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Obstructive Sleep Apnea Syndrome: Links Between Pathophysiology and Cardiovascular Complications

Abstract

Purpose: The prevalence of obstructive sleep apnea syndrome (OSAS) is increasing, especially in the middle-aged population. OSAS is associated with an elevated risk of cardiovascular morbidity and mortality. Arterial hypertension is often the first consequence of OSAS, but the most severe complications are coronary artery disease, stroke and arrhythmias. The aim of this review was to analyze the several mechanisms involved in the development of the cardiovascular events, such as endothelial dysfunction accompanied by a pro-inflammatory and pro-oxidant status, hemorheological alterations, hypercoagulability and imbalance between matrix metalloproteases and their inhibitors.

Source: A search on PubMed was carried out using the following terms: obstructive sleep apnea syndrome; endothelial dysfunction; oxidative stress; inflammation; rheology; matrix metalloproteases.

Principal findings: OSAS severity strongly influenced cardiovascular risk factors and, furthermore, it was correlated with the incidence of fatal and non-fatal events.

Conclusions: The treatment with continuous positive airways pressure (cPAP) is the gold standard for OSAS and was able to positively influence all the pathophysiological mechanisms responsible for cardiovascular diseases. Long-term cPAP improved endothelial function and hemorheology, reduced oxidative stress and inflammation, and decreased the levels of metalloproteases.

*Manuscript submitted 9th August, 2015
Manuscript accepted 27th October, 2015*

Clin Invest Med 2015; 38 (6): E362-E370.

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The obstructive sleep apnea syndrome (OSAS) is a sleep disorder characterized by repeated airflow obstructions during sleep with consequent episodes of apnea or hypopnea and intermittent arterial oxygen desaturation [1,2]. OSAS affects especially middle-aged and elderly subjects and its prevalence is increasing worldwide [3]. About 9% of women and 24% of men in the general population are affected by sleep breathing disorders and misdiagnosis is common [4]. Clinically, OSAS is characterized by snoring, choking, awakening due to gasping and by the effects of sleep fragmentation: morning headache, sleepiness, decreased concentration and memory loss [1]. In the last decades, attention has been directed towards the consequences of OSAS on the cardiovascular system [5] and the overall mortality attributed to OSAS [4,6]. OSAS subjects often develop arterial hypertension and OSAS is now considered the most common secondary cause of hypertension [7]. OSAS-associated hypertension is frequently severe, resistant to treatment and accompanied by a non-dipper profile [8]. The blood pressure increase seems to be associated with OSAS severity in a dose-response manner and the treatment induces a reduction in blood pressure values [8].

Some researchers have demonstrated early atherosclerotic lesions in OSAS subjects, suggesting that this clinical condition is an independent risk factor for coronary artery disease (CAD) [9]. In OSAS subjects, a higher incidence of myocardial infarction during night-time has been reported [10]; therefore, OSAS could precipitate myocardial ischemia during sleep in patients with CAD. Untreated, OSAS may also worsen the prognosis of CAD subjects by increasing cardiovascular death [11]. Subjects with severe OSA have a 67% increased risk of all-cause mortality and a 265% increased risk of cardiovascular mortality [12]. The OSAS severity seems to be responsible for the incidence of cardiovascular events. The apnea/hypopnea index (AHI) and the time spent with oxygen saturation <90% have been shown to be predictors of cardiovascular outcome [13]. In particular, subjects with severe OSAS have a 2.5-fold increased risk for cardiovascular events and a 2-fold increased risk for stroke, in comparison with the general population [14]. The incidence of fatal and non-fatal cardiovascular events is higher in subjects with severe OSAS in comparison with the healthy population and the rates of mortality seem to increase in subjects with pre-existing cardiovascular disease [15]; however, Yaggi *et al.* have demonstrated an elevated risk of stroke and death in patients with OSAS for any cause, independent of other risk factors, including arterial hypertension [16]. OSAS has a prevalence of 44-72% in subjects with stroke and those with an AHI >20 have a 4-fold increased risk of cerebral events [11]. The Sleep Heart Health Study revealed an association

between severe sleep-disordered breathing and arrhythmias, such as atrial fibrillation, non-sustained ventricular tachycardia and complex ventricular ectopy, which often occur during the nocturnal hours [17]; in addition, the incidence of atrial fibrillation and ventricular ectopy increases with the increasing severity of the disorder [18].

The pathogenesis of cardiovascular events in OSAS subjects is complex and depends on several factors. The sleep fragmentation is the first consequence of OSAS and is considered to be responsible for hypertension development as it stimulates the renin angiotensin system and increases the cytokine production, leading to endothelial dysfunction [19]. The intermittent hypoxia induces increased sympathetic activity, which exerts effects on blood pressure and heart rate, promotes free radical production and adhesion molecules expression and induces insulin resistance and leptin resistance [19,20,21]. The cyclic airway occlusion also causes the intermittent generation of an elevated negative intrathoracic pressure and increases ventricular transmural pressure and ventricular after-load with a parallel raise in venous return; this latter mechanism leads to a reduction in left ventricular stroke volume [21]. During the apneic event, hypoxia may cause an imbalance between the myocardial oxygen demand and supply, which may induce myocardial ischemia or arrhythmias [22].

The aim of this review was to analyze the pathophysiological links between OSAS and cardiovascular complications, such as endothelial dysfunction, nitric oxide availability, systemic inflammation, oxidative stress, hypercoagulability, hemorheological alterations, and matrix metalloproteinases profile.

Results and Discussion

Endothelial dysfunction

Several researchers showed that OSAS is associated with an altered endothelial function [23,24]. In a small group (8 patients) of obese subjects with OSAS, some authors observed reduced vasodilation in response to intra-arterial infusion of acetylcholine but not in response to sodium nitroprusside, suggesting an impaired endothelial-dependent vasodilation [25]. Others described a negative correlation between the flow-mediated dilation and the degree of nocturnal hypoxemia in 20 subjects with a mean AHI of 23.7 per hour [26]. In a larger population of OSAS (1,037 subjects, age $\geq$ 68 years, mean AHI 9.9 per hour, range 0–149 per hour), higher AHI values were associated with an increased brachial artery baseline diameter and with a reduced FMD in a dose-dependent manner [27].

Repetitive hypoxia-reoxygenation episodes are presumed to play a pivotal role in the genesis of endothelial dysfunction. Intermittent hypoxia induces the production of reactive oxygen species (ROS), which contribute to the generation of adhesion molecules with leukocyte activation and an enhanced systemic inflammation, and also to the decreased circulating nitric oxide (NO) [28,29]. Untreated sleep apnea is associated with high levels of endothelin, which contribute to vasoconstriction, with a reduced number of endothelial progenitor cells and with an increased endothelial cell apoptosis [30]. Long-term continuous positive airways pressure (cPAP), the gold standard for OSAS treatment [3], improves endothelial function as it attenuates oxidative stress and inflammation, reduces the leukocyte activation and the adhesion to the endothelium and enhances the endothelial repair capacity [31].

Nitric oxide bioavailability

The reduced availability of nitric oxide (NO) may be involved in the pathogenesis of arterial hypertension and cardiovascular diseases, especially in severe OSAS. Plasma NO metabolites (nitrites and nitrates), usually expressed as NOx, are reduced in OSAS subjects [29, 32-35], especially in those with severe OSAS [41]. Noda *et al.* observed lower plasma NO concentrations in OSAS subjects with an AHI ≥ 20 in comparison with controls or OSAS subjects with an AHI ≤ 20 per hour [29]. Alonso-Fernandez *et al.* found lower NOx levels in 31 men with new diagnosed OSAS than in healthy subjects [32]. Similarly, Cifci *et al.* in 69 OSAS subjects with an AHI ≥ 15 [33]. Canino *et al.* [36] found decreased NOx in subjects with AHI >30 in comparison with subjects with AHI <30 , but not in comparison with normal controls. They also observed a negative correlation between NOx and AHI and a positive correlation between NOx and mean nocturnal SO₂. Other authors [35] evaluated 36 subjects with mild-moderate OSAS and 31 subjects with severe OSAS and demonstrated, in both groups, reduced NOx levels during the nocturnal hours. The intermittent hypoxia induces a down-regulation of the NO synthase (NOS) expression, reducing the NO production [37,38]. As oxygen is a substrate of NOS, the frequent desaturation in OSAS subjects could reduce NOS activity; in addition, hypoxia is responsible for alterations in gene regulation, so it could suppress the transcription of endothelial NOS (eNOS) gene [33]. Zhao *et al.* [38] examined the effects of intermittent hypoxia on cultured human umbilical vein endothelial cells, and observed significantly lower levels of NO, NOS activity and NOS mRNA expression. Furthermore, Jelic *et al.* [39] reported reduced expression of eNOS, lower levels of phosphorylated eNOS and an increased expression of inducible NOS

(iNOS) in venous endothelial cells of newly diagnosed OSAS subjects. Treatment with cPAP for 4 weeks increased eNOS and phosphorylated eNOS, and decreased iNOS expression, improving flow-mediated dilation [39]. In animal models, chronic intermittent hypoxia induced NF- κ B activity leading to overproduction of inflammatory mediators that are able to inhibit eNOS expression, such as TNF- α [37]. Moreover, the increased production of ROS in OSAS subjects might cause an uncoupling of the eNOS and a decreased activity of this enzyme [40]. cPAP therapy seemed to improve the endothelial function as it increased NOx levels in the long-term [32,34,41], even after one overnight application [28]. Oyama *et al.* examined 32 subjects with metabolic syndrome and OSAS (mean AHI 56.2 ± 21.6 per hour) and observed a significant increase in NOx levels after 3 months of cPAP treatment [34].

Systemic inflammation

C-reactive protein (CRP) is an acute-phase protein produced by the liver and is one of the most studied biomarkers of low-grade inflammation in cardiovascular diseases, as it is a risk factor for atherosclerosis and cardiovascular morbidity and mortality [42]. Several researchers have evaluated the role of CRP in OSAS with conflicting results, probably due to the presence of obesity or pre-existing CAD [42]. In OSAS subjects (30 patients with a median AHI of 25 per hour), increased levels of circulating inflammatory molecules, such as CRP and cytokines, have been found, as well as increased leukocyte activation and adhesion molecules expression [39]. A recent meta-analysis showed that OSAS is associated with elevated levels of CRP, IL-6, TNF- α , IL-8 and adhesion molecules [43]. CRP levels were higher in OSAS subjects (22 untreated subjects) than in controls and significantly correlated with OSAS severity, expressed as AHI [44]. It has been suggested that the increased CRP production is induced by cytokines, such as IL-6, the synthesis of which is stimulated by the repeated hypoxia [44]. Some authors demonstrated elevated circulating levels of TNF- α and IL-6 in subjects with sleep apnea, positively associated with the presence of excessive daytime sleepiness, suggesting that also the sleep deprivation might be responsible for cytokine synthesis [45]. There is a strong interdependence between cytokines and sleep: cytokines are involved in the sleep regulation as they induce somnolence during the acute-phase response. In contrast, sleep deprivation induces biological changes in immune and endocrine systems, increasing cytokine production and activity [46]. Furthermore, the cytokine dysregulation seems to influence respiratory mechanisms, as the inflammatory status may alter the characteristics of the upper

airway, inducing pharyngeal edema and impairing the proprioceptive reflexes necessary to maintain the airways patency [47].

Kritikou *et al.* [48] reported some difference between apneic males and females: male OSAS subjects showed increased levels of CRP, IL-6 and TNF-receptor 1, and female OSAS subjects also showed higher CRP levels than controls. The short-term cPAP treatment did not improve the markers of inflammation [48]; at least 3 months of treatment were needed to reduce CRP levels [42].

Leukocyte production and expression of adhesion molecules was also increased in OSAS: circulating soluble adhesion molecules (ICAM-1, VCAM-1, E-selectin) were elevated in OSAS subjects with cardiovascular disease, and E-selectin levels seemed to be associated with OSAS severity [49]. The cPAP treatment also reduced these parameters of inflammation [49]. It has been suggested that even lymphocytes are involved in the atherosclerotic development and that, in OSAS, increased cytotoxicity and a cytokine imbalance in CD4 and CD8 T cells might contribute to the vascular injury [50].

Oxidative stress

Several reports have evaluated the oxidative/antioxidant status of OSAS subjects, observing an increase in lipid [51,52,53] and protein oxidation [53,54] and a decrease in antioxidant defenses [55]. ROS, such as the superoxide anion (O_2^-), can oxidize NO, producing peroxynitrite and other radicals and leading to the oxidation of carbohydrates, lipids and proteins [56]. In OSAS, superoxide anions are produced mostly by inflammatory leukocytes, by mitochondria during respiration and by hypoxia/reoxygenation events [56].

Celec *et al.* demonstrated elevated plasma levels of thiobarbituric acid-reacting substances (TBARS), markers of lipid peroxidation, in 89 subjects with severe OSAS (AHI > 30 per hour) [51]. The same finding has been observed by Barcelò *et al.* in a small group (n=14) of subjects with severe OSAS [57] and by Murri *et al.* in 28 subjects with sleep apnea/hypopnea syndrome requiring cPAP [58]. Papandreu *et al.* showed a positive correlation between TBARS and AHI and a negative correlation between TBARS and the lowest nocturnal oxygen saturation in 21 obese subjects with OSAS (mean AHI 45.5 ± 31.4 per hour) [59]. In a previously published study [60], we showed that TBARS was positively correlated with AHI and ODI (oxygen desaturation index) and negatively correlated with mean nocturnal oxygen saturation in 48 OSAS subjects (mean AHI 38.47 ± 25.66).

Vatansever *et al.* [61] showed that plasma concentration of malondialdehyde (MDA) was higher in subjects with severe OSAS, but not in those with mild OSAS, in comparison with

normal controls, and was significantly correlated with AHI values, while Chen *et al.* demonstrated a positive correlation between AHI and MDA in 44 subjects with mild to moderate OSA [62]. In contrast, Ntalapascha *et al.* found no increase in TBARS levels in 18 patients with severe OSAS (AHI > 30) [63]. Svatikova *et al.* in 41 subjects with moderate or severe OSAS (mean AHI 47 ± 3 per hour) [64] and demonstrated no significant differences in MDA levels between controls and OSAS subjects and these results were corroborated by other research groups [65,66].

Plasma TBARS [51,57,67] and MDA [61] levels may be improved by cPAP therapy. The levels of plasmatic 8-isoprostane are also found to be higher in subjects with OSAS without pulmonary or cardiac diseases, than in normal controls and these levels were reduced by cPAP therapy [68].

Regarding protein oxidation, an increase in advanced oxidation protein product (AOPP) levels has been found in subjects with severe OSAS [51], and a positive correlation between AOPP and AHI was demonstrated by Yang *et al.* in a group of 67 patients [69], but not by other authors [70]; however, protein carbonyl content was elevated in subjects with severe OSAS and significantly correlated with AHI value [60,61].

The total antioxidant status (TAS) was reduced in 32 OSAS subjects without comorbidity and mostly in subjects with mild-moderate disease (AHI < 30) [55]. Lloret *et al.* [71] observed lower levels of reduced glutathione in subjects with severe OSAS and an increase in oxidized glutathione levels during sleep apnea. Long-term cPAP increased TAS to normal levels [72]. Also the total antioxidant capacity (TAC) was reduced in subjects with OSAS; it was negatively correlated with OSAS severity and it increased after a 1-month cPAP treatment [58].

Blood coagulation and hemorheological abnormalities

In OSAS subjects, increased platelet activation and aggregation have been observed, especially during the nocturnal hours [73,74]. This activated platelet phenotype was correlated with the increased sympathetic tone and the consequent elevated values of circulating catecholamines [75]. Other authors found an overexpression of P-selectin on platelet surfaces, influenced by OSAS severity [73]. Chin *et al.* showed increased levels of fibrinogen and hematocrit in the morning in a small group of OSAS subjects, suggesting elevated blood viscosity [76]. Also, Nobili *et al.* showed increased plasma fibrinogen, which was correlated with AHI value and with nocturnal minimal oxygen saturation (SO_2), and increased blood viscosity [77]. Steiner *et al.* [78] observed a correlation between

plasma fibrinogen and AHI value and between fibrinogen and nocturnal minimal oxygen saturation (SO₂). Tazbirek *et al.* found elevated blood viscosity and erythrocyte aggregation in obese men with OSAS, in comparison with those without OSAS [79] while Sinnapah *et al.* [80] found a correlation between erythrocyte aggregation and AHI and between erythrocyte aggregation and BMI in overweight OSAS subjects. The erythrocyte deformability, obtained with a diffractometric method and expressed as elongation index (EI), was significantly reduced in 48 OSAS subjects (mean AHI 38.47±25.66) [36], even if other authors [79,81] did not find this alteration in erythrocyte deformability when employing other techniques. It has been demonstrated that treatment with cPAP reduces plasma fibrinogen [76] and blood and plasma viscosity [79].

Matrix metalloproteinases and their inhibitors

Matrix metalloproteinases (MMPs) form a large family of endopeptidases (collagenases, gelatinases, stromelysin and matrilysin), which are produced in the vascular wall and able to degrade several extracellular matrix proteins [82]. In particular, gelatinases A and B (MMP-2 and -9) are responsible for IV type collagen, gelatin and laminin degradation, vasculature remodelling, angiogenesis and inflammation, and they are also involved in the atherosclerotic process [83-85]. Once MMPs are secreted in the extracellular space, they may be activated by several proteases, including other MMPs, and ROS, in particular, peroxynitrite [86,87]. MMPs activity is also regulated by the four tissue inhibitors of MMP (TIMPs): TIMP-1 inhibits especially MMP-9 while TIMP-2 inhibits especially MMP-2 [88]. An altered expression of MMPs and TIMPs has been observed in OSAS, and in particular an increase in MMP-9 levels and activity [89-92].

Tazaki *et al.* observed a significant increase in MMP-9, but not in TIMP-1, serum levels in 44 obese OSAS subjects in comparison with obese controls [90]. They also found higher serum level and activity of MMP-9 in subjects with moderate to severe OSAS in comparison with subjects with only mild disease [90]. MMP-9 level and activity were also found to be significantly correlated with AHI, BMI and inflammatory cytokines, such as IL-6 and TNF- α [90]. Similarly, Ye *et al.* [41] described elevated serum levels of MMP-9 in 51 overweight OSAS subjects in comparison with control subjects, which correlated with OSAS severity and CRP. Chuang *et al.* [92] reported elevated plasma levels of MMP-1, -2, -3 and -9 and of TIMP-1 and also plasma MMP-9 activity in a small group of subjects (n=8) with mild to moderate OSAS. Chuang ob-

served elevated concentration and activity of MMP-9 but not MMPs or TIMP-1. In a group of 48 OSAS subjects, increased plasma concentrations of gelatinases (MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) have been found, as well as higher values of MMP-9 and TIMP-1 in subjects with AHI>30 in comparison with subjects with AHI<30 [93]. MMP-9 is correlated with some polysomnographic parameters, such as AHI, oxygen desaturation index (ODI) and mean oxygen saturation [93,94]. Long-term cPAP treatment decreased MMP-9 levels [89] as does uvulopalatal flap surgery [95].

Conclusions

OSAS is associated with an elevated cardiovascular risk, as demonstrated by several researchers, who have investigated different aspects of endothelial and vascular impairment. Intermittent hypoxia seems to be the pathophysiological link between oxidative stress, inflammation, NO bioavailability and MMP profile. cPAP treatment is the only therapeutic strategy able to modify all the mechanisms involved in the development of arterial hypertension, endothelial dysfunction and to reduce the risk of cardiovascular events.

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