVD, +OSA/+CVD, and controls. Categorical data were analyzed by χ\textsuperscript{2} or Fisher exact probability tests. Continuous variables were analyzed by the Kruskal-Wallis test. Spearman rank-order correlations were calculated between the lipid-peroxidation biomarkers and the demographic and clinical data. Correlations with sleep-apnea variables were calculated only for patients with OSA. Then, stepwise regression analysis using the SAS software package version 8.1 was used to determine the contribution of RDI and SaO\textsubscript{2} levels to the lipid oxidation biomarkers concentrations. The natural log (Ln) of TBARS, PD, and RDI and the square root of BMI were used in order to improve normality of TBARS, PD, RDI, and BMI. In Experiment 2, independent and dependent t tests were used to compare the mean nocturnal levels of LnTBARS and LnPD between patients with OSA and controls before and after nCPAP treatment.

RESULTS

Univariate Analysis

Univariate analysis revealed that the 3 groups differed significantly in age (P < .0001), BMI (P < .0003), diabetes mellitus (P < .0004), hypercholesterolemia (P < .03), and drug use (P < .0001). The groups also differed significantly in cholesterol (P < .02), glucose (P < .004), TBARS (P < .0001), PD (P < .0003), and PON1 (P < .002) (Table 1a and 1b). The +OSA/-CVD group was more obese (P < .01); had higher cholesterol (P < .006), triglyceride (P < .03), TBARS (P < .0003), and

Blood Collection

Ten milliliters of venous blood were obtained from each patient after an overnight fast. Blood samples were collected in precooled EDTA-containing tubes (vacutainers; Beckton-Dickinson, Franklin Lakes, NJ, US) and were kept on ice. Serum was obtained by centrifugation at 1,000 x g for 10 minutes. The absorbance of the supernatant was read spectrophotometrically at 532 nm against the blank. The data are expressed as nmol PD/mL plasma.

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Lipid Peroxidation Assays

Lipid peroxidation for TBARS and PD assays was induced by incubating diluted plasma (at 1:4 with PBS final volume of 1 mL), with 100 µL of 67 mmol of AAPH (2,2-azobis[2-amidinopropane] dihydrochloride) for 2 hours at 37°C. The control tube was incubated without the AAPH. The samples were then refrigerated, after which TBARS and PD were determined.

Experiment 2

Participants

Fifteen male subjects participated in this experiment: 9 had OSA, and 6 were of similar age and BMI as the men with OSA and were referred to the sleep laboratory because of suspected OSA but were found to be only habitual snorers with a few scattered hypopneas. Subjects were instructed to avoid consuming alcohol and coffee beverages during the day. At 7:00 PM, the room lights were turned off. Blood samples were collected from 11:00 PM until 6:20 AM, the room lights were turned on. Blood samples were collected in precooled EDTA-containing tubes (vacutainers; Beckton-Dickinson, Franklin Lakes, NJ, US) and were kept on ice. Plasma was separated within an hour in a refrigerated centrifuge at 1,000 x g for 10 minutes. The absorbance of the supernatant was read spectrophotometrically at 365 nm against the color reagent (CHOD-iodide-Merk cat. no 14106) was added to 100 µL diluted plasma samples, vortexed, and let stand for 30 minutes in the dark. Absorbance was read at 365 nm against the color reagent as the blank. Data are expressed as nmol PD/mL plasma.

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Serum arylesterase activity was measured spectrophotometrically at 270 nm with phenyl acetate as the substrate. The assay mixture consisted of 1 mmol/L of phenyl acetate and 0.9 mmol/L CaCl\textsubscript{2} in 20 mmol/L Tris HCl, pH of 8.0, at 25°C. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The data are presented as Units per minute per milliliter of serum. One unit of arylesterase activity is equal to 1 µmol of phenyl acetate hydrolyzed per minute per milliliter of serum.\textsuperscript{23}
used statins, aspirin, diuretics, and antihypertensive medications.

and aspirin (n = 1); drugs used in group +OSA/-CVD included statins (n = 30) and aspirin (n = 1); drugs used in group +OSA/+CVD were significantly older (P < .002), TBARS (P < .001), and PD (P < .05). They also had higher rates of diabetes mellitus (P < .002), drug use (P < .001), and hypercholesterolemia (P < .03). Patients with CVD also had higher concentrations of glucose (P < .04) and tended to have lower levels of PON1 (P < .06).

Drug use by the controls included hypnotics (n = 3), statins (n = 2), and aspirin (n = 1); drugs used in group +OSA/-CVD included statins (n = 8) and aspirin (n = 3). Most of the patients in the +OSA/+CVD group used statins, aspirin, diuretics, and antihypertensive medications.

### Table 1a—Demographic and clinical data for control subjects and patients with obstructive sleep apnea, with and without cardiovascular disease

<table>
<thead>
<tr>
<th>Controls (n = 30)</th>
<th>Subject Group</th>
<th>+OSA-CVD (n = 55)</th>
<th>+OSA+CVD (n = 59)</th>
<th>Controls vs +OSA-CVD vs +OSA+CVD</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean ± SD</td>
<td>42.9 ± 13.8</td>
<td>46.8 ± 10.2</td>
<td>58.5 ± 11.3</td>
<td>.0001</td>
<td>.0001</td>
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<td>BMI, kg/m², mean ± SD</td>
<td>26.0 ± 3.2</td>
<td>28.4 ± 3.5</td>
<td>30.6 ± 5.5</td>
<td>.0003</td>
<td>.05</td>
</tr>
<tr>
<td>Men, %</td>
<td>90.0</td>
<td>85.5</td>
<td>83.0</td>
<td>NS</td>
<td>.0001</td>
</tr>
<tr>
<td>HHD, %</td>
<td>3.3</td>
<td>0.0</td>
<td>0.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MI, %</td>
<td>0.0</td>
<td>3.4</td>
<td>.40</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HTN, %</td>
<td>13.3</td>
<td>0.0</td>
<td>7.95</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>1.8</td>
<td>0.0</td>
<td>20.3</td>
<td>.0004</td>
<td>.002</td>
</tr>
<tr>
<td>Drug use, %</td>
<td>40.0</td>
<td>25.4</td>
<td>93.1</td>
<td>.0001</td>
<td>.0001</td>
</tr>
<tr>
<td>Smoking history, %</td>
<td>Never</td>
<td>54.6</td>
<td>55.9</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td></td>
<td>Current</td>
<td>27.3</td>
<td>18.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>13.3</td>
<td>23.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterol, %</td>
<td>16.7</td>
<td>20.0</td>
<td>39.0</td>
<td>.03</td>
<td>.03</td>
</tr>
<tr>
<td>Other diseases, %</td>
<td>23.3</td>
<td>14.1</td>
<td>35.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RDI, mean ± SD</td>
<td>8.2 ± 2.6</td>
<td>26.9 ± 13.8</td>
<td>31.3 ± 18.5</td>
<td>.0001</td>
<td>.0005</td>
</tr>
<tr>
<td>% time O₂&lt;90, mean ± SD 1.1 ± 2.8</td>
<td>13.1 ± 23.6</td>
<td>13.4 ± 19.9</td>
<td>.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*OSA-CVD/Refers to patients with obstructive sleep apnea and without cardiovascular disease; +OSA/CVD, patients with obstructive sleep apnea and with cardiovascular disease; BMI, body mass index, calculated as weight in kilograms divided by the square of height in meters; HHD, ischemic heart disease; MI, history of myocardial infarction; HTN, hypertension; RDI, respiratory disturbance index—the total number of apneas plus hypopneas divided by the hours of sleep; % time O₂<90, percentage of time asleep with an SaO₂ less than 90%.

*Categorical variables were compared by χ² tests and continuous variables by the Kruskal-Wallis test. All P values are 2-tailed. Ellipses indicate P value not computed.

Other diseases include, control group: osteoporosis (n = 1), back problems (n = 2), vasomotor rhinitis (n = 2), liver disease (n = 1), and asthma (n = 2); +OSA/CVD group: Parkinson disease (n = 1), asthma (n = 4), liver disease (n = 2), kidney disease (n = 3), other (n = 11); +OSA+CVD group: asthma (n = 3), kidney disease (n = 1), cancer (n = 1), back problems (n = 2), vasomotor rhinitis (n = 2)

### Table 1b—Biochemical data for control subjects and patients with obstructive sleep apnea, with and without cardiovascular disease

<table>
<thead>
<tr>
<th>Controls (n = 30)</th>
<th>Subject Group</th>
<th>+OSA-CVD (n = 55)</th>
<th>+OSA+CVD (n = 59)</th>
<th>Controls vs +OSA-CVD vs +OSA+CVD</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/dL</td>
<td>181.3 ± 30.5</td>
<td>204.4 ± 34.9</td>
<td>195.7 ± 37.5</td>
<td>.02</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>135.4 ± 57.5</td>
<td>174.4 ± 75.5</td>
<td>166.2 ± 89.9</td>
<td>.10</td>
<td>NS</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>41.6 ± 10.3</td>
<td>43.1 ± 7.5</td>
<td>44.9 ± 12.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>112.5 ± 27.1</td>
<td>126.4 ± 32.4</td>
<td>118.7 ± 33.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>92.9 ± 7.9</td>
<td>98.6 ± 12.6</td>
<td>109.1 ± 24.7</td>
<td>.004</td>
<td>.04</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TBARS, nmol MDA/mL</td>
<td>12.9 ± 3.5</td>
<td>17.2 ± 6.3</td>
<td>18.6 ± 7.3</td>
<td>.0001</td>
<td>.0003</td>
</tr>
<tr>
<td>PD, nmol/mL</td>
<td>818.0 ± 126.7</td>
<td>901.2 ± 103.9</td>
<td>906.5 ± 132.1</td>
<td>.0003</td>
<td>.0001</td>
</tr>
<tr>
<td>PON1, U/min·mL⁻¹</td>
<td>92.1 ± 14.4</td>
<td>86.7 ± 17.6</td>
<td>79.5 ± 13.6</td>
<td>.002</td>
<td>.06</td>
</tr>
</tbody>
</table>

HDL refers to high-density lipoprotein; LDL, low-density lipoprotein; TBARS, thiobarbituric reactive substances; PD, peroxides; PON1, paraoxonase-1

*Data are presented as mean ± SD. Variables were compared by the Kruskal-Wallis test. All P values are 2-tailed.

### Correlations and Stepwise Regression Analysis

Both TBARS and PD significantly increased with increasing RDI values (TBARS: r = .43, P < .0001 and PD: r = .36, P < .0002), while PON1 showed the opposite (r = -.24, P < .01). The corresponding correlations with percentage of time below 90% SaO₂ and with minimum nocturnal SaO₂ were r = .30 (P < .002), r = .24 (P < .01), and r = -.14 (P < .14) and r = -.24 (P < .01), r = -.18 (P < .06), and r = .16 (P < .09), respectively. In addition, TBARS was significantly correlated with age (r = .22, P < .02), glucose (r = .29, P < .002) and cholesterol (r = .21, P < .03) levels; PD was only significantly correlated with glucose (r = .19, P < .04); and PON1 was only correlated with cholesterol (r = .19, P < .04).

Logistic regression analysis using the STEPSWISE option of the SAS package was performed to determine the predictors of lipid peroxidation biomarkers. Using the INCLUDE option, the variables sex, age, and the square root of BMI were first forced into the model, and the following variables were then added—RDI, drug use; hypercholesterolemia; and cholesterol, triglyceride, creatinine, LDL, and glucose levels—to predict Ln of TBARS, Ln of PD, and PON1. The retention criterion was .05 (Table 2). Sex, age, and the square root of BMI accounted together for 5.45% of the total variance in Ln TBARS. In addition, Ln RDI, hypercholesterolemia, and creatinine were entered into the model. The Ln RDI accounted for 19.60% of the total variance in Ln TBARS. For Ln PD, age, sex, and the square root of BMI accounted for 3.33% of the variance, and from the added variables, only Ln RDI was retained in the model, accounting for an additional 5.34% of the variance. Similarly, only Ln RDI was retained in the PON1 model, accounting for 6.45% of the variance. Similar analysis was performed after replacing RDI with percentage of time spent below 90% SaO₂ and with minimal nocturnal SaO₂ level, modeled as either continuous variables or in quartiles. While these analyses confirmed that SaO₂ is a significant predictor of lipid peroxidation biomarkers, neither of these analyses improved upon the models that used RDI (data not shown).
Treatment with nCPAP

Demographic and clinical data of patients with OSA and controls are presented in Table 3. There were no significant differences between patients and controls in age (mean ± SD, 43.70 ± 9.20 years, NS) or in BMI (29.90 ± 4.02 kg/m² vs 27.40 ± 2.09 kg/m², NS). Figure 1 presents the overnight TBARS and PD concentrations in patients with OSA and controls and in the 5 patients with OSA who were treated with nCPAP. Patients with OSA had significantly higher nocturnal plasma TBARS and PD concentrations than controls (whole night mean ± SD, TBARS: 21.00 ± 6.70 nmol MDA/mL vs 9.90 ± 1.10 nmol MDA/mL, P < .003; PD: 1086.40 ± 67.00 nmol PD/mL vs 826.40 ± 102.50 nmol PD/mL, P < .00008). Treatment with nCPAP significantly decreased both TBARS (17.00 ± 6.10 nmol MDA/mL vs 9.60 ± 2.70 nmol MDA/mL, P < .0001) and PD (1125.80 ± 56.50 nmol PD/mL vs 946.00 ± 64.00 nmol PD/mL, P < .0003) concentrations. Only the 5 patients tested twice with and without nCPAP were included in this comparison. We should also note that sleep efficiency, that is, the percentage of total sleep time out of total time in bed, was about the same in all groups, ranging from 67.3% to 76.0%. Posttreatment TBARS and PD levels were not significantly different than the controls levels (P < .36 and P < .11, respectively).

DISCUSSION

The following are the major findings of our study: the lipid peroxidation biomarkers TBARS and PD were significantly increased in patients with OSA compared with controls, the concentration of the protective enzyme PON1 was decreased in patients with OSA and cardiovascular morbidity in comparison with nonapneic controls, and plasma concentrations of TBARS and PD were lowered by nCPAP treatment. Obstructive sleep apnea has been repeatedly demonstrated to be associated with an increased prevalence of CVD, but the mechanisms underlying this association are not yet fully understood. Oxidative stress, which is an imbalance between oxidant production and antioxidant defenses in favor of the former, plays an important role in the pathogenesis of atherosclerosis. Recent evidence has demonstrated greater oxidative stress in OSA due to increased production of oxygen-reactive species by granulocytes and monocytes and by polymorphonuclear cells obtained from patients with OSA. A decrease in the plasma concentration of nitric oxide, which promotes oxidative stress, is in accord with this line of evidence. Moreover, the increased production of oxygen-reactive species in patients with OSA has been shown to be associated with increased expression of adhesion molecules, resulting in increased avidity of monocytes and lymphocytes to endothelial cells in culture. In agreement with these findings, we showed that the plasma lipid biomarkers TBARS and PD were higher in patients with OSA than in controls independent of all possible confounders such as age, BMI, smoking status, drug use, etc. Similar findings have been previously reported in a small group of patients with OSA.

In addition to the increased free-radical production by leukocytes and possibly from other sources, a decrease in endogenous antioxidant concentrations could also contribute to enhance oxidative stress in OSA. Here we measured the activity of the endogenous antioxidant enzyme PON1 that has been reported to be physically associated with HDL and to protect both LDL and HDL from oxidation and, consequently, from the development of atherosclerosis. Furthermore, PON1 has been shown to reduce the adhesion of monocytes to endothelial cells, an early atherosclerotic event that we showed to be augmented in OSA. The PON1 level was significantly lower in the +OSA/+CVD group than in controls and tended to be lower than in +OSA-/CVD group by approximately 10%. Stepwise regression analysis demonstrated an independent significant negative association between RDI and PON1, yet a cardiovascular condition was significantly associated with down regulation of PON1 activity.

Based on the assumption that the apnea-related hypoxia-reoxygenation cycles play a major role in exaggerating oxidative stress in OSA, we expected that lipid peroxidation levels would be best correlated with measures of SaO₂. Correlating percentage of time below an SaO₂ of 90%, minimum nocturnal SaO₂, and RDI with the levels of the 3 lipid peroxidation biomarkers revealed that RDI was the best predictor of the 3. This observation was also corroborated by the logistic regression analysis. It seems reasonable that RDI emerged as the best predictor for oxidative stress would be best associated with parameters that take into account the intermittency in blood reoxygenation.

By separately examining OSA patients with and without cardiovascular morbidity, we attempted to clarify whether cardiovascular sequelae in OSA are associated with an even greater increase in oxidative stress. Our
results provide some support for this hypothesis. Patients with cardiovascular and OSA showed a tendency toward having lower PON1 concentrations when compared with the +OSA/-CVD patients, which may further exacerbate their oxidative stress condition. Of note, the same tendency was found when we separated the +OSA/+CVD group into patients with hypertension only and those who had IHD or a history of MI or stroke. Thus, the mean levels of TBARS, PD, and PON1 for the 2 subgroups of +OSA/+CVD patients were 17.70 ± 6.50 nmol MDA/mL, 930.40 ± 143.90 nmol/mL, and 81.10 ± 15.28 U·min⁻¹·mL⁻¹, and 20.74 ± 8.32 nmol MDA/mL, 914.0 ± 103.5 nmol/mL, and 78.94 ± 11.98 U·min⁻¹·mL⁻¹, respectively. This observation is in agreement with a recent finding from our laboratory demonstrating a significant association between the susceptibility of patients with OSA to CVD and polymorphism of the antioxidant and immunomodulatory protein haptoglobin, which is encoded by 2 alleles with profoundly different biophysical and biochemical properties.35 Patients with OSA who are younger than 55 years of age and had a haptoglobin 2-2 phenotype had a 2.32-fold higher risk of having CVD than did their counterparts with haptoglobin 2-1. Since the protein product of the haptoglobin-1 allele is a superior antioxidant, it suggests that patients who are homozygotic for the haptoglobin-2 allele have a lower antioxidant protection, which makes them more susceptible to cardiovascular morbidity. We should also add that we previously reported that +OSA/+CVD patients had higher concentrations of plasma homocysteine than did patients who were +OSA/-CVD and patients with CVD but without OSA.36 Homocysteine is another independent risk factor for CVD that is thought to have deleterious effects that involve endothelial dysfunction mediated by oxidative stress.37 Taken together, independent markers for cardiovascular morbidity as decreased PON1 activity increased TBARS and PD, as well as increased homocysteine levels and a 2-2 haptoglobin phenotype, may have a predictive value in identifying patients with OSA who are prone to having cardiovascular morbidity.

It is well established that lipid peroxidation and oxidative stress are key features in the development of atherosclerosis, which is an underlying cause of MI, stroke, and other CVD.38 Therefore, it is highly likely that the high rates of lipid peroxidation observed in patients with OSA play an important role in the pathogenesis of cardiovascular morbidity in this syndrome. Patients with OSA, even without any evidence for CVD, are nightly exposed to oxidative stress and thus to accelerated atherosclerosis. Hence, even without any evidence of CVD, patients with OSA have been shown to have endothelial dysfunction.39-41 This subclinical condition of atherosclerosis has been shown to be associated with oxidative stress.38,42

Our study has several potential limitations that should be acknowledged. First, in view of the increased state of oxidative stress with age and the potential confounding effects of BMI and sex, comparing patients with OSA to an age-, BMI-, and sex-matched group would be a more appropriate method than the case series study used here. However, the fact that nCPAP treatment lowered the concentrations of TBARS and PD suggests that the differences between sleep apneics and controls were not due to differences in confounding variables but due to the syndrome itself. In view of the fact that many covariates showed significant differences between the groups, however, we cannot completely dismiss the possibility that subjects’ characteristics other than those related to OSA have at least partially contributed to the observed differences in lipid peroxidation.

Another potential criticism is that assays to detect lipid peroxidation are dependent not only on the extent of oxidative stress, but also on the amounts of serum lipid substrate present and on the concentrations of protective endogenous antioxidants. Attempts to account for the total amount of serum lipid substrates would require our subjects to consume a controlled diet, which was not possible in such a study. When we normalized the levels of the lipid peroxidation biomarkers to serum LDL concentrations, however, we obtained the same results. We attempted to

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**Figure 1**—Mean (± SD) hourly (a) thiobarbituric reactive substances (TBARS) and (b) peroxides (PD) concentrations measured throughout the night in patients with obstructive sleep apnea (OSAS) before and after treatment with nasal continuous positive airway pressure (nCPAP) and in nonapneic controls.
examine the effects of protective antioxidants by measuring PON1, which showed significantly lower values in patients with OSA and cardiovascular morbidity in comparison with controls. Further studies should replicate and extend these findings to other endogenous antioxidants such as plasma activity of superoxide dismutase, glutathione peroxidase, and catalase, and plasma levels of vitamins C and E. Another potential criticism is that TBARS has been claimed to be a nonspecific marker of lipid peroxidation.43 However, we also measured lipid peroxides by an iodometric method, which is considered to be more specific than TBARS measurements. Moreover, both assays, TBARS and PD, utilized in vitro oxidized plasma, which is considered to be a more relevant model of lipoprotein oxidation in the arterial wall than is the in vitro oxidation of isolated LDL.45 In addition, it has been shown that under conditions of induced oxidative stress, which is presumably characteristic of patients with OSA, there was a good correlation between TBARS and the levels of isoprostane, which is a more specific marker of lipid peroxidation.46

In conclusion, our present findings demonstrate a significant increase in biomarkers of oxidative stress, and a strong tendency toward a decrease in the concentration of the endogenous antioxidant PON1, in patients with OSA, which was further attenuated in patients with OSA who also have CVD. Treatment with nCPAP effectively lowered the concentrations of PD and TBARS. This suggests the involvement of oxidative stress in the cardiovascular sequelae in OSA.

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