European Review for Medical and Pharmacological Sciences 2020; 24: 6939-6948

Obstructive Sleep Apnea, oxidative stress, inflammation and endothelial dysfunction- An overview of predictive laboratory biomarkers

G. ORRÙ[1], M. STORARI[2], A. SCANO[1], V. PIRAS[2], R. TAIBI[3], D. VISCUSO[2]

[1]Department of Surgical Sciences, Molecular Biology Service (MBS), University of Cagliari, Cagliari, Italy
[2]Department of Surgical Science, Institute of Dentistry, University of Cagliari, Cagliari, Italy
[3]Department of Medical Oncology, Istituto Nazionale Tumori, Centro di Riferimento Oncologico di Aviano (PN), Italy

Abstract. – OBJECTIVE: Obstructive Sleep Apnea (OSA) represents an emerging public health concern with great impact on cardiovascular state. Oxidative stress (OS), inflammation and altered Nitric Oxide (NO) production are recognized as prominent mechanisms of many acute and chronic diseases and even of the normal aging process. They are investigated as major pathophysiological processes in OSA through the analysis and comparison of significative and validated biomarkers.

MATERIALS AND METHODS: The review is developed using as key terms "sleep apnea", "oxidative stress", "inflammation", and "endothelial dysfunction". Included studies must have followed the American Academy of Sleep Medicine guidelines according to the diagnosis and classification of OSA. Lipid, protein and DNA oxidation products, PCR, IL-6, IL-8, TNF-α, NO and nitrosative stress compounds, and endothelial functioning tests have been detected for their contribution in OSA along the last 3 decades.

RESULTS: Nocturnal intermittent hypoxia has emerged to be significantly associated to oxidative/nitrosative stress, increase in pro-inflammatory markers, imbalance in NO production, and endothelium impairment. Body Mass Index (BMI) contribution needs further clarifications. Continuous Positive Airway Pressure (CPAP) therapy has demonstrated beneficial effects on vascular function and pro-inflammatory milieu in OSA.

CONCLUSIONS: Oxidative stress and Inflammation significantly correlate with OSA; similarly, vascular functioning is impaired in accordance to unregulated levels of NO and derived compounds. Continuous Positive Airway Pressure markedly improves oxidative stress, inflammation and endothelial dysfunction in OSA.

Key Words:
Sleep apnea, Oxidative stress, Inflammation, Endothelial dysfunction.

Introduction

Obstructive Sleep Apnea (OSA) is a common Sleep Breathing Disorders (SBD) recognized as a major public health concern. Approximately, 1 of every 5 adults suffer from at least mild OSA and 1 of every 15 from moderate up to severe OSA[1-4]. Men are 2 to 3 times more prone to develop OSA[1,5] and the risk tends to increase after the middle age[2-4,6]. OSA consists in repetitive episodes of upper airways' collapse, especially at the level of oropharynx. It causes the complete or partial and intermittent airflow obstruction during sleep associated to respiratory efforts. Obstructions result from variable combinations of anatomic factors which predispose to airways' collapse during inspiration and neuromuscular compensations unable to maintain the patency[7]. The subsequent alveolar hypoventilation induces sleep fragmentation *via* repeated Central Nervous System (CNS) activations, called arousals, responsible for brief awakenings[8]. Airways' collapse and intermittent hypoxia contribute to develop alterations in metabolism and immune system[9]. Some studies[10,11] support an increased infarction of pro-inflammatory molecules and cells in upper airways' tissues. An imbalance in oxidative processes may also emerge; beside mechanical trauma, cycling hypoxia-reoxygenation events are referred as possible causative factors[12]. Such phenomena augment both the expression of adhesion molecules and the release of free radical species and inflammatory cytokines. Systemic inflammation and endothelial damage are unavoidable consequences[9]. OSA is therefore recognized as an independent risk factor for several cardiovascular diseases (CVDs), including hypertension and coronary artery disease[13,14]. A 10-unit average increase in the Apnea-Hypopnea Index (AHI) relates to a 17% greater risk in such conditions[15]. Furthermore, it is also associated with

Corresponding Author: Domenico Viscuso, MD; e-mail: d.viscuso@libero.it

obesity, metabolic syndrome (MetS), dyslipidemia and insulin-resistance[16-18]. Inflammation and oxidative stress represent primary pathogenic factors for all these diseases, whether their causal or propagating role remains unresolved[19,20].

Materials and Methods

The current review examines the presence of oxidative stress, inflammation and imbalance in Nitric Oxide (NO) production in patients suffering from OSA. For this purpose, a search in PubMed has been performed using as key terms "sleep apnea", "oxidative stress", "inflammation" and "endothelial dysfunction". Only full-length original publications dealing with human subjects affected from OSA since 1990 have been taken into consideration. Reviews, meta-analysis and case reports/series have been excluded. Lipid, protein and DNA oxidative products, C-Reactive Protein (CRP), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-α (TNF-α), NO and nitrosative stress compounds have been selected as representative biomarkers (Table I). Endothelial dysfunction has been further investigated *via* the study of Arterial Flow-Mediated Dilation (FMD), Endothelial Progenitor Cells (EPCs) and Morning Reactive Hyperemia (MRH) tests. Other inclusion criteria refer to: a) diagnosis of OSA performed *via* an overnight full polysomnography (PSG) [including: electroencephalography, electrooculography, electromyography, electrocardiography, upper airways ventilatory flow, chest and abdomen movements, O2 saturation (SaO2)]; b) apnea defined as a complete cessation of airflow for at least 10 seconds; c) hypopnea defined as a substantial reduction in airflow (> 50%) for at least 10 seconds or a moderate reduction in airflow for at least 10 seconds associated with electroencephalographic arousals or oxygen desaturation (= 4%); d) the average number of apnea plus hypopnea events/h of sleep defines the AHI: 0-4.9 no OSA, 5-14.9 mild OSA, 15-29.9 moderate OSA, and > 30 severe OSA; e) Continuous Positive Airway Pressure (CPAP) administered when AHI ≥ 15 events/h. A total of 33 studies met the inclusion criteria.

Results

Oxidative Stress

Free radicals are defined as atoms or molecules with at least one uncoupled electron in the outer orbit, thus prone to chemical reactions[21]. For over 60 years their role in human pathology has been recognized[21-23]. Although considered initially merely toxic by-products of the oxidative metabolism, they have been progressively described as important mediators of several pathophysiological processes. Reactive radicals are released by leukocytes as defensive mechanism against microorganisms[24], promote ischemia-reperfusion injurie[25] and take part in numerous signal pathways[26]. However, as normally produced during aerobic respiration, protective endogenous mechanisms arose in order to maintain them under rigorous control. Oxidative stress consists therefore in the excessive accumulation of free radicals due to an imbalance between antioxidant defenses and oxidants' productive system. Predominant Reactive Oxygen Species (ROS) are Superoxide (O_2^-), Hydrogen Peroxide (H_2O_2), Hydroxyl Radical (OH^-) and Lipid Peroxides. Additionally, Reactive Nitrogen Species (RNS) can cooperate or overlap ROS, such as Peroxynitrite ($OONO^-$) which formed during the reaction between O_2^- and NO. Excessive ROS and RNS damage important cellular components, such as lipids, proteins, carbohydrates and even nucleic acids, altering their overall functioning. Thus, they participate in the onset and progression of numerous diseases[27], OSA included[28]. However, ROS have an extremely short half-life by virtue of their high reactivity. Compounds formed from the reaction between ROS and organic biomolecules show more stability and thus allow more objective measurements of oxidative stress in tissues or fluids[29].

Lipid Peroxidation

Lipid peroxidation (LPO) is one of the most well-known forms of oxidative damage. It affects lipidic membranes, lipoproteins and other molecules that contain lipids. Malondialdehyde (MDA) is the prototypical LPO end-product and derives from the peroxidative decomposition of unsaturated fatty acids. MDA reacts with proteins, mainly with Lys residues, and alters their physiological properties forming MDA-modified protein adducts which act as autoantibodies[29]. ThioBarbituric Acid Reactive Substances (TBARS) is the oldest and widest assay used to measure MDA[29]. The relevance of MDA is further underlined because of its contribution in Diabetes Mellitus (DM)[30] and atherosclerosis[31]. Higher levels of MDA have been also observed in asthma[32] and neurological diseases[33]. MDA role in OSA still remains controversial.

Table I. Relationship of different biomarkers studied in OSA patients and relative trend with the disease severity (AHI) and after CPAP treatment.

Biomarker	Authors/ reference	nOSA	OSA	AHI	Controls/OSA biomarker value	After CPAP
TBARS	Jurado-Gamez et al[34]	23	46	n.a.	↑*	↓*
	Oyama et al[35]	n.a.	32	+*	n.a.	↓*
	Hopps et al[36]	n.a	48	+*	n.a.	n.a.
	Kang et al[37]	7	44	-	↔	n.a.
	Svatikova et al[38]	35	41	n.a.	↔	n.a.
IsoPs	Carpagnano et a[139]	12	18	+*	↑*	↓*
	Passali et al[40]	20	20	n.a.	↑*	↓*
	Minoguchi et al[41]	30	40	+*	v	↓#
	Karamanli et al[42]	n.a.	35	n.a.	n.a.	↓#
PCs	Jurado-Gamez et al[34]	23	46	n.a.	↔	↓#
	Hopps et al[36]	n.a.	48	+*	n.a.	n.a.
	Passali et al[40]	20	20	n.a.	↑*	n.a.
8-OHdG)	Jurado-Gamez et al[34]	23	46	n.a.	↑*	↓*
	Teramoto et al[43]	160	160	+#	↓*	n.a.
Comet assay	Kang et al[37]	7	44	n.a.	↔	n.a.
CRP	Minoguchi et al[41]	30	40	+*	↑*	↓#
	Karamanli et al[42]	n.a.	35	n.a.	n.a.	↔
	Taheri et al[49]	623	284	-	↔	n.a.
	Chami et al[50]	542	358	-	↑*	n.a.
	Chung et al[51]	22	68	-	↔	n.a.
	Peled et al[52]	9	89	-	↑*	n.a.
	Korktmaz et al[53]	40	107	-	↔	n.a.
	Guilleminault et al[54]	54	146	-	↔	n.a.
	Shamsuzzaman et al[55]	20	22	+#	↑*	n.a.
	Panoutsopoulos et al[56]	18	20	+*	↑*	↓*
	Yüksel et al[57]	15	51	+*	↑*	n.a.
	Andaku et al[58]	10	25	+#	↑#°	n.a.
IL-6	Oyama et al[35]	n.a.	32	n.a.	n.a.	↓*
	Karamanli et al[42]	n.a.	35	n.a.	n.a.	↓°°
	Chami et al[50]	542	358	+*	↑*	n.a.
	Vgontzas et al[63]	10	12	n.a.	↑#	n.a.
	Vgontzas et al[64]	28	16	n.a.	↑#	↔
	Zhang et al[65]	30	75	+*	↑*	n.a.
IL-8	Oyama et al[35]	n.a.	32	n.a.	n.a.	↓*
	Zhang et al[65]	30	75	+*	↑*	n.a.
TNF-α/r1/r2	Oyama et al[35]	n.a.	32	+#	n.a.	↓#
	Karamanli et al[42]	n.a.	35	n.a.	n.a.	↓°°
	Chami et al[50]	542	358	-	↔	n.a.
	Vgontzas et al[63]	10	12	n.a.	↑*	n.a.
	Vgontzas et al[64]	28	16	n.a.	↑*	↔
NO-Tyrs	Karamanli et al[42]	n.a.	35	n.a.	n.a.	↓#
	Jelic et al[81]	15	30	+	↑*	↓*
NO	Oyama et al[35]	n.a.	32	+*	n.a.	↑*
	Yüksel et al[57]	15	51	-	↓#	n.a.
	Ip et al[83]	40	30	+*	↓#	↑*
eNO	Zhang et al[65]	30	75	+#	↑*	n.a.
	Olopade et al[85]	8	20	n.a.	↑#	n.a.
	JalilMirmohammadi et al[86]	7	47	n.a.	↑#°°°	n.a.
	Duong-Quy et al[87]	30	52	+#	↑*	n.a.
eNOS	Jelic et al[81]	15	30	+*	↓*	↑*
iNOS	Jelic et al[81]	15	30	-	↑#	↓*
ADMA	Oyama et al[35]	n.a.	32	+*	n.a.	↓*

* = p-value≤0.01; # = p-value<0.05; + = Presence of correlation; - = Absence of correlation; ↑ = Increased; ↓: Decreased; ↔ = Unvaried; ° = In presence of Excessive Daytime Sleepiness; °° = In EBC and not in plasma; °°° = In obese patients.

Significative statistical differences (p=0.001) have emerged between healthy individuals, 1.6 µM (1.5-1.8), and OSA patients, 2.6 µM (1.9-3.7)[34]. The same study has also demonstrated improvements after 3 months of CPAP therapy, worn at least 4 hours/night, [p=0.001, MDA 1.9 µM (1.6-2.2)]. Similar results have been obtained by other authors[35]. Positive correlations have been found between TBARS and Apnea-Hypopnea Index (AHI) (p<0.01)[35,36] and Oxygen Desaturation Index (ODI) (p<0.0001), while negative with Mean Oxygen Saturation (mSO_2) (p<0.0003)[35]. On the contrary, other trials have not found out any relevant difference related to OSA severity (p>0.05)[37,38].

Isoprostanes (IsoPs) are compounds generated by a nonenzymatic and free radicals-catalyzed peroxidation of esterified arachidonic acid. Widespread in human tissues, they act as vasoconstrictors in lungs and kidneys and regulate platelets' functions. They are also useful markers of inflammation, ischemia-reperfusion injury, atherosclerosis and DM[29]. IsoPs appear meaningful in OSA. 8-Isoprostane (8-IsoP) has demonstrated to be significantly higher in OSA patients than healthy subjects in both plasma and Exhaled Breath Condensate (EBC) (p<0.0001, both)[39]. Such evidence has been further confirmed[40]. Overnight urinary excretion of 8-IsoP has shown positive correlations with OSA severity (p=0.0004), independently to the Body Mass Index (BMI) (p<0.0001 and p<0.001 in relation to lean and obese subjects without OSA, respectively) [41]. CPAP has proven to be able to restore 8-IsoP (p<0.03)[41]. A significant lowering of 8-IsoP has been detected after CPAP treatment in both serum (p=0.019)[42] and EBC[39]. Conversely, plasma levels remained comparable between affected patients and controls in another trial (p=0.74)[38].

Protein Oxidation

The carbonyls compounds measurement represents the most used technique to study protein oxidation. Protein Carbonyls (PCs) are Advanced Oxidation Protein Products (AOPPs) and they originate *via* the oxidation of several amino acids. They are chemically stable and tend to accumulate in tissues during normal aging, age-related diseases, chronic inflammation and ischemia-reperfusion injury[29]. PCs impact on OSA remains debatable. No significant differences have been found in plasma between non OSA and OSA patients, respectively 0.09 nmol/mg (0.04-0.12) and 0.07 nmol/mg (0.05–0.15) (p=0.498)[34]. Such evidence has been contradicted by other authors[40] (p<0.0001) who have further attributed to PCs 100% specificity and 100% sensitivity as oxidative stress biomarkers in OSA. Negative correlations have been observed with mSO2 (p<0.001) while positive with AHI (p<0.0001) and ODI (p<0.0001)[36]. Neck and waist circumferences have been also appeared to influence PCs levels (p<0.0001 and p<0.02, respectively)[36]. However, a notable decrease has been reported after 3 months in patients with severe OSA treated with CPAP (p=0.021)[34].

DNA Oxidation

Numerous techniques have been developed to measure the oxidatively modified nucleic acids. However, such methods need further validations. Additionally, limitations exist regarding which tissues provide accurate samples[29]. 8-hydroxy-2-deoxyguanosine (8-OHdG) may be considered a reliable index of oxidative DNA damage[29]. Recently, higher plasma levels of 8-OHdG have been reported in patients affected from OSA, 107 ng/ml (104-111), in contrast to normal individuals, 103 ng/ml (88-105) (p=0.001)[34]. Such evidence has been further corroborated. Urinary excretion of 8-OHdG has appeared significantly greater in middle-aged and elderly OSA patients compared to age and BMI-matched controls[43]. Positive relationships have been also found with AHI and ODI[43]. CPAP has revealed effective in lowering 8-OHdG plasma levels after 3 months (p=0.001), from 107 ng/ml (105-114) to 102 ng/ml (101-106)[34]. Another useful technique to study DNA damage is Alkaline Single-Cell Gel Electrophoresis, or Comet Assay[44], albeit, when used, no correlations have emerged among OSA and DNA oxidative damage[37].

Inflammatory Markers

Sleeping and breathing modifications in OSA may predispose to increased local and systemic inflammation with a great impact on related comorbidities outcomes.

CRP is an acute-phase protein. It is highly stable and does not undergo diurnal variations[45]. Therefore, it represents an important measure of inflammation[46]. CRP has also emerged to be strongly predictive of cardiovascular risk[47,48]. CRP may be considered a suggestive marker of the association between sleep architecture modifications in SBD and related comorbidities. The Wisconsin Sleep Cohort Study (WSCS) performed a large population-based study demonstrating that higher CRP serum levels in OSA

patients were dependent to BMI (p<0.0001) rather than AHI severity (p=0.32, 5<AHI<15; p=0.76, AHI>15)[49]. Similar results have been reported by other authors after introducing BMI-based adjusted models[50-52]. BMI has been moreover found significantly associated to higher CRP levels if considered both OSA and control patients together (p<0.01) and OSA patients alone (p<0.01)[53]. On the contrary, although one study[54] has reported no correlations, other trials[55,56] have shown independent associations between CRP and OSA severity. CRP has appeared significantly greater in moderate to severe OSA, considering both lean and obese control subjects (p<0.01, both)[41]. CRP has been also found positively correlated with OSA independently to CVDs (1.0±0.7 mg/dl, control; 6.0±3.6 mg/dl, OSA without CVDs; 6.2±3.9 mg/dl, OSA with CVDs; p<0.001)[57], while Excessive Daytime Sleepiness (EDS) has emerged to influence it (p<0.05)[58]. In addition, the appropriate use of CPAP seems to ameliorate significantly CRP levels (p<0.01)[55], albeit there are no univocal results[42].

Intermittent hypoxemia and sleep deprivation are associated with increased levels of IL-6[59,60]. IL-6 is a pro-inflammatory cytokine greatly involved in the genesis and progression of CVDs[61,62]. Findings of the association between IL-6 and OSA (p=0.02) have been shown in a large population study based on a demographic multivariable and BMI adjusted model[50]. Patients with an AHI>30 events/ hours have revealed mean IL-6 circulating levels 0.69 pg/ml greater than healthy subjects[50]. A similar difference has been reported between smokers/non-smokers and in the development of myocardial infarction[61]. However, Vgontzas et al[63] evidenced a significant relationship between IL-6 and BMI (p<0.001). IL-6 values have been found higher in patients with OSA, intermediate in obese controls and lower in non-obese controls (linear trend p<0.05)[64]. Visceral fat has emerged to be a strong predictor of increased IL-6 in OSA (p<0.01)[64], albeit EDS (p<0.044) and mSO_2 (p=0.001) contributions should not be neglected[63]. Additionally, among non-smokers individuals, IL-6 has recently appeared notably greater in nasal lavage samples of OSA patients than controls (p<0.001), likewise *IL-8* (p<0.001)[65]. After 3 months, CPAP markedly improves plasma levels of both IL-6 (p<0.01) and IL-8 (p<0.01)[35]. Oppositely, Karamanli et al[42] have demonstrated a significant decreased of IL-6 only in EBC (p<0.001) but not in serum (p=0.074).

TNF-α represents one of the major regulators of inflammation. TNF-α contributes to vasodilatation, edema formation, leukocytes' adhesion and coagulation. It is also significantly implicated into oxidative stress[66]. TNF-α has been positively related to OSA (p<0.01) and to EDS (p<0.001), but not to BMI[63]. Furthermore, obese OSA patients have shown an increased expression of TNF Receptor 1 (TNF-rl) if compared to both obese and lean controls (1641.4±78.1 pg/ml, 1489.5±88.8 pg/ml and 1307.3±57.2 pg/ml, respectively; linear trend p<0.01)[64]. Thus, OSA, but not fat, has appeared to be associated with TNF-rl levels (p<0.05)[64]. A similar trend regards TNF-α itself, albeit without statistical significance (1.7±0.3pg/ml, 1.5±0.2 pg/ml and 1.4±0.2 pg/ml; linear trend p=0.09)[64]. On the contrary, Chami et al[50] proved the absence of correlations between OSA and TNF-α. CPAP significantly decreases TNF-α in EBC (p<0.001) after 3 months[41] whereas controversial results have appeared for serum levels[35,42,64].

Nitric Oxid-Related Compounds

NO is an endogenous signaling molecule generated from L-Arginine by Nitric Oxide Synthases (NOSs). Several NOS-independent pathways exist, too[67]. 3 different isoforms of NOS have been mainly described: Neuronal NOS (nNOS or NOS-1)[68], Inducible NOS (iNOS or NOS-2)[69,70] and Endothelial NOS (eNOS or NOS3)[71-73]. nNOS and eNOS are constitutively expressed (cNOS). They produce small amounts of NO on a moment-to-moment principle[74] and regulate neuronal impulses and the vascular smooth musculature tone. iNOS, by contrast, belongs to innate immunity. It is synthetized *ex novo* under pro-inflammatory stimuli and is associated to a greater and prolonged NO production[75-77]. The altered production of NO has been implicated in several pathological conditions, such as cancer, DM, neurologic, immune and vascular diseases[67]. NO is a gas characterized by a very short half-life because it is rapidly converted into nitrites ($NO2^-$) and nitrates ($NO3^-$). Although several scavenging mechanisms exist, NO can form very unstable and highly reactive species, such as NO^- (Nitrosonium), NO^- (Nitroxyl anion) or $ONOO^-$. $NO2^-$ are themselves major oxidation products capable of inducing proteins' nitration[67]. NO dysregulations may affect OSA long-term complications.

Nitrated proteins can be considered reliable markers of nitrosative stress because of the association with numerous pathological cellular models, including ischemia-reperfusion injury[78]. Little is known about Nitrotyrosins' (NO-Tyrs) impact on OSA. However, studies[79,80] suggest a key role in

CVDs, important comorbidities of SBD. NO-Tyrs have demonstrated to be 5- up to 7-fold higher, according to the severity, in patients affected from OSA than healthy individuals (p=0.001)[81]. Such evidence is further validated through improvements reported in both blood (p=0.032) and EBC (p=0.037) levels after CPAP[42].

NO itself regulates the airways' patency *via* both muscular and nervous pathways. As most stable endogenous metabolites of NO, $NO2^-$ and $NO3^-$ assess indirectly NO production in laboratory measurements[82]. $NO2^-$ and $NO3^-$ have been found 2-fold higher in healthy individuals measuring the AHI (p=0.001) and the time with SaO2<90% (p=0.004)[83]. Similar results have been recently confirmed (28.1±24.1 μM in OSA patients and 43.4±32.1 μM in controls; p<0.05)[57]. On the contrary, iNOS has resulted to be highly expressed in OSA (p=0.04)[81]. CPAP seems to markedly increase serum $NO2^-$ and $NO3^-$[35,83]. After one night, patients diagnosed with moderate to severe OSA have shown comparable levels to that of control subjects[83]. CPAP has moreover demonstrated to notably restore eNOS and iNOS expression in circulating EPCs, marker of endothelial repair capacity (p<0.001, for both)[81]. CPAP decreases also blood levels of Asymmetrical Dimethylarginine (ADMA), an endogenous NOS inhibitor (p<0.01)[35].

Exhaled forms of NO have been suggested as noninvasive biomarkers of airways' inflammation by virtue of NO influence in vasodilation and chemotaxis[84]. Various authors have investigated the relationship between Exhaled Nitric Oxide (eNO) and OSA. Increased oral and nasal eNO has been reported after sleep, if compared with pre sleep levels, in patients affected from moderate to severe OSA (p<0.05)[85]. However, a significant difference in oral eNO has been reported even in non OSA individuals (p<0.05)[85]. After sleep nasal eNO has appeared higher in both OSA (p<0.001) and control (p=0.034) patients whereas the difference has remained significantly between the 2 groups before (p<0.001) and after sleep (p=0.001)[65]. In addition, eNO has been revealed greater only in obese patients after sleep (p<0.05), suggesting the role of BMI as confounder in airways inflammation[86]. Exhaled Fraction of NO (FENO), Maximal Bronchial Production Rate of NO (J'awNO) and Alveolar Concentration of NO (CANO) are additional parameters to measure eNO. The correlation with AHI has been demonstrated for FENO (p=0.007), CANO p=0.03) and J'awNO (p<0.0001)[87]. All these 3 parameters have shown significant differences between patients and controls[87].

Endothelial Dysfunction

Chronic intermittent hypoxia-reoxygenation affects the vascular micromilieu *via* morphological and functional modifications. Sympathetic over-activation, insulin-resistance, oxidative stress, inflammation and alteration in NO-based vascular vasodilation are the predominating forces responsible for the subsequent endothelium impairment[88]. Injuries of the endothelial wall are well-established major pathogenic mechanisms of CVDs. Thus, they may explain the increased cardiovascular morbidity and mortality in OSA[89-91] (Figure 1). FMD represents a validated noninvasive method to quantify endothelial dysfunction and endothelial NO-mediated reactivity[92,93]. It consists in measuring the brachial artery diameter after having induced reactive hyperemia[92,93]. Patients affected from OSA have shown significantly lower FMD percentages when compared to non OSA individuals[81,94,95]. BMI seems not to influence the response[51,56]. Chung et al[51] support important differences only in severe OSA (p<0.05). The WSCS conversely has demonstrated a reduction of 0.55% in FMD for each 2-fold increase in AHI only in individuals suffering from MetS (p=0.015)[96]. No correlations have emerged in no-MetS patients (p=0.42)[96]. However, MetS has been positively associated with both SBD (p<0.001) and lower FMD (4.3% median in MetS; 5.5% median in no-MetS; p=0.028)[96]. CPAP has demonstrated to be effective in ameliorating FMD[56,81,97], especially in proportion to hours of use (p<0.001)[97]. CPAP has further shown to significantly increase MRH. Significative results have emerged *via* a laser doppler analysis (p<0.001)[34] and measuring the Forearm Blood Flow (FBF) response to induced venous occlusion (p<0.01)[35]. Lastly, as markers of endothelial repair, reduced EPCs levels have been associated with vascular impairment and increased cardiovascular risk[98,99]. EPCs have appeared lower in OSA patients than controls (p<0.001)[81,94]. Daily use of CPAP has markedly improved their blood levels (p=0.03)[94], equaling those of controls (p=0.15)[81].

Conclusions

In summary, OSA greatly affects the quality of life of those who suffer from, *per* se and *via* the numerous comorbidities associated. Increased morbidity and mortality are well-documented in OSA patients, whereas the pathogenesis is not yet fully understood, likewise the linkage with cardiovascular and metabolic diseases. Oxidative

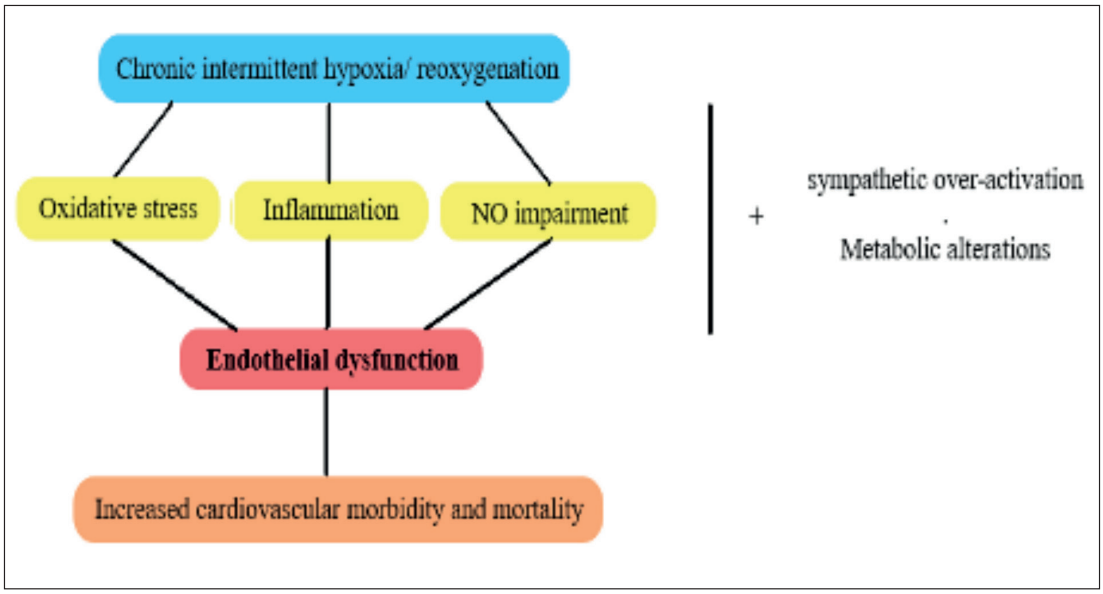

Figure 1. Schematic representation of the pathogenic processes responsible for the endothelial morphological and functional impairment in OSA.

stress, pro-inflammatory state and NO-dependent endothelial dysregulation have been undoubtedly recognized as main promoter for the onset and the progression of such affections. They have been suggested to play a key role even in breathing disorders. In the current review, OSA has emerged as a potential independent milieu responsible for the augmented production of free radicals and inflammatory cytokines. Additionally, it seems to cause vascular dysfunction. However, the contribution of body weight remains debatable. Furthermore, CPAP has demonstrated to significantly ameliorate biomarkers values and endothelial responsiveness to induced hyperemia.

Conflict of Interests

All authors disclose no financial and personal relationships with people or organizations that could inappropriately influence this manuscript.

References

1) Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328: 1230-1235.
2) Bixler E, Vgontzas A, Ten Have T, Tyson K, Kales A. Effects of age on sleep apnea in men. Am J Respir Crit Care Med 1998; 157: 144-148.
3) Bixler E, Vgontzas A, Lin H, Ten Have T, Rein J, Vela-Bueno A, Kales A. Prevalence of sleep-disordered breathing in women. Am J Respir Crit Care Med 2001; 163: 608-613.
4) Durán J, Esnaola S, Rubio R, Iztueta A. Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. Am J Respir Crit Care Med 2001; 163: 685-689.
5) Strohl K, Redline S. Recognition of obstructive sleep apnea. Am J Respir Crit Care Med 1996; 154: 274-289.
6) Redline S. Epidemiology of sleep-disordered breathing. Semin Respir Crit Care Med 1998; 19: 113-122.
7) Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002; 165: 1217-1239.
8) Parish JM, Somers VK. Obstructive sleep apnea and cardiovascular disease. Mayo Clin Proc 2004; 79: 1036-1046.
9) Faraut B, Boudjeltia KZ, Vanhamme L, Kerkhofs M. Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery. Sleep Med Rev 2012; 16: 137-149.
10) Kimoff RJ, Hamid Q, Divangahi M, Hussain S, Bao W, Naor N, Payne RJ, Ariyarajah A, Mulrain K, Petrof BJ. Increased upper airway cytokines and oxidative stress in severe obstructive sleep apnoea. Eur Respir J 2011; 38: 89-97.
11) Boyd JH, Petrof BJ, Hamid Q, Fraser R, Kimoff RJ. Upper airway muscle inflammation and denervation changes in obstructive sleep apnea. Am J Respir Crit Care Med 2004; 170: 541-546.
12) Lavie P. Obstructive sleep apnea syndrome: an oxidative stress disorder. Sleep Med Rev 2003; 7: 35-51.

13) Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000; 342: 1378-1384.
14) Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. Lancet 2005; 365: 1046-1053.
15) Wang X, Ouyang Y, Wang Z, Zhao G, Liu L, Bi Y. Obstructive sleep apnea and risk of cardiovascular disease and all-cause mortality: a meta-analysis of prospective cohort studies. Int J Cardiol 2013; 169: 207-214.
16) Peled N, Kassirer M, Shitrit D, Kogan Y, Shlomi D, Berliner AS, Kramer MR. The association of OSA with insulin resistance, inflammation and metabolic syndrome. Respir Med 2007; 101: 1696-1701.
17) Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E. Sleep loss: a novel risk factor for insulin resistance and type 2 diabetes. J Appl Physiol 2005; 99: 2008-2019.
18) Calvin AD, Albuquerque FN, Lopez-Jimenez F, Somers VK. Obstructive sleep apnea, inflammation, and the metabolic syndrome. Metab Syndr Relat Disord 2009; 7: 271-277.
19) Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84: 1381-1478.
20) Ischiropoulos H, Beckman JS. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J Clin Invest 2003; 111: 163-169.
21) Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.
22) Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11: 298-300.
23) Harman D. Atherosclerosis: a hypothesis concerning the initiating steps in pathogenesis. J Gerontol 1957; 12: 199-202.
24) Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 1973; 52: 741-744.
25) Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterology 1981; 81: 22-29.
26) Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. Free Radic Biol Med 1997; 22: 269-285.
27) McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000; 108: 652-659.
28) Eisele HJ, Markart P, Schulz R. Obstructive sleep apnea, oxidative stress, and cardiovascular disease: Evidence from Human Studies. Oxid Med Cell Longev 2015; 2015: 608438.
29) Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006; 52: 601-623.
30) Slatter DA, Bolton CH, Bailey AJ. The importance of lipid-derived malondialdehyde in diabetes mellitus. Diabetologia 2000; 43: 550-557.
31) Berliner J, Heinecke JW. The role of oxidized lipoproteins in atherogenesis. Free Radic Biol Med 1996; 20: 707-727.
32) Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. Eur Respir J 2003; 21: 177-186.
33) Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004; 3: 205-214.
34) Jurado-Gamez B, Fernandez-Marin MC, Gomez-Chaparro JL, Munoz-Cabrera L, Lopez-Barea J, Perez-Jimenez F, Lopez-Miranda J. Relationship of oxidative stress and endothelial dysfunction in sleep apnoea. Eur Respir J 2011; 37: 873-879.
35) Oyama JC, Yamamoto H, Maeda T, Ito A, Node K, Makino N. Continuous positive airway pressure therapy improves vascular dysfunction and decreases oxidative stress in patients with the metabolic syndrome and obstructive sleep apnea syndrome. Clin Cardiol 2012; 35: 231-236.
36) Hopps E, Canino B, Calandrino V, Montana M, Lo Presti R, Caimi G. Lipid peroxidation and protein oxidation are related to the severity of OSAS. Eur Rev Med Pharmacol Sci 2014; 18: 3773-3778.
37) Kang IG, Jung JH, Kim ST. The Effect of obstructive sleep apnea on DNA damage and oxidative stress. Clin Exp Otorhinolaryngol 2013; 6: 68-72.
38) Svatikova A, Wolk R, Lerman LO, Juncos LA, Greene EL, McConnell JP, Somers VK. Oxidative stress in obstructive sleep apnoea. Eur Heart J 2005; 26: 2435-2439.
39) Carpagnano GE, Kharitonov SA, Resta O, Foschino-Barbaro MP, Gramiccioni E, Barnes PJ. 8-Isoprostane, a marker of oxidative stress, is increased in exhaled breath condensate of patients with obstructive sleep apnea after night and is reduced by continuous positive airway pressure therapy. Chest 2003; 124: 1386-1392.
40) Passali D, Corallo G, Yaremchuk S, Longini M, Proietti F, Passali GC, Bellussi L. Oxidative stress in patients with obstructive sleep apnoea syndrome. Acta Otorhinolaryngol Ital 2015; 35: 420-425.
41) Minoguchi K, Yokoe T, Tanaka A, Ohta S, Hirano T, Yoshino, O'Donnell CP, Adachi M. Association between lipid peroxidation and inflammation in obstructive sleep apnoea. Eur Respir J 2006; 28: 378-385.
42) Karamanli H, Ozol D, Ugur KS, Yildirim Z, Armutc¸ u F, Bozkurt B, Yigitoglu R. Influence of CPAP treatment on airway and systemic inflammation in OSAS patients. Sleep Breath 2014; 18: 251-256.
43) Teramoto S, Yamaguchi Y, Yamamoto H, Hanaoka Y, Ishii M, Shinichiro H, Kume H, Akishita M, Ouchi Y. Increase in oxidative stress levels in elderly patients with obstructive sleep apnea syndrome: effects of age and sex. J Am Geriatr Soc 2008; 56: 569-571.
44) Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184-191.
45) Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. Clin Chem 2001; 47: 426-430.

46) Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem 2004; 279: 48487-48490.
47) Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001; 103: 1813-1818.
48) Bassuk SS, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. Curr Probl Cardiol 2004; 29: 439-493.
49) Taheri S, Austin D, Lin L, Nieto J, Young T, Mignot E. Correlates of serum C-reactive protein (CRP)--no association with sleep duration or sleep disordered breathing. Sleep 2007; 30: 991-996.
50) Chami HA, Fontes JD, Vasan RS, Keaney Jr JF, O'Connor GT, MD, Larson MT, Benjamin EJ, Gottlieb DJ. Vascular inflammation and sleep disordered breathing in a community-based cohort. Sleep 2013; 36: 763-768.
51) Chung S, Yoon IY, Shin YK, Lee CH, Kim JW, Lee T, Choi DJ, Ahn HJ. Endothelial dysfunction and C-reactive protein in relation with the severity of obstructive sleep apnea syndrome. Sleep 2007; 30: 997-1001.
52) Peled N, Kassirer M, Shitrit D, Kogan Y, Shlomi D, Berliner AS, Kramer RM. The association of OSA with insulin resistance, inflammation and metabolic syndrome. Respir Med 2007; 101: 1696-1701.
53) Guilleminault C, Kirisoglu C, Ohayon MM. C-reactive protein and sleep disordered breathing. Sleep 2004; 27: 1507-1511.
54) Korkmaz M, Korkmaz H, Kucuker F, Ayyildiz S, Cankaya S. Evaluation of the association of sleep apnea- related systemic inflammation with CRP, ESR, and neutrophil-to-lymphocyte ratio. Med Sci Monit 2015; 21: 477-481.
55) Shamsuzzaman ASM, Winnicki M, Lanfranchi P, Wolk R, Kara T, Accurso V, Somers VK. Elevated C-reactive protein in patients with obstructive sleep apnea. Circulation 2002; 105: 2462-2464.
56) Panoutsopoulos A, KalllianoA, Kostopoulos K, Seretis C, Koufogiorga E, Protogerou A, Trakada G, Kostopoulos C, Zakopoulos N, Nikolopoulos I. Effect of CPAP treatment on endothelial function and plasma CRP levels in patients with sleep apnea. Med Sci Monit 2012; 18: 747-751.
57) Yüksel M, Okur HK, Pelin Z, Öğünç AV, Öztürk L. Arginase activity and nitric oxide levels in patients with obstructive sleep apnea syndrome. Clinics (Sao Paulo) 2014; 69: 247-252.
58) Andaku DK, D'almeida V, Carneiro G, Hix S, Tufik S, Togeiro SM. Sleepiness, inflammation and oxidative stress markers in middle-aged males with obstructive sleep apnea without metabolic syndrome: a cross-sectional study. Respiratory Research 2015; 16: 3.
59) Greenberg H, Ye X, Wilson D, Htoo AK, Hendersen T, Liu SF. Chronic intermittent hypoxia activates nuclear factor-kappa-B in cardiovascular tissues in vivo. Biochem Biophys Res Commun 2006; 343: 591-596.
60) Vgontaz AN, Zoumakis E, Bixler EO. Adverse effects of modest sleep deprivation on sleepiness performance and inflammatory cytokines. J Clin Endocrinol Metab 2004; 89: 2119-2126.
61) Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000; 101: 1767-1772.
62) Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000; 148: 209-214.
63) Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 1997; 82: 1313-1316.
64) Vgontzas AN, Zoumakis E, Bixler EO, Lin HM, Collins B, Basta M, Pejovic S, Chrousos GP. Selective effects of CPAP on sleep apnoea-associated manifestations. Eur J Clin Invest 2008; 38: 585-595.
65) Zhang D, Xiao Y, Luo J, Wang X, Qiao Y, Huang R, Wu W. Measurement of fractional exhaled nitric oxide and nasal nitric oxide in male patients with obstructive sleep apnea. Sleep Breath 2019; 23: 785-793.
66) Zelová H, Hošek J. TNF-α signalling and inflammation: interactions between old acquaintances. Inflamm Res 2013; 62: 641-651.
67) Bian K, Murad F. Nitric oxide (NO)-biogeneration, regulation, and relevance to human diseases. Front Biosci 2003; 8: d264-278.
68) Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 1991; 351: 714-718.
69) Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. Cell 1994; 78: 915-918.
70) Nathan C. Inducible nitric oxide synthase: regulation subserves function. Curr Top Microbiol Immunol 1995; 196: 1-4.
71) Janssens SP, Shimouchi A, Quertermous T, Bloch DB, Bloch KD. Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase. J Biol Chem 1992; 267: 14519-14522.
72) Marsden PA, Schappert KT, Chen HS, Flowers M, Sundell CL, Wilcox JN, Lamas S, Michel T. Molecular cloning and characterization of human endothelial nitric oxide synthase. FEBS Lett 1992; 307: 287-293.
73) Sessa WC, Harrison JK, Barber CM, Zeng D, Durieux EM, D'Angelo DD, Lynch KR, Peach MJ. Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. J Biol Chem 1992; 267: 15274-15276.
74) Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. J Cardiovasc Pharmacol 1999; 34: 879-886.
75) Xie QW, Nathan C. Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon-gamma and bacterial lipopolysaccharide. Trans Assoc Am Physicians 1993; 106: 1-12.
76) Gottesfeld Z, Maier M, Mailman D, Lai M, Weisbrodt NW. Splenic sympathetic response to endotoxin is blunted in the fetal alcohol-exposed rat: role of nitric oxide. Alcohol 1998; 16: 19-24.

77) Weisbrodt NW, Lai M, Gottesfeld Z. Ileal nitric oxide formation is altered in the young rat in response to endotoxin: effects of exposure to alcohol in utero. Alcohol 1999; 17: 247-251.
78) Nakazawa H, Fukuyama N, TakizawSa, Tsuji C, Yoshitake M, Ishida H. Nitrotyrosine formation and its role in various pathological conditions. Free Radic Res 2000; 33: 771-784.
79) Shishehbor MH, Aviles RJ, Brennan ML, Fu X, Goormastic M, Pearce GL, Gokce N, Keaney JF Jr, Penn MS, Sprecher DL, Vita JA, Hazen SL. Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. JAMA 2003; 289: 1675-1680.
80) Pennathur S, Bergt C, Shao B, Byun J, Kassim SY, Singh P, Green PS, McDonald TO, Brunzell J, Chait A, Oram JF, O'Brien K, Geary RL, Heinecke JW. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. J Biol Chem 2004; 279: 42977-42983.
81) Jelic S, Padeletti M, Kawut SM, Higgins C, Canfield SM, Onat D, Colombo PC, Basner RC, Factor P, LeJemtel TH. Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. Circulation 2008; 117: 2270-2278.
82) Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, Ghirlanda G. Oxidative stress, nitric oxide, and diabetes. Rev Diabet Stud 2010; 7: 15-25.
83) Ip MSM, Lam B, Chan LY, Zheng L, Tsang KWT, Fung PCW, Lam WK. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. Am J Respir Crit Care Med 2000; 162: 2166-2171.
84) Zhang D, Luo J, Qiao Y, Xiao Y, Huang R, Zhong X. Measurement of exhaled nitric oxide concentration in patients with obstructive sleep apnea: a meta-analysis. Medicine (Baltimore) 2017; 96: e6429.
85) Olopade CO, Christon JA, Zakkar M, Hua C, Swedler WI, Scheff PA, Rubinstein I. Exhaled pentane and nitric oxide levels in patients with obstructive sleep apnea. Chest 1997; 111: 1500-1504.
86) JalilMirmohammadi S, Mehrparvar AH, Safaei S, Samimi E, Jahromi MT. The association between exhaled nitric oxide and sleep apnea: The role of BMI. Respir Med 2014; 108: 1229-1233.
87) Duong-Quy S, Hua-Huy T, Tran-Mai-Thi HT, Le-Dong NN, Craig TJ, Dinh-Xuan AT. Study of exhaled nitric oxide in subjects with suspected obstructive sleep apnea: a pilot study in Vietnam. Pulm Med 2016; 3050918.
88) Schultz R. The vascular micromilieu in obstructive sleep apnea. Eur Respir J 2005; 25: 780-782.
89) Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000; 342: 1378-1384.
90) Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Roccella EJ. The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA 2003; 289: 2560-2572.
91) Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto FJ, O'Connor JT, Boland LL, Schwartz JE, Samet JM. Sleep-disordered breathing and cardiovascular disease. Cross sectional results of the Sleep Heart Health Study. Am J Respir Crit Care Med 2001; 163: 19-25.
92) Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Vidlansky ME, Tschakovsky ME, Green DJ. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011; 300: H2-12.
93) Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, for the International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257-265.
94) Jelic S, Lederer DJ, Adams T, Padeletti M, Colombo PC, Factor P, Le Jemtel TH. Endothelial repair capacity and apoptosis are inversely related in obstructive sleep apnea. Vasc Health Risk Manag 2009; 5: 909-920.
95) El Solh AA, Akinnusi ME, Baddoura FH, Mankowski CR. Endothelial cell apoptosis in obstructive sleep apnea. A link to endothelial dysfunction. Am J Respir Crit Care Med 2007; 175: 1186-1191.
96) Korcarz CE, Stein JH, Peppard PE, Young TB, Barnet JH, Nieto FJ. Combined effects of sleep disordered breathing and metabolic syndrome on endothelial function: the Wisconsin Sleep Cohort Study. Sleep 2014; 37: 1707-1713.
97) Bayram NA, Ciftci B, Keles T, Durmaz T, Turhan S, Bozkurt E, Peker Y. Endothelial function in normotensive men with obstructive sleep apnea before and 6 months after CPAP treatment. Sleep 2009; 32: 1257-1263.
98) Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003; 348: 593-600.
99) Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353: 999-1007.