Plasma Homocysteine Levels in Obstructive Sleep Apnea*  
Association With Cardiovascular Morbidity

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Objectives: Obstructive sleep apnea (OSA) is associated with cardiovascular morbidity and mortality. Plasma levels of homocysteine are also associated with cardiovascular morbidity and mortality. We therefore investigated homocysteine and conventional cardiovascular risk factors in OSA patients with and without cardiovascular morbidity in comparison with normal control subjects and ischemic heart disease (IHD) patients without OSA.

Setting: Technion Sleep Medicine Center, Haifa, Israel.

Methods and participants: Levels of homocysteine, cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, creatinine, vitamins B₁₂ and B₆, and folic acid were determined in 345 participants after overnight fasting. These included OSA patients with IHD (n = 49), with hypertension (n = 61), or without any cardiovascular disease (n = 127). Two control groups were employed: IHD patients without or with low likelihood for sleep apnea (n = 35), and healthy control subjects (n = 73).

Results: After adjustment for age, body mass index, creatinine, and existence of diabetes mellitus, OSA patients with IHD had significantly higher homocysteine levels (14.6 ± 6.77 μmol/L) than all other groups including the IHD-only patients. Hypertensive OSA patients had comparable homocysteine levels to IHD patients (11.80 ± 5.28 μmol/L and 11.92 ± 5.7 μmol/L, respectively), while patients with OSA only had comparable levels to normal control subjects (9.85 ± 2.99 μmol/L and 9.78 ± 3.49 μmol/L, respectively). No differences in conventional cardiovascular risk factors or in vitamin levels were found between groups.

Conclusions: Patients with the combination of IHD and OSA have elevated homocysteine levels. We hypothesize that these results may be explained by endothelial dysfunction combined with excess free-radical formation in OSA patients.

Key words: homocysteine; hypertension; ischemic heart disease; obstructive sleep apnea

Abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CON = control subjects without obstructive sleep apnea and free of any major disease; HDL = high-density lipoprotein; HPLC = high-performance liquid chromatography; HT-OSA = obstructive sleep apnea patients with hypertension only; IHD = ischemic heart disease; IHD-only = patients with ischemic heart disease only; IHD-OSA = ischemic heart disease patients with obstructive sleep apnea; LDL = low-density lipoprotein; NO = nitric oxide; OSA = obstructive sleep apnea; OSA-only = obstructive sleep apnea patients without any cardiovascular morbidity

Obstructive sleep apnea (OSA) syndrome is a common health problem affecting as much as 4 to 9% of the adult population. It is characterized by repeated breathing arrests during sleep, leading to repeated arterial oxygen desaturations. The syndrome is associated with sleep fragmentation and excessive daytime sleepiness that may result in intellectual deterioration and mood changes. A strong independent association was established between OSA and cardiovascular morbidity, such as systemic hypertension, coronary heart disease, atherosclerosis, and stroke. Furthermore, OSA patients were shown to have elevated levels of circulating endothelin-1, as well as vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and L-selectin, and platelet activation and aggregation, all established markers of cardiovascular morbidity. Lavie et al and He et al reported increased cardiovascular mortality risk in OSA patients, particularly in middle-aged patients. Therefore, identifying possible risk factors involved in OSA cardiovascular morbidity is of great clinical importance.
Since the pioneering observation of McCully in infants with inborn errors of metabolism, linking elevated levels of the nonprotein, sulfur-containing amino acid homocysteine in the plasma with vascular diseases, many clinical and epidemiologic studies have shown a clear correlation between mildly elevated total blood homocysteine concentrations and premature coronary artery diseases, peripheral artery diseases, stroke, or venous thrombosis. In a large-scale study based on the European Concerted Action Project, Graham et al concluded that a 5% increment elevates cardiovascular risk by as much as cholesterol increases of 20 mg/dL (0.5 mmol/L). Nygard et al prospectively investigated the relation between plasma total homocysteine concentration and mortality among 557 patients with angiographically confirmed coronary artery disease. They found a strong graded relation between plasma homocysteine and overall mortality. Anderson et al further emphasized the importance of homocysteine as a significant predictor of mortality independent of traditional risk factors in 1,412 patients with severe, angiographically defined coronary artery disease.

In view of the strong associations between OSA and cardiovascular morbidity and mortality, and between homocysteine and cardiovascular morbidity and mortality, we investigated the levels of homocysteine as well as conventional cardiovascular risk factors such as total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in OSA patients with and without ischemic heart disease (IHD), and in OSA patients with hypertension. We compared them to normal control subjects and to patients with IHD but without OSA. Since sleep apnea is much more prevalent in men and homocysteine levels are more prevalent in men and homocysteine levels are higher in women, the gender factor was also taken into consideration. In the analysis of homocysteine metabolism, we used the method approved by the local ethical committee, and all participants signed an informed consent before being enrolled in the study. We used a 5% increment to evaluate the cardiovascular risk.

Materials and Methods

Participants

We investigated fasting levels of homocysteine in a total of 345 male participants. These included three groups of OSA patients: (1) OSA patients with IHD (IHD-OSA; n = 49), of whom 21 patients had a history of myocardial infarction, 3 patients had a history of cerebrovascular accident, and 1 patient had a history of peripheral vascular disease; (2) OSA patients with hypertension only (HT-OSA; n = 61), defined as either having a history of antihypertensive treatment or BP > 140/90 mm Hg (1 patient had a history of cerebrovascular accident); and (3) OSA patients without any cardiovascular morbidity (OSA-only; n = 127). The control subjects consisted of two groups: (1) a group of patients with IHD only (IHD-only; n = 35), of whom 13 patients had a history of myocardial infarction; and (2) control subjects without OSA and free of any major disease (CON; n = 73). The study was approved by the local ethical committee, and all participants signed an informed consent before being enrolled in the study. Sleep apnea patients were recruited from the patients’ population of the Technion Sleep Medicine Center laboratory in Haifa during 1997–98. This eight-bed laboratory serves the northern part of Israel. The total number of patients studied during that period was 3,000, most of them because of suspected OSA. Consecutive patients were recruited for the study. Their assignment to the study groups was based on medical history and sleep laboratory findings. A diagnosis of OSA was based on at least 1 night of polysomnographic recording. This included monitoring of respiration using chest and abdomen respiratory belts and oronasal temperature sensors, as substitute measurements of respiratory effort and flow, and oxygen saturation. From these measures, we obtained the apnea-hypopnea index (AHI; the total number of apneas plus hypopneas divided by the hours of sleep), and lowest and mean nocturnal oxygen saturation; the percentage of sleep time spent with oxygen saturation <90% was also recorded. Apnea was defined as a cessation in airflow of at least 10 s, and hypopnea was defined as a decrease in the amplitude of the respiratory signal of at least 50% for a minimum of 10 s followed by either a decrease in oxygen saturation of 4%, or signs of physiologic arousal. Height and weight were recorded, and the body mass index (BMI) was calculated. Each patient was also interviewed by one of the Sleep Medicine Center physicians regarding their sleep-related complaints and medical history. A sleep laboratory finding of at least AHI > 10 plus characteristic symptoms such as excessive daytime sleepiness, chronic fatigue, and restless sleep established the diagnosis of OSA. Inclusion in the HT-OSA and IHD-OSA groups was based on a documented clinical history. The history of IHD was based on the results of either angiography or thallium single-photon emission CT. Patients in the IHD-only group were recruited from two sources: patients with documented IHD referred to the sleep laboratory because of suspected OSA who were found to have normal sleep (n = 8), and from the patients’ population of a large cardiology department (n = 27). Approximately 60 patients with IHD diagnosed in this department either by angiography or thallium single-photon emission CT during a 6-month period were contacted by telephone and offered to participate in the study. All consenting patients were interviewed about their sleep, and those with low risk for OSA were included in the study. Normal control subjects were recruited from the population referred to the sleep laboratory and found to have normal sleep (n = 22), and from the hospital, sleep laboratory, and university personnel (n = 51). In both groups, a decision on a low likelihood of OSA was based on lack of characteristic symptoms such as snoring, witnessed episodes of nocturnal apneas, and excessive daytime sleepiness. An exclusion criterion applied to all groups was regular use of vitamins.

Blood Collection

Twelve milliliters of venous blood was obtained from each participant after overnight fasting. For homocysteine determinations, blood samples were collected to precooled ethylenediaminetetra-acetic acid-containing specimen tubes (Vacutainers; Becton-Dickinson; Plymouth, UK) and kept on ice. Plasma was
separated within 2 h in a refrigerated centrifuge, aliquoted, and stored at −70°C until assayed. Serum was obtained for the determination of B12, folate, cholesterol, triglycerides, LDL, HDL, and creatinine. B6 was determined in randomly selected plasma samples (n = 186) of the patients and control subjects.

**Homocysteine Determination**

Total plasma homocysteine analysis was carried out on a Biochrom 20 Amino-Acid analyzer (Pharmacia BioTech; Cambridge, UK) using a high-performance column and a modified physiologic sample separation according to manufacturers’ instructions,31,32 as published.33 Briefly, a 200-μL plasma sample was mixed with 10 μL of sodium tetraborate (0.14 mol/L; pH 9) and 10 μL of dithiothreitol (1.08 mol/L), and let stand for 25 min at room temperature to ensure complete reduction of all S-S bonds. Norleucine (100 nmol in 50 μL of double-distilled water) was added as an internal standard. Proteins were precipitated with 130 μL of 15% sulfosalicylic acid and centrifuged. The pH of the clear supernatant was adjusted to 2.5 by adding 50 μL of lithium hydroxide at 0.75 mol/L. One-hundred microliters of the treated sample (40 μL of plasma) was loaded by programmed autosampler to the column. Data were collected and calculated by EZChrom chromatography data system (EZchrom Scientific Software; San Ramon, CA), using five concentrations of D,L-homocysteine for age, BMI, creatinine levels, and the presence of diabetes mellitus. In addition, they had statistically significant different homocysteine and prevalence of diabetes mellitus. In addition, they had statistically significant different homocysteine and creatinine levels. Post hoc testing revealed that the IHD-OSA and IHD-only groups were of similar age

**Blood Chemistry**

Cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and creatinine were analyzed from serum with a Vitros 250 Chemistry system (Johnson & Johnson, Clinical Diagnostics; Rochester, NY) by the American Medical Laboratories, Israel.

**Statistical Analysis**

Categorical data were analyzed by χ² with Fisher Exact Probability Tests. The continuous values were analyzed by one-way analysis of variance followed by Duncan’s multiple-range test, or by the Kruskal-Wallis test for the variables that were not normally distributed. This was followed by analysis of the covariance on ranked homocysteine levels after adjusting homocysteine for age, BMI, creatinine levels, and the presence of diabetes mellitus. Planned post hoc comparisons were made to compare homocysteine levels among the five groups. Spearman correlation analysis was used to examine the relationship among homocysteine, folate, B12, B6, and creatinine, within groups; p < 0.05 was considered statistically significant. Scatter diagrams to demonstrate these relationships are also provided.

**RESULTS**

The demographic, clinical, and biochemical data of the five groups are presented in Tables 1, 2. Overall, the three OSA groups were of similar severity, with mean AHI and minimum oxygen saturation varying from 33.74 to 30.1 and from 85.09 to 79.95%, respectively, with large variability within groups. The five groups were statistically significantly different in age and BMI and had a different prevalence of diabetes mellitus. In addition, they had statistically significant different homocysteine and creatinine levels. Post hoc testing revealed that the IHD-OSA and IHD-only groups were of similar age.

**Table 1—Demographic and Clinical Data of Study Groups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>CON† (n = 73)</th>
<th>OSA-Only (n = 127)</th>
<th>HT-OSA (n = 61)</th>
<th>IHD-OSA (n = 49)</th>
<th>IHD-Only (n = 35)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>47.52 (11.15)</td>
<td>47.75 (11.64)</td>
<td>55.15 (9.33)</td>
<td>60.75 (10.80)</td>
<td>59.69 (8.51)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.59 (3.21)</td>
<td>30.35 (5.50)</td>
<td>30.98 (5.44)</td>
<td>30.15 (4.32)</td>
<td>28.67 (7.23)</td>
<td>0.0001</td>
</tr>
<tr>
<td>AHI, events/h</td>
<td>5.75 (2.99)</td>
<td>31.5 (21.20)</td>
<td>33.74 (19.53)</td>
<td>30.15 (16.80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MINSO2, %</td>
<td>93.35 (3.71)</td>
<td>84.06 (12.63)</td>
<td>79.95 (12.22)</td>
<td>85.09 (7.71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPMI, %</td>
<td>0.00</td>
<td>0.00</td>
<td>40.52</td>
<td>37.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD, %</td>
<td>0.79</td>
<td>8.06</td>
<td>10.2</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>2.70</td>
<td>11.20</td>
<td>20.41</td>
<td>20.00</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Never smoke, %</td>
<td>67.57</td>
<td>69.17</td>
<td>70.18</td>
<td>57.14</td>
<td>53.88</td>
<td>N/S</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD), or mean. MINSO2 = minimum oxygen saturation; SPMI = status postmyocardial infarction; DM = diabetes mellitus; NS = not significant.
†AHI was available for 22 of 73 subjects in the CON group.
(60.75 ± 10.8 years vs 59.69 ± 8.51 years, respectively); the HT-OSA group (55.15 ± 9.33 years) was approximately 5 years younger than the IHD-OSA and IHD-only groups, and older than the CON group (47.82 ± 11.15 years) and the OSA-only group (47.78 ± 11.64 years). The CON and OSA-only groups were also of similar age. The CON and IHD-only groups had borderline significantly different BMI (26.59 ± 3.21 kg/m² vs 28.67 ± 7.23 kg/m²; p < 0.06); they were less obese than the three OSA groups (OSA-only, 30.35 ± 5.5 kg/m²; HT-OSA, 30.98 ± 5.44 kg/m²; and IHD-OSA, 30.15 ± 4.32 kg/m²). The CON and OSA-only groups had lower prevalence of diabetes (2.7% and 0.79%, respectively) than the three cardiovascular groups (HT-OSA, 11.29%; IHD-OSA, 20.41%; and IHD-only, 20%). The IHD-only group had a higher usage of medications than the other groups (97.14%). The CON and OSA-only groups had similar medication usage (13.5% and 7.87%, respectively); the IHD-OSA and the HT-OSA groups had similar medication usage (73.47% and 62.9%, respectively). There were no significant differences, however, between the IHD-OSA and IHD-only groups with respect to the type of medications used: antiaggregants, 69.4% vs 88.6%; β-blockers, 34.7% vs 62.8%; calcium channel blockers and angiotensin-converting enzyme inhibitors, 45% vs 42.8%; nitrates, 24.5% vs 45.7%; lipid-lowering drugs, 28.6% vs 42.8%; and antidiabetic medications, 12.2% vs 20%, respectively.

Post hoc testing of the unadjusted homocysteine levels revealed that the CON and OSA-only groups had similar homocysteine levels (9.78 ± 3.49 μmol/L and 9.85 ± 2.99 μmol/L, respectively), which were lower than the HT-OSA group (11.8 ± 5.28 μmol/L) and the IHD-only group (11.92 ± 5.77 μmol/L). The latter two groups had similar homocysteine levels. The IHD-OSA group had statistically significantly higher homocysteine levels (14.6 μmol/L) than all the other groups (by 49%, 48.2%, 23.7%, and 22.4% higher than CON, OSA-only, HT-OSA, and IHD-only; p < 0.05 for each, or better). Analysis of the covariance on the ranks of homocysteine with age, BMI, creatinine level, and the existence of diabetes mellitus as covariates revealed statistically significant differences in homocysteine (p < 0.002).

Again, the IHD-OSA group differed from all the groups (p < 0.0001, p < 0.03, p < 0.03, and p < 0.001 against CON, IHD-only, HT-OSA, and OSA-only, respectively). The CON group differed from the HT-OSA group (p < 0.03) and tended to differ from the IHD-only group (p < 0.10). The percentages of patients having homocysteine levels above the 90th percentile of the control subjects (13.2 μmol/L) were 40.82%, 22.86% 12.5%, and 11.81% in the IHD-OSA, IHD-only, OSA-HT, and OSA-only groups, respectively. The IHD-OSA group had significantly higher percentage than all other groups (χ² = 24.42; p < 0.00001).

**Correlation Analysis**

Figures 1-4 present the scatter diagrams for homocysteine and folic acid, B₁₂, B₆, and creatinine for all groups combined. As can be seen, there was an overall negative relationship between homocysteine and folic acid and B₁₂, no relationship with B₆, and a positive relationship with creatinine. These relationships held for each of the groups separately as was revealed by Spearman rank-order correlation analysis (Table 3). Significant or nearly significant correlations between homocysteine and B₁₂ were found in the CON group (−0.22, p < 0.05), the IHD-OSA group (−0.32, p < 0.02), the HT-OSA group (−0.22, p < 0.08), and the IHD-only group (−0.32, p < 0.06), and for all groups combined (−0.20, p < 0.0006). Folate and homocysteine were significantly or nearly significantly correlated in the CON group (−0.40, p < 0.0004), the OSA-only group (−0.31, p < 0.0003), the IHD-OSA group (−0.25, p < 0.07), and the HT-OSA group (−0.29, p < 0.02), and for all groups

![Table 2—Biochemical Data of Study Groups*](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21966/)
combined (−0.26, p<0.0001). Creatinine and homocysteine were significantly or nearly significantly correlated in the CON group (0.20, p<0.08), the OSA-only group (0.22, p<0.02), the HT-OSA group (0.48, p<0.003), and the IHD-only group (0.58, p<0.0007), and for all groups combined (0.31, p<0.0001).

**Discussion**

The major finding of the present study is that the IHD-OSA group had significantly higher homocysteine levels than the IHD-only group after adjustment for major potential confounding factors. Homocysteine levels in the IHD-OSA group were also higher than those of the HT-OSA and OSA-only groups, as well as the CON group. Furthermore, the HT-OSA group had comparable levels of homocysteine to the IHD-only group.

In comparison with literature data on large-scale epidemiologic studies in nonselected cardiovascular patients, homocysteine levels in the IHD-OSA group were very high. The mean homocysteine level in
seven studies investigating large groups of nonselected cardiovascular patients was $12.2 \pm 1.3 \mu\text{mol/L}$. This is similar to the level of homocysteine observed in our IHD-only group ($11.92 \pm 5.77 \mu\text{mol/L}$) but 20% lower than the mean level in the IHD-OSA group ($14.6 \pm 6.77 \mu\text{mol/L}$). Likewise, the mean percentage of nonselected IHD patients having abnormal fasting levels of homocysteine in seven studies with a total of 1,201 patients was 21.1%, which is similar to the percentage (22%) of abnormal homocysteine levels in the IHD-only group of this study. This, however, is almost half the percentage of IHD-OSA having homocysteine levels higher than the 90th percentiles of normal control subjects. The fact that homocysteine levels in the control group ($10.8 \mu\text{mol/L}$) and in the OSA-only group ($10.6 \mu\text{mol/L}$) were within the range of the control values in the above studies (10.5 to 11.9 $\mu\text{mol/L}$) rules out the possibility that the results obtained for the IHD-OSA group were due to methodologic differences in homocysteine determination. Furthermore, a 1999 Israeli study reported...
homocysteine levels of 12.1 ± 5.8 μmol/L in non-selected hypertensive patients with documented cardiovascular disease as compared to 11.1 ± 4.7 μmol/L for age- and gender-matched hypertensive patients without cardiovascular disease, which closely agree with the present results. Finally, for quality control, we reanalyzed 186 randomly selected samples using HPLC and obtained identical results.

None of the conventional risk factors for cardiovascular diseases, ie, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, were significantly different between the groups. This, most likely, reflects the fact that 45.7% of the IHD-only group and 28.5% of the IHD-OSA group regularly used lipid-lowering medications.

Homocysteine, a nonprotein, sulfur-containing amino acid, and an intermediate in the metabolism of the essential amino acid methionine, was implicated in the development and progression of cardiovascular disease. The mechanisms by which it exerts its effects have not yet been fully elucidated, but cumulative data clearly demonstrate that it affects multiple vascular functions in vitro and in vivo, such as promoting prothrombotic phenotype of the endothelium by increasing platelet aggregation and activation, stimulating vascular smooth-muscle cell proliferation, and altering endothelial function. Currently, endothelial injury and dysfunction are among the leading mechanisms proposed to contribute to the development of atherothrombotic vascular disease due to mildly and chronically increased homocysteine levels. The leading mechanism suggested for the adverse vascular effects of homocysteine on endothelial function involves oxidative stress and depletion of bioactive nitric oxide (NO).

Although we do not have an immediate explanation why the IHD-OSA group had such high levels of homocysteine, we can rule out the possibility that it results from folate or vitamins B12 or B6 deficiency or from increased creatinine levels. Although within-group individual differences in homocysteine levels were moderately related to folate and B12 levels, which is in agreement with several reports in the literature, there were no significant differences in vitamin levels between groups. The lack of correlation between B6 and homocysteine levels is explained by the fact that B6 participates in homocysteine metabolism only in cases where there are extremely high levels of homocysteine, such as after methionine loading. Likewise, neither age nor the rate of hypertension, diabetes, history of myocardial infarction, smoking, or usage of medications could account for these differences. Also, the potential contribution of BMI, which was significantly higher in the IHD-OSA group, was controlled by the statistical analysis. We would like to propose that the high levels of homocysteine in sleep apnea patients who also have IHD may be explained by a specific impairment in their ability to neutralize excess homocysteine resulting from impaired endothelial function. Homocysteine, like other thiols, is a reactive molecule. It is auto-oxidized in the plasma, forming in the process hydrogen peroxide, and accompanying free radicals that are toxic to endothelial cells. Also, homocysteine specifically inhibits glutathione peroxidase activity, leading to further increase in hydrogen peroxide. Normally, endothelial cells detoxify homocysteine by releasing NO, which forms S-nitroso-homocysteine adducts by binding to homocysteine. This protective effect of NO is eventually compromised, as long-term exposure to high homocysteine concentrations damages the endothelium, and thus limits NO production. In addition, homocysteine may also decrease the bioavailability of NO by impairing its synthesis.

Recent studies have shown that OSA syndrome is associated with decreased levels of circulating NO. In both studies, this was reversed by effective treatment with nasal continuous positive airway pressure. In addition, elevated plasma levels of the endogenous
NO-synthase inhibitor, asymmetric dimethylarginine, were reported in normotensive OSA patients as compared to normotensive control subjects.\textsuperscript{45} Asymmetric dimethylarginine was shown\textsuperscript{46} to be a novel marker of atherosclerosis. Furthermore, sleep apnea was also shown to be associated with endothelial dysfunction. Kato et al\textsuperscript{47} and Carlson et al\textsuperscript{48} reported that endothelium-dependent but not endothelium-independent vasodilation was impaired in OSA patients. Kraiczi et al\textsuperscript{49} reported on an increased vasoconstrictor sensitivity to angiotensin II, and Duchna et al\textsuperscript{50} reported on a decreased vasodilator response to bradykinin in sleep apnea patients. These findings point to an impairment in endothelial functioning in sleep apnea patients. Similarly, utilizing a mouse model with chronic mild hyperhomocysteinemia due to heterozygous cystathionine-\(\beta\) synthase deficiency leads to endothelial dysfunc-
tion, partly due to increased oxidative stress and depletion in NO bioactivity.\textsuperscript{51} More interestingly though, these hyperhomocysteinemic mice exhibited a paradoxical vasoconstriction to bradykinin as observed in OSA patients.\textsuperscript{50}

Although speculative at this stage, it is possible that endothelial impairment is much more profound in OSA patients who also suffer from cardiovascular diseases. This may be responsible for the diminution in NO production or bioavailability, and consequently for the accelerated accumulation of plasma homocysteine.

The diminution in NO may be accelerated in OSA patients by yet another mechanism. The apnea-related recurrent hypoxia/reoxygenation occurring throughout sleep in OSA may be analogous to the well-documented ischemia/reoxygenation injury as suggested by Dean and Wilcox.\textsuperscript{52} This leads to an excess formation of oxygen-free radicals through depletion of adenosine triphosphate and activation of xanthine oxidase. This possibility is supported by the findings that adenosine triphosphate metabolic products such as adenosine and uric acid in the plasma are increased in OSA patients.\textsuperscript{53,54} Moreover, hypoxia/reoxygenation can also cause increased free-radical formation via activation of inflammatory cells as observed for neutrophils and monocytes in OSA patients.\textsuperscript{55,56} Excess in superoxide production due to hypoxia/reoxygenation may also inactivate NO resulting in the formation of the toxic peroxynitrite. This may further prevent the inactivation of homocysteine leading to an increase in its levels,\textsuperscript{38,40} putting OSA patients with cardiovascular morbidity at a greater risk. Further studies are needed in order to determine what is the exact mechanism by which OSA patients with cardiovascular diseases have such exceptional plasma levels of homocysteine, and if this can be reversed by effective nasal continuous positive airway pressure treatment.

In view of the increased mortality risks associated with increasing levels of homocysteine,\textsuperscript{29} its high levels in cardiovascular OSA patients may confer an added risk in addition to the risk conferred by the repeated apneic and hypoxemic events. This may at least partially explain the high cardiovascular mortality risk reported in sleep apnea patients.\textsuperscript{17,57} Future studies should determine if supplementary treatment with vitamins (ie, folic acid, B\textsubscript{12}, and B\textsubscript{6}) that were reported to normalize homocysteine levels\textsuperscript{29,58} may have beneficial effects in ameliorating cardiovascular risks in OSA patients.

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