Serum Amyloid A in Obstructive Sleep Apnea

Anna Svatikova; Robert Wolk, MD, PhD; Abu S. Shamsuzzaman, MBBS, PhD; Tomas Kara, MD; Eric J. Olson, MD; Virend K. Somers, MD, PhD

Background—Patients with severe obstructive sleep apnea (OSA) may have increased risk for cardiovascular and cerebrovascular diseases. Serum amyloid A (SAA) protein has recently been linked to the development of atherosclerosis, stroke, diabetes, and dementia. We tested the hypothesis that plasma SAA levels are increased in otherwise healthy subjects with OSA.

Methods and Results—Plasma SAA levels were measured in 10 male patients with moderate to severe OSA before sleep, after 5 hours of untreated OSA, and in the morning after effective continuous positive airway pressure treatment. SAA levels were also measured in 10 closely matched control subjects at similar time points. Baseline plasma SAA levels before sleep were strikingly higher in patients with moderate to severe OSA than in controls (18.8 ± 2.6 versus 7.2 ± 2.6 μg/mL, respectively; P = 0.005) and remained unchanged in both groups throughout the night. SAA levels in 10 male patients with mild OSA were comparable with controls (P = 0.46). Plasma SAA in 7 female patients with moderate to severe OSA was also markedly higher compared with matched control female subjects (24.1 ± 2.4 versus 10.2 ± 2.4 μg/mL, respectively; P = 0.0013) but was not different from male patients with moderate to severe OSA (P = 0.3). There was a significant positive correlation between SAA and apnea-hypopnea index (r = 0.40, P = 0.03).

Conclusions—Plasma SAA levels are more than 2-fold greater in patients with moderate to severe OSA compared with subjects with mild OSA or healthy controls regardless of gender. Elevated SAA may contribute to any increased risk for cardiovascular and neuronal dysfunction in patients with OSA. (Circulation. 2003;108:1451-1454.)

Key Words: amyloid ■ sleep ■ cardiovascular diseases

Obstructive sleep apnea (OSA) is associated with increased cardiovascular morbidity,1–3 In addition, OSA may be related to dementia.4 Inflammatory mechanisms, implicated in cardiovascular and cerebral degenerative diseases,4,5 may be activated in sleep disorders (including OSA)6–9 and thus contribute to the association between OSA, cardiovascular disease, and dementia.

Serum amyloid A (SAA) is one of the major acute-phase proteins in humans that is upregulated by inflammatory cytokines, including interleukin-1 and interleukin-6.6,10 It is synthesized predominantly by the liver and secreted as a major component of the apolipoproteins in the HDL particle.10 Elevated SAA is associated with increased risk for coronary heart disease.10–12 SAA may accumulate in the brain of patients with Alzheimer disease, a condition that may be associated with chronic brain inflammation and may contribute to neuronal loss and white matter damage.13,14

Any sustained elevation of SAA in patients with OSA might help explain their increased incidence of cardiovascular disease as well as dementia. We therefore tested the hypothesis that plasma SAA levels are increased in otherwise healthy subjects with OSA.

Methods
We compared subjects with moderate to severe OSA (apnea-hypopnea index [AHI] ≥ 20) with those with mild OSA (AHI 6 to 19) and those without OSA (AHI ≤ 5). We studied 20 male and 7 female patients with newly diagnosed OSA who were free of other diseases, had never been treated for OSA, and were taking no medications, and 10 healthy male and 7 healthy female control subjects matched for age and body mass index, in whom occult OSA was excluded by overnight polysomnography. All participants were nonsmokers.

The presence and severity of sleep apnea were determined by standard overnight polysomnography. AHI was calculated as the total number of apneas and hypopneas per hour of sleep. Sleep studies followed a split-night protocol according to the standard of care in our institution. The first half of the study was for the diagnosis of OSA. A therapeutic continuous positive airway pressure (CPAP) trial followed in the second half of the night. SAA levels in 10 male patients with moderate to severe OSA were measured at 9:00 PM (before sleep), at 2:00 AM (after 5 hours of untreated OSA, before CPAP therapy started), and at 6:00 AM (after waking in the morning, after 4-hour CPAP treatment). Measurements were obtained at similar times in 10 control subjects. Plasma SAA was also measured at baseline, before sleep, in 10 male patients with mild OSA, 7 female patients with moderate to severe OSA, and 7 matched healthy female control subjects. The study was approved by the Human Subjects Review Committee.

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Plasma SAA concentrations were measured using ELISA (SAA kit, BioSource International, Inc) using a monoclonal antibody specific for SAA. The minimum detectable concentration of SAA was 1.3 μg/mL. Intraassay coefficients of variation were 3.1%, 6.0%, and 12% at 15.7, 71.5, and 192 μg/mL, respectively. Interassay coefficients of variation were 12%, 13%, and 11% at 7.2, 36.1, and 70.5 μg/mL, respectively. Spike recovery of SAA yielded an average recovery of 94%.

Results are reported as mean±SEM. Continuous variables were compared between groups using one-way ANOVA. Differences between various time points (9:00 PM, 2:00 AM, and 6:00 AM) within each group were assessed using ANOVA for repeated measures, followed by the Newman-Keuls post-hoc tests. Statistical significance was defined as P<0.05.

Results
The OSA and control groups were very similar with regard to demographics, hemodynamics, and metabolic characteristics (Table). OSA subjects suffered more severe hypoxic burden than control subjects (Table). Treatment with CPAP in the OSA group reduced AHI from 46±4 to 10±4 events per hour (P=0.002) and increased O2 nadir from 81±1% to 92±1% (P=0.0002). Time spent at O2 saturation below 90% decreased from 12±3 to 0±3 minutes (P=0.02).

Baseline plasma SAA levels before sleep were strikingly higher in male patients with moderate to severe OSA than in control subjects (18.8±2.6 versus 7.2±2.6 μg/mL, respectively; P=0.005). These SAA levels remained markedly higher but stable throughout the night in the OSA group (between group comparison, F=7.8; P=0.01) and were not affected by several hours of untreated OSA or by acute CPAP treatment (Figure 1). SAA levels also remained stable through the night in the control subjects. Baseline SAA level in 10 male patients with mild OSA was comparable with the control group (8.7±2.6 versus 7.2±2.6 μg/mL, respectively; P=0.46) (Figure 2).

Baseline Characteristics of Patients With OSA and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Male Subjects</th>
<th>Female Subjects</th>
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<tr>
<td></td>
<td>Moderate to</td>
<td>Moderate to</td>
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<td></td>
<td>Severe OSA</td>
<td>Severe OSA</td>
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<td>Mild OSA</td>
<td>Controls</td>
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<tr>
<td>Demographics</td>
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<tr>
<td>Age, y</td>
<td>51±3</td>
<td>47±3</td>
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<tr>
<td>BMI, kg/m²</td>
<td>31±1</td>
<td>32±1</td>
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<tr>
<td>Mean BP, mm Hg</td>
<td>92±8</td>
<td>95±8</td>
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<tr>
<td>Biochemical measurements</td>
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<tr>
<td>HDL, mg/dL</td>
<td>34±2</td>
<td>33±2</td>
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<td>LDL, mg/dL</td>
<td>91±15</td>
<td>108±15</td>
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<tr>
<td>Triglycerides, mg/dL</td>
<td>348±80</td>
<td>194±80</td>
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<tr>
<td>Creatinine, mg/dL</td>
<td>1.2±0.1</td>
<td>0.93±0.1</td>
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<tr>
<td>Insulin, μU/mL</td>
<td>11.8±2.3</td>
<td>8.0±2.3</td>
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<td>Glucose, mg/dL</td>
<td>99.7±3.5</td>
<td>96.7±3.5</td>
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<tr>
<td>Diagnostic sleep study</td>
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<td>AHI, events per h</td>
<td>46±4</td>
<td>14±4</td>
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<tr>
<td>Time with O₂ saturation &lt;90%, min</td>
<td>12±3</td>
<td>4±3</td>
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<tr>
<td></td>
<td>3±4†</td>
<td>1±3*</td>
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<tr>
<td></td>
<td>34±2</td>
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<tr>
<td></td>
<td>24±7</td>
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| Values are mean±SEM. *P<0.05 compared with male patients with moderate to severe OSA. †P<0.05 compared with male patients with mild OSA. ‡P<0.05 compared with female patients with moderate to severe OSA.

Discussion
The novel findings of our present study include, first, that patients with moderate to severe OSA have elevated SAA levels, ~2.5-fold greater than measurements in healthy subjects or patients with mild OSA. The mean level of SAA in patients with OSA without other disease is even higher than the 75th percentile of SAA measurements obtained in patients with unstable angina or non-Q-wave myocardial infarction enrolled in the Thrombolysis In Myocardial Infarction 11A study.12 Second, untreated OSA, CPAP treatment, or normal sleep did not acutely affect SAA levels. Effects of chronic treatment with CPAP on SAA are unknown and remain to be established.

The mechanisms whereby OSA affects SAA levels are unknown. One possibility is the effect of hypoxia/reoxygenation related to sleep apnea. Hypoxia stimulates the genes of acute-phase proteins, as well as cytokines known to induce these proteins.13 Hypoxemia at high altitude may increase plasma levels of several inflammatory mediators.16 Sleep deprivation or fragmentation may also influence systemic inflammation, including elevation of circulating cytokine levels.6–8 Patients with OSA in our study were characterized not only by severe apnea (Table) but also by a much higher arousal index, suggesting that both hypoxemia and sleep fragmentation may be implicated in elevated SAA levels. Nevertheless, these perturbations do not seem to have any
acute effects on SAA through the night but may be linked to chronic SAA elevations evident even before sleep.

The association between OSA and elevated SAA may have important pathophysiological implications. Compelling epidemiological evidence implicates increased SAA in cardiovascular and neurological diseases, suggesting that SAA may be an important link between OSA and conditions such as atherosclerosis, diabetes, and dementia.

**Atherosclerosis, Inflammation, and Metabolic Dysfunction**

Low-grade inflammation contributes importantly to the initiation and the progression of atherogenic processes. Elevated SAA constitutes part of a general inflammatory response and may also exert direct proatherogenic effects. For example, the association of SAA with HDL might alter the metabolism of this lipoprotein particle, thereby compromising its protective effect against atherosclerosis. Recent observations suggest that SAA can predict early mortality in patients with stable and unstable angina, myocardial infarction, and coronary artery disease as well as in patients with other coronary and peripheral vascular disease.

Sleep loss and hypoxemia may also be causally associated with metabolic derangements. Patients with sleep-disordered breathing have insulin resistance, with higher fasting glucose and insulin levels. SAA tissue expression and a hepatic receptor for SAA (Tanis) are dysregulated in association with glucose intolerance. Whether elevated SAA is related to glucose intolerance in OSA remains unclear.

**Dementia**

An etiological link between OSA and dementia, although unproven, is suggested by the observation that CPAP treatment can reduce the level of dementia. Amyloid deposition is thought to play an important role in many forms of dementia. Elevated levels of SAA may be related to tissue amyloid deposition. Proteolysis of SAA to amyloid A may
lead to formation of amyloid fibrils, which can be seen on histological examinations of brain tissues obtained from subjects with dementia. In Alzheimer disease, amyloid β (the main component of amyloid plaque) can be found in biological fluids as a soluble protein. Elevated SAA in patients with OSA may conceivably be related to some types of dementia seen in OSA.

**Strengths of the Study**

First, we included only normotensive, newly diagnosed, and untreated OSA patients who had no coexisting diseases apart from OSA. Second, patients and controls were not taking any medications. Third, the control subjects were matched for age and body mass index, thus ruling out any potential confounding influence of age and especially obesity on our data. Fourth, complete overnight polysomnography excluded any possibility of occult sleep apnea in our obese control subjects.

**Conclusion**

SAA levels are chronically elevated in patients with OSA. SAA has been associated with cardiovascular and neuronal diseases and may be an important potential mechanism to explain any increased risk for cardiovascular and neuronal dysfunction in patients with OSA.

**Acknowledgments**

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**References**


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