Cardiovascular Morbidity in Obstructive Sleep Apnea Oxidative Stress, Inflammation, and Much More

David Gozal¹ and Leila Kheirandish-Gozal¹

¹Kosair Children's Hospital Research Institute, and Division of Pediatric Sleep Medicine, Department of Pediatrics, University of Louisville, Louisville, Kentucky

Sleep-disordered breathing and obstructive sleep apnea (OSA) are highly prevalent disorders throughout the lifespan, which may affect up to 2–10% of the population, and have now been firmly associated with an increased risk for cardiovascular and neurobehavioral complications. Nevertheless, the overall pathophysiologic mechanisms mediating end-organ injury in OSA remain undefined, particularly due to the very frequent coexistence of other disease states, such as obesity, that clearly complicate the potential cause–effect relationships. Two major, and to some extent overlapping, mechanisms have been proposed to explain the morbid consequences of OSA, namely increased generation and propagation of reactive oxygen species and initiation and amplification of inflammatory processes. The evidence supporting the validity of these concepts as well as that detracting from such mechanisms will be critically reviewed in the context of clinical and laboratory-based approaches. In addition, some of the contradictory issues raised by such evaluation of the literature will be interpreted in the context of putative modifications of the individual responses to OSA, as determined by genetic variants among susceptibility-related genes, and also by potential environmental modulators of the phenotypic expression of any particular end-organ morbidity associated with OSA.

Keywords: inflammation; oxidative stress; reactive oxygen species; intermittent hypoxia; sleep fragmentation

The increasing prevalence of obstructive sleep apnea (OSA) across the lifespan has prompted attention to the morbid consequences of this rather frequent condition. Of particular interest, there has been quite conclusive epidemiologic data indicating that OSA is pathophysiologically linked to cardiovascular disorders, such as hypertension, ischemic heart disease, and cerebrovascular disease. Furthermore, when such conditions are preexisting, their clinical course and the progression of those conditions will be accelerated in the presence of coexisting OSA.

Despite the extensive body of literature linking OSA and cardiovascular disease, the mechanisms underlying this association remain incompletely identified. OSA-induced biological changes could involve any of the various physiologic disturban-

ces associated with the disease, namely intermittent hypoxia, intermittent hypercapnia, sleep fragmentation, and intrathoracic pressure changes. Among these, intermittent hypoxia is most likely the preponderant factor causing the cardiovascular alterations that may manifest in patients with OSA. Indeed, patients with OSA may suffer from repeated episodes of hypoxia and normoxia, which are in many ways reminiscent of ischemia–reperfusion events, and are currently believed to promote the production of reactive oxygen species (ROS) and the promotion of oxidative stress (1–5), which in turn may adversely affect endothelial regulation through NO-mediated pathways (6). In addition, OSA has been implicated in the induction and propagation of inflammatory cascades that in turn can both promote and exacerbate atherogenesis and vascular dysfunction.

In this article, we briefly, yet critically, review the evidence supporting or refuting the existence of oxidative stress and inflammatory processes as the putative mechanisms linking the increased prevalence and severity of cardiovascular involvement to OSA. Whenever possible, we allude to studies in the pediatric population, because, in many ways, children are less likely to be “contaminated” by coexisting confounders, such as obesity, smoking, other comorbidities, and medications. We will further examine whether these assumptions can be validated by more rigorous experimental models, and ultimately propose a model that incorporates these concepts into a unifying paradigm (Figure 1).

Question 1: Is OSA Associated with Increased Systemic Oxidative Stress?

The evidence for increased oxidative stress in patients with OSA, although very attractive, is somewhat controversial. Barcelo and collaborators (7, 8) reported that thiobarbituric acid–reactive substance (TBARS) formation was higher in patients with severe OSA compared with healthy subjects, and that continuous positive airway pressure (CPAP) treatment improved the abnormal lipid peroxidation events. In another study of 114 patients, morning levels of TBARS and peroxides were also significantly higher in patients with OSA (with or without cardiovascular disease) compared with control subjects (9), and similar to Barcelo and colleagues' study (7), CPAP treatment decreased nocturnal levels of TBARS and peroxides in patients, and such beneficial effect has been more recently accomplished using mandibular prosthesis treatment of OSA (10). Of note, increased oxidized low-density lipoprotein levels were also detected in patients with OSA (11). Christou and colleagues (12) also found that the levels of diacron-reactive oxygen metabolites (i.e., the ability of transition metals to catalyze the formation of free radicals in the presence of peroxide, as monitored by oxidized alchilamine products) were elevated in the blood of 21 patients with OSA compared with control subjects. In two other recent studies, El-Sohl and coworkers showed that inhibition of xanthine oxidase by allopurinol led to improved endothelial function

(Received in original form August 21, 2006; accepted in final form October 24, 2007)

D.G. is supported by the National Institutes of Health (grants HL-65270, HL-69932, and SCOR 2P50HL60296-06), the Children's Foundation Endowment for Sleep Research, and the Commonwealth of Kentucky Challenge for Excellence Trust Fund. L.K.-G. receives support from an investigator-initiated grant from AstraZeneca Ltd and from a grant from the National Aeronautics and Space Administration (NNJ05HF 06G).

Correspondence and requests for reprints should be addressed to David Gozal, M.D., Kosair Children's Hospital Research Institute, University of Louisville School of Medicine, 570 South Preston Street, Suite 204, Louisville, KY 40202. E-mail: david.gozal@louisville.edu

Am J Respir Crit Care Med Vol 177, pp 369–375, 2008

Originally Published in Press as DOI: 10.1164/rccm.200608-1190PP on November 1, 2007
Internet address: www.atsjournals.org

the severity of any of the OSA-related polysomnographic measures in these children (19). One of the potential explanations for the discrepant findings may reside in the patient selection methodology (inclusion or exclusion of patients with concomitant morbidities, such as obesity or diabetes, which are traditionally associated with oxidative pathophysiologic mechanisms), as well as in the overall severity and duration of the disease before testing. Indeed, when obese or overweight children with OSA were studied using serum uric acid as the biomarker for oxidative stress, significant correlations between disease severity and uric acid emerged (20). Furthermore, as shown by Jordan and colleagues, assessments of oxidative markers in serum may be methodologically insensitive or exhibit a great degree of circadian variability, and therefore multiple markers may need to be determined simultaneously to improve the robustness of the findings (21). Taken together, the data would support the assumption that OSA is indeed associated with increased oxidant stress and other associated responses that modulate inflammation and proliferation (22). Notwithstanding such considerations, the implications of such findings need to be integrated with the now considerable evidence supporting a major role for oxidative stress in the initiation and propagation of events that ultimately promote atherosclerosis and vascular dysfunction (23). Finally, a recent study by El Sohl and colleagues suggested that OSA is associated with increased endothelial cell apoptosis, and that the latter, which could be mediated by oxidative stress, correlates with the degree of vascular dysfunction (24). Although concerns were raised regarding the methodologies used in this latter study in subsequent letters to the editor, this article nevertheless provides the initial step toward exploration of endothelial cell loss as being involved in the vascular deficits seen in patients with OSA.

Question 2: Does OSA Affect NO-dependent Endothelial Function via an Oxidative Stress Mechanism?

One of the major consequences of OSA is the emergence of endothelial dysfunction, as evidenced by the presence of altered NO-dependent vasodilation responses in patients with OSA, and its reversal after effective implementation of treatment for OSA (6, 25–28). The reduced NO release from the endothelium appears to involve the down-regulation and uncoupling of endothelial NO synthase as well as the parallel increase in circulating levels of endogenous inhibitors of NO synthase, such as asymmetric NG-dimethylarginine (ADMA) (29–31). One of the potential mechanisms underlying the down-regulation of endothelial NO synthase expression and function involves oxidative stress, whereby the latter will readily modify the activity of endothelial NO synthase (32–35), and conversely, enhanced NO availability will attenuate oxidative stress and protect the endothelium as well as endothelial repair mechanisms, such as recruitment and proliferation of endothelial progenitor cells (36–39). Furthermore, the interaction between NO and free radicals, such as the superoxide anion, will lead to increased formation of peroxynitrite, and promote a variety of biological cascades that promote atherogenesis (40). Taken together, the cumulative evidence would point toward a reduction in NO availability in the context of OSA, and the latter has indeed been recently verified when measuring the exhaled alveolar NO fraction (41, 42)

Question 3: Is There Evidence for Increased Systemic Inflammation in OSA?

Increases in systemic oxidative stress are traditionally implicated in the activation of immune cells, which, in turn, are

primary generators of ROS and inflammation. Following this assumption, Schultz and colleagues examined systemic inflammatory responses in 18 patients with OSA, and found markedly enhanced release of superoxide from stimulated polymorphonuclear neutrophils (measured by superoxide dismutase-inhibitable reduction of cytochrome *c*) compared with control subjects, and further showed that CPAP therapy resulted in decreased superoxide release (43). Studies from Dyugovskaya and colleagues have further elucidated potential immune mechanisms that appear to be activated by OSA. These investigators have based their approach on the premise that T cells play a significant role in atherogenesis, both through cytokine production and/or by directly contributing to vascular injury. They reported that CD4 and CD8 T cells of patients with OSA undergo phenotypic and functional changes, and acquire cytotoxic activity, with a shift in CD4 and CD8 T cells toward type 2 cytokine dominance and increased IL-4 expression. Conversely, IL-10 expression in T cells was negatively correlated with the severity of OSA, whereas tumor necrosis factor (TNF)- α was positively correlated with the apnea-hypopnea index. Furthermore, CD8 T cells of patients with OSA exhibited marked increases in TNF- α and CD40 ligand, and were particularly cytotoxic against endothelial cells. All these findings were markedly improved or reversed by treatment with CPAP (44–46). Elevated systemic TNF- α levels were found in OSA, and improved after treatment (47), whereas in contrast with such findings, no evidence of increased TNF- α levels was found in a large group of patients with OSA and control subjects ($n = 155$) (48), suggesting that the increased expression and activity of this cytokine may be restricted to localized effects within the endothelial surface. Notwithstanding these findings, a recent publication in the *Journal* (49) suggests that not only TNF- α levels are increased but they correlate with the degree of sleepiness and the severity of hypoxia (49). We have recently reported very similar findings in children with OSA (50), whereby TNF- α serum levels were not only elevated in a significant proportion of children with polysomnographic evidence of OSA but that the magnitude of TNF- α levels was primarily correlated with the degree of respiratory event-induced sleep fragmentation (50).

An important circulating marker of inflammation, C-reactive protein (CRP), which is produced in the liver through IL-6 activity, is one of the best predictors for future cardiovascular morbidity (28, 29), and directly participates in atheromatous lesion formation (51). Increased circulating levels of CRP have been rather consistently reported in both adults (52–65), as well as in children with OSA (66, 67), and are reduced on effective treatment (54, 68). However, similar to previous studies examining oxidative stress, some investigators have failed to identify CRP increases in OSA (69–74), suggesting that the determinants of CRP elevation in the presence of OSA cannot be exclusively accounted for the severity of the condition but are also dictated by multiple other circumstances. Among the latter, the presence of concurrent risk factors (e.g., obesity, medications, diabetes, cigarette smoking) may be indeed a major determinant of the variance in CRP responses. In fact, the interactions between the severity of OSA, lifestyle and environmental conditions, and genetically derived individual susceptibility have now been proposed as the major potential players involved in the magnitude of the oxidative stress and inflammatory responses associated with OSA (75, 76). Of interest, a recent study has identified the TNF- α –308 polymorphism as being associated with OSA, thereby linking inflammatory responses to the sleep-associated respiratory disturbance (77). Similarly, a recent study in the *Journal* indicates that decreases in CRP will occur with effective CPAP treatment of adult patients with OSA

in conjunction with an amelioration of the carotid intima-media thickness (78).

Expression of adhesion molecules on circulating monocytes may serve as yet another indication of the activation of systemic inflammation in OSA. Using 18 patients with moderate to severe OSA and 26 healthy control subjects, Dyugovskaya and colleagues found that OSA was associated with increased expression of adhesion molecules CD15 and CD11c, and increased adherence of monocytes in culture to human endothelial cells (44–46, 79). Similar findings regarding circulating levels of adhesion molecules and their down-regulation upon treatment have been reported by several groups of investigators in both adult and pediatric cohorts (80–83). Overall, these findings suggest that OSA elicits activation of cell–cell interactions involving the endothelial cellular substrate that, in turn, may promote atherogenesis, and that treatment of OSA is effective in reducing the systemic inflammatory responses, including those associated with generation of free radicals.

Question 4: Do Experimental Models Confirm the Presence of Increased Oxidative Stress and Inflammatory Responses?

Recent development of animal and cell-based models mimicking components of OSA has allowed for more systematic exploration of some of the mechanisms associated with end-organ dysfunction in patients with OSA (84–87). Although we are still far from gaining comprehensive insights into the most likely complex multiplicity of interactions occurring between the various cellular elements involved in the process of OSA-induced vascular disease, conclusive evidence now supports the observation that repetitive hypoxic events during sleep are associated with increased sympathetic activity and hypertension (88). Furthermore, episodic hypoxia appears to induce abnormal arteriolar function, possibly via altered NO release (89).

Recently, in a series of elegant *in vitro* experiments, Lattimore and colleagues showed that application of episodic hypoxia (IH) elicited increased cholesterol uptake by macrophages, one of the cardinal events associated with atherogenesis (90). Furthermore, IH during sleep induced marked alterations in lipid regulation in both obese and nonobese mice, a finding that is compatible with the notion of acceleration of atherogenesis (91, 92).

Similarly, when HeLa cells were exposed to IH, luciferase reporter assays uncovered a maladaptive process to the repeated hypoxic events, whereby hypoxia-inducible factor (HIF)-1 α was not up-regulated (in contrast with sustained hypoxia); instead, there was a marked up-regulation of the transcriptional activity of nuclear factor (NF)- κ B, a critical proinflammatory regulator (93). These findings were further reproduced *in vivo*, whereby mice exposed to IH demonstrated increased NF- κ B activity and inducible NO synthase (a downstream gene) in monocytes and cardiovascular tissues (94). Of note, remarkably similar findings occur in the central nervous system, and indeed inducible NO synthase plays a mechanistic role in cognitive and neural deficits (95, 96). Thus, induction of cellular processes culminating in the activation and propagation of the inflammatory response appears to occur in response to IH. However, for the sake of completeness, some contradictory evidence has also emerged in regard to the absence of IH-induced changes on HIF-1 α expression and activity. Indeed, IH appears to modify HIF-1 α expression in the pheochromocytoma (PC)-12 cell line when exposed to IH, possibly through a calcium calmodulin kinase-dependent mechanism (97). Such changes in HIF-1 α may be of importance for *in vivo* regulation of circulating triglycerides and in the modulation of inflammatory responses to IH, because transgenic

mice with partial deficiency of HIF-1 α developed no increases in IL-6 plasma levels when exposed to IH (98). Thus, considering the role of oxidative stress in the modulation of the proinflammatory activity of NF- κ B and the antiinflammatory actions of HIF-1 α (99), the stage is clearly ready for future studies aiming to assess the impact of such transcription factors on the cardiovascular system in the context of OSA.

The causal link between IH and oxidative stress is also gradually emerging from these *in vitro* and *in vivo* models (100). Indeed, increased production of ROS as elicited by IH may play a role in the sympathetic dysregulation traditionally seen in patients with OSA, possibly through increased efflux of catecholamines from the adrenal medulla (101). Furthermore, chronic exposures to IH in rats will increase cardiac muscle lipid peroxidation and left ventricular dysfunction (102). In both rats and mice, as well as in cell culture systems, IH is associated with evidence of oxidative stress and up-regulation of one of the key elements underlying the production of ROS, namely the expression and activity of NADPH oxidase (103–107). Indeed, virtually all types of vascular cells can produce O₂^{•−} and H₂O₂, two of the most significant ROS in the vessel wall (108). Production of O₂^{•−} occurs via the one-electron reduction of molecular oxygen, a reaction that is mediated by several enzymatic systems and the mitochondria. Among the enzymes capable of O₂^{•−} production are xanthine oxidase and the NADPH oxidases, with the latter enzymes playing a critical role in ROS production within the vasculature (109, 110). O₂^{•−} itself may directly impinge on vascular signaling cascades, but more importantly, can produce other reactive species. For example, the reaction of O₂^{•−} with NO⁺ inactivates NO⁺, a primary regulator of vascular relaxation and vasodilation, causing the generation of peroxynitrite, which itself has deleterious consequences. Alternatively, dismutation of O₂^{•−} by superoxide dismutase produces H₂O₂, a more stable ROS. H₂O₂ is implicated in the regulation of signaling pathways leading to vascular smooth muscle growth, contraction, migration, and inflammation (111, 112). Considering the rather extensive and intricate roles played by the NADPH oxidase family of enzymes in both inflammatory cell and vascular cell physiologic and pathologic states (113–116), we propose that the increased expression and activity of this enzyme in various end organs after IH is likely to play a major role in the cardiovascular morbidity associated with OSA.

Conclusions

In summary, the last decade has clearly allowed for rather extensive evidence to accumulate, and to justify our belief on the existence of a causative link between OSA and cardiovascular disease. However, the exact mechanisms for such association remain thus far elusive. Carefully conducted studies in the future, including well-randomized interventional trials, are likely to unravel the central role played by oxidative stress and inflammatory cascades in the end-organ injury associated with OSA. Although it is highly likely that no single gene will account for all the proposed processes involved in vascular dysfunction, we propose that the nature and magnitude of interactions between several cellular populations, namely the endothelium, platelets, T cells, and macrophages, will be key determinants in the extent and progression rate of the cardiovascular morbidity that can be ascribed to OSA. At the molecular level, altered NADPH oxidase expression and activity as induced by OSA will emerge as a key player in the deleterious cardiovascular consequences of OSA, and potentially provide therapeutic targets aiming to minimize the rather detrimental consequences of OSA. However, we should not forget the large array of proinflammatory genes, which are either activated or modulated in the various

cellular subsets by the increased transcriptional activity of NF- κ B, AP1, HIF-1 α , and possibly other transcriptional factors, and their interactions to potentially affect many other genes, including anti- and prooxidant systems; cellular survival, proliferation, and differentiation; and lipid metabolism and raft signaling. Furthermore, the potential interactions between gene polymorphisms (conferring individual susceptibility determinants), lifestyle components modulating overall end-organ vulnerability, and the phenotypic expression of OSA and its consequences will have to be identified and incorporated into future prediction schemes of morbidity risks associated with OSA.

Conflict of Interest Statement: D.G. serves as part of the National Speaker Bureau for Merck Co. L.K.-G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Lavie L. Obstructive sleep apnea syndrome: an oxidative stress disorder. *Sleep Med Rev* 2003;7:35–51.
- Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during obstructive sleep apnea. *Sleep* 1993;16:S15–S21.
- Prabhakar NR. Sleep apneas: an oxidative stress? *Am J Respir Crit Care Med* 2002;165:859–860.
- Budhiraja R, Parthasarathy S, Quan SF. Endothelial dysfunction in obstructive sleep apnea. *J Clin Sleep Med*. 2007;3:409–415.
- Caples SM, Garcia-Touchard A, Somers VK. Sleep-disordered breathing and cardiovascular risk. *Sleep* 2007;30:291–303.
- Ip MS, Lam B, Chan LY, Zheng L, Tsang KW, Fung PC, 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.
- Barcelo A, Miralles C, Barbe F, Vila M, Pons S, Agustí AG. Abnormal lipid peroxidation in patients with sleep apnoea. *Eur Respir J* 2000;16:644–647.
- Barceló A, Barbé F, de la Peña M, Vila M, Pérez G, Piérola J, Durán J, Agustí AG. Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. *Eur Respir J* 2006;27:756–760.
- Lavie L, Vishnevsky A, Lavie P. Evidence for lipid peroxidation in obstructive sleep apnea. *Sleep* 2004;27:123–128.
- Itzhaki S, Dorchin H, Clark G, Lavie L, Lavie P, Pillar G. The effects of 1-year treatment with a Herbst mandibular advancement splint on obstructive sleep apnea, oxidative stress, and endothelial function. *Chest* 2007;131:740–749.
- Tan KC, Chow WS, Lam JC, Lam B, Wong WK, Tam S, Ip MS. HDL dysfunction in obstructive sleep apnea. *Atherosclerosis* 2006;184:377–382.
- Christou K, Markoulis N, Moulas AN, Pastaka C, Gourgoulis KI. Reactive oxygen metabolites (ROMs) as an index of oxidative stress in obstructive sleep apnea patients. *Sleep Breath* 2003;7:105–110.
- El Solh AA, Saliba R, Bosinski T, Grant BJ, Berbari E, Miller N. Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. *Eur Respir J* 2006;27:997–1002.
- Grebe M, Eisele HJ, Weissmann N, Schaefer C, Tillmanns H, Seeger W, Schulz R. Antioxidant vitamin C improves endothelial function in obstructive sleep apnea. *Am J Respir Crit Care Med* 2006;173:897–901.
- Tan KC, Chow WS, Lam JC, Lam B, Bucala R, Betteridge J, Ip MS. Advanced glycation endproducts in nondiabetic patients with obstructive sleep apnea. *Sleep* 2006;29:329–333.
- Yamauchi M, Nakano H, Maekawa J, Okamoto Y, Ohnishi Y, Suzuki T, Kimura H. Oxidative stress in obstructive sleep apnea. *Chest* 2005;127:1674–1679.
- Christou K, Moulas AN, Pastaka C, Gourgoulis KI. Antioxidant capacity in obstructive sleep apnea patients. *Sleep Med* 2003;4:225–228.
- Alzogaibi MA, Bahammam AS. Lipid peroxides, superoxide dismutase and circulating IL-8 and GCP-2 in patients with severe obstructive sleep apnea: a pilot study. *Sleep Breath* 2005;9:119–126.
- Montgomery-Downs HE, Krishna J, Roberts LJ II, Gozal D. Urinary F2-isoprostane metabolite levels in children with sleep-disordered breathing. *Sleep Breath* 2006;10:211–215.
- Verhulst SL, Van Hoeck K, Schrauwen N, Haentjens D, Romain R, Van Gaal L, De Backer WA, Desager KN. Sleep-disordered breathing and uric acid in overweight and obese children and adolescents. *Chest* 2007;132:76–80.
- Jordan W, Cohrs S, Degner D, Meier A, Rodenbeck A, Mayer G, Pilz J, Ruther E, Kornhuber J, Bleich S. Evaluation of oxidative stress measurements in obstructive sleep apnea syndrome. *J Neural Transm* 2006;113:239–254.
- Hoffmann MS, Singh P, Wolk R, Romero-Corral A, Raghavakaimal S, Somers VK. Microarray studies of genomic oxidative stress and cell cycle responses in obstructive sleep apnea. *Antioxid Redox Signal* 2007;9:661–669.
- Jacobi J, Kristal B, Chezard J, Shaul SM, Sela S. Exogenous superoxide mediates pro-oxidative, pro-inflammatory, and pro-coagulatory changes in primary endothelial cell cultures. *Free Radic Biol Med* 2005;39:1238–1248.
- 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.
- Schulz R, Schmidt D, Blum A, Lopes-Ribeiro X, Lucke C, Mayer K, Olschewski H, Seeger W, Grimminger F. Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnoea: response to CPAP therapy. *Thorax* 2000;55:1046–1051.
- Kato M, Roberts-Thomson P, Phillips BG, Haynes WG, Winnicki M, Accurso V, Somers VK. Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation* 2000;102:2607–2610.
- Lattimore JL, Wilcox I, Skilton M, Langenfeld M, Celermajor DS. Treatment of obstructive sleep apnoea leads to improved microvascular endothelial function in the systemic circulation. *Thorax* 2006;61:491–495.
- Duchna HW, Orth M, Schultze-Werninghaus G, Guilleminault C, Stoohs RA. Long-term effects of nasal continuous positive airway pressure on vasodilatory endothelial function in obstructive sleep apnea syndrome. *Sleep Breath* 2005;9:97–103.
- Lavie L, Hefetz A, Luboshitzky R, Lavie P. Plasma levels of nitric oxide and L-arginine in sleep apnea patients: effects of nCPAP treatment. *J Mol Neurosci* 2003;21:57–63.
- Ohike Y, Kozaki K, Iijima K, Eto M, Kojima T, Ohga E, Santa T, Imai K, Hashimoto M, Yoshizumi M, et al. Amelioration of vascular endothelial dysfunction in obstructive sleep apnea syndrome by nasal continuous positive airway pressure—possible involvement of nitric oxide and asymmetric NG, NG-dimethylarginine. *Circ J* 2005;69:221–226.
- Svatikova A, Wolk R, Wang HH, Otto ME, Bybee KA, Singh RJ, Somers VK. Circulating free nitrotyrosine in obstructive sleep apnea. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R284–R287.
- Coyle CH, Martinez LJ, Coleman MC, Spitz DR, Weintraub NL, Kader KN. Mechanisms of H2O2-induced oxidative stress in endothelial cells. *Free Radic Biol Med* 2006;40:2206–2213.
- Thomas SR, Schulz E, Keaney JF Jr. Hydrogen peroxide restrains endothelium-derived nitric oxide bioactivity—role for iron-dependent oxidative stress. *Free Radic Biol Med* 2006;41:681–688.
- Miyagawa K, Ohashi M, Yamashita S, Kojima M, Sato K, Ueda R, Dohi Y. Increased oxidative stress impairs endothelial modulation of contractions in arteries from spontaneously hypertensive rats. *J Hypertens* 2007;25:415–421.
- Silver AE, Beske SD, Christou DD, Donato AJ, Moreau KL, Eskurza I, Gates PE, Seals DR. Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. *Circulation* 2007;115:627–637.
- Kotamraju S, Matalon S, Matsunaga T, Shang T, Hickman-Davis JM, Kalyanaraman B. Upregulation of immunoproteasomes by nitric oxide: potential antioxidative mechanism in endothelial cells. *Free Radic Biol Med* 2006;40:1034–1044.
- Kotamraju S, Kalivendi S, Shang T, Kalyanaraman B. Nitric oxide, proteasomal function, and iron homeostasis—implications in aging and neurodegenerative diseases. *Methods Enzymol* 2005;396:526–534.
- Thum T, Fraccarollo D, Thum S, Schultheiss M, Daiber A, Wenzel P, Munzel T, Ertl G, Bauersachs J. Differential effects of organic nitrates on endothelial progenitor cells are determined by oxidative stress. *Arterioscler Thromb Vasc Biol* 2007;27:748–754.
- Thum T, Fraccarollo D, Schultheiss M, Froese S, Galuppo P, Widder JD, Tsikas D, Ertl G, Bauersachs J. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes* 2007;56:666–674.

40. Dickhout JG, Hossain GS, Pozza LM, Zhou J, Lhotak S, Austin RC. Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis. *Arterioscler Thromb Vasc Biol* 2005;25:2623–2629.
41. Foresi A, Leone C, Olivieri D, Cremona G. Alveolar-derived exhaled nitric oxide is reduced in obstructive sleep apnea syndrome. *Chest* 2007; 132:860–867.
42. Lattimore JD, Wilcox I, Adams MR, Kilian JG, Celermajor DS. Treatment of obstructive sleep apnoea leads to enhanced pulmonary vascular nitric oxide release. *Int J Cardiol* [Epub ahead of print 2007 Jun 28].
43. Schulz R, Mahmoudi S, Hattar K, Sibelius U, Olschewski H, Mayer K, Seeger W, Grimminger F. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea: impact of continuous positive airway pressure therapy. *Am J Respir Crit Care Med* 2000;162:566–570.
44. Dyugovskaya L, Lavie P, Hirsh M, Lavie L. Activated CD8+ T-lymphocytes in obstructive sleep apnoea. *Eur Respir J* 2005;25:820–828.
45. Dyugovskaya L, Lavie P, Lavie L. Phenotypic and functional characterization of blood $\gamma\delta$ T cells in sleep apnea. *Am J Respir Crit Care Med* 2003;168:242–249.
46. Dyugovskaya L, Lavie P, Lavie L. Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. *Am J Respir Crit Care Med* 2002;165:934–939.
47. Kataoka T, Enomoto F, Kim R, Yokoi H, Fujimori M, Sakai Y, Ando I, Ichikawa G, Ikeda K. The effect of surgical treatment of obstructive sleep apnea syndrome on the plasma TNF- α levels. *Tohoku J Exp Med* 2004;204:267–272.
48. Imagawa S, Yamaguchi Y, Ogawa K, Obara N, Suzuki N, Yamamoto M, Nagasawa T. Interleukin-6 and tumor necrosis factor- α in patients with obstructive sleep apnea-hypopnea syndrome. *Respiration* 2004;71:24–29.
49. Ryan S, Taylor CT, McNicholas WT. Predictors of elevated nuclear factor κ B-dependent genes in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2006;174:824–830.
50. Serpero LD, Kheirandish L, Sans Capdevila O, Tauman R, Gozal D. Sleep fragmentation and circulating TNF α levels in children with sleep-disordered breathing [abstract]. *Proc Am Thorac Soc* 2006;3: A555.
51. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836–843.
52. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in prediction of first cardiovascular events. *N Engl J Med* 2002;347: 1557–1565.
53. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000;102: 2165–2168.
54. Shamsuzzaman AS, 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.
55. Minoguchi K, Yokoe T, Tanaka A, Ohta S, Hirano T, Yoshino G, O'Donnell CP, Adachi M. Association between lipid peroxidation and inflammation in obstructive sleep apnoea. *Eur Respir J* 2006;28: 378–385.
56. Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Tanaka A, Oda N, Okada S, Ohta S, Naito H, Adachi M. Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005;172:625–630.
57. Zouaoui Boudjeltia K, Van Meerhaeghe A, Doumit S, Guillaume M, Cauchie P, Brohee D, Vanhaeverbeek M, Kerkhofs M. Sleep apnoea-hypopnoea index is an independent predictor of high-sensitivity C-reactive protein elevation. *Respiration* 2006;73:243–246.
58. Kageyama N, Nomura M, Nakaya Y, Watanabe T, Ito S. Relationship between adhesion molecules with hs-CRP and changes therein after ARB (Valsartan) administration in patients with obstructive sleep apnea syndrome. *J Med Invest* 2006;53:134–139.
59. Saletu M, Nosiska D, Kapfhammer G, Lalouschek W, Saletu B, Benesch T, Zeitlhofer J. Structural and serum surrogate markers of cerebrovascular disease in obstructive sleep apnea (OSA): association of mild OSA with early atherosclerosis. *J Neurol* 2006;253:746–752.
60. Can M, Acikgoz S, Mungan G, Bayraktaroglu T, Kocak E, Guven B, Demirtas S. Serum cardiovascular risk factors in obstructive sleep apnea. *Chest* 2006;129:233–237.
61. Punjabi NM, Beamer BA. C-reactive protein is associated with sleep disordered breathing independent of adiposity. *Sleep* 2007;30:29–34.
62. Ye J, Liu H, Li Y, Liu X, Zhu JM. Increased serum levels of C-reactive protein and matrix metalloproteinase-9 in obstructive sleep apnea syndrome. *Chin Med J (Engl)* 2007;120:1482–1486.
63. Peled N, Kassirer M, Kramer MR, Rogowski O, Shlomi D, Fox B, Berliner AS, Shtrit D. Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome. *Thromb Res* [Epub ahead of print 2007].
64. 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.
65. Friedman M, Bliznikas D, Vidyasagar R, Woodson BT, Joseph NJ. Reduction of C-reactive protein with surgical treatment of obstructive sleep apnea hypopnea syndrome. *Otolaryngol Head Neck Surg* 2006; 135:900–905.
66. Tauman R, Ivanenko A, O'Brien LM, Gozal D. Plasma C-reactive protein among children with sleep-disordered breathing. *Pediatrics* 2004;113:564–569.
67. Larkin EK, Rosen CL, Kirchner HL, Storer-Isser A, Emancipator JL, Johnson NL, Zambito AMV, Tracy RP, Jenny NS, Redline S. Variation of C-reactive protein levels in adolescents association with sleep-disordered breathing and sleep duration. *Circulation* 2005;111: 1978–1984.
68. Kheirandish-Gozal L, Sans Capdevila O, Tauman R, Gozal D. Plasma C-reactive protein in non-obese children with obstructive sleep apnea before and after adenotonsillectomy. *J Clin Sleep Med* 2006;2:301–304.
69. Guilleminault C, Kirsoglu C, Ohayon MM. C-reactive protein and sleep-disordered breathing. *Sleep* 2004;27:1507–1511.
70. Kaditis AG, Alexopoulos EI, Kalampouka E, Kostadima E, Germanis A, Zintzaras E, Gourgouliannis K. Morning levels of C-reactive protein in children with obstructive sleep-disordered breathing. *Am J Respir Crit Care Med* 2005;171:282–286.
71. Barcelo A, Barbe F, Llompert E, Mayoralas LR, Ladaria A, Bosch M, Agustí AG. Effects of obesity on C-reactive protein level and metabolic disturbances in male patients with obstructive sleep apnea. *Am J Med* 2004;117:118–121.
72. Taheri S, Austin D, Lin L, Nieto FJ, 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.
73. Sharma SK, Mishra HK, Sharma H, Goel A, Sreenivas V, Gulati V, Tahir M. Obesity, and not obstructive sleep apnea, is responsible for increased serum hs-CRP levels in patients with sleep-disordered breathing in Delhi. *Sleep Med* [Epub ahead of print 2007 Jul 16].
74. Ryan S, Nolan GM, Hannigan E, Cunningham S, Taylor C, McNicholas WT. Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. *Thorax* 2007;62:509–514.
75. Gozal D, Kheirandish L. Oxidant stress and inflammation in the snoring child: confluent pathways to upper airway pathogenesis and end-organ morbidity. *Sleep Med Rev* 2006;10:83–96.
76. Baldwin CM, Bootzin RR, Schwenke DC, Quan SF. Antioxidant nutrient intake and supplements as potential moderators of cognitive decline and cardiovascular disease in obstructive sleep apnea. *Sleep Med Rev* 2005;9:459–476.
77. Riha RL, Brander P, Vennelle M, McArdle N, Kerr SM, Anderson NH, Douglas NJ. Tumour necrosis factor- α (–308) gene polymorphism in obstructive sleep apnoea-hypopnoea syndrome. *Eur Respir J* 2005;26:673–678.
78. Drager LF, Bortolotto LA, Figueiredo AC, Krieger EM, Lorenzi-Filho G. Effects of continuous positive airway pressure on early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med* 2007;176:706–712.
79. Lavie L, Dyugovskaya L, Lavie P. Sleep-apnea-related intermittent hypoxia and atherogenesis: adhesion molecules and monocytes/ endothelial cells interactions. *Atherosclerosis* 2005;183:183–184.
80. Ohga E, Nagase T, Tomita T, Teramoto S, Matsuse T, Katayama H, Ouchi Y. Increased levels of circulating ICAM-1, VCAM-1, and L-selectin in obstructive sleep apnea syndrome. *J Appl Physiol* 1999;87:10–14.
81. O'Brien LM, Serpero LD, Tauman R, Gozal D. Plasma adhesion molecules in children with sleep-disordered breathing. *Chest* 2006;129: 947–953.
82. Chin K, Nakamura T, Shimizu K, Mishima M, Nakamura T, Miyasaka M, Ohi M. Effects of nasal continuous positive airway pressure on soluble cell adhesion molecules in patients with obstructive sleep apnea syndrome. *Am J Med* 2000;109:562–567.

83. El-Solh AA, Mador MJ, Sikka P, Dhillon RS, Amsterdam D, Grant BJ. Adhesion molecules in patients with coronary artery disease and moderate-to-severe obstructive sleep apnea. *Chest* 2002;121:1541–1547.
84. Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J Neurosci* 2001;21:2442–2450.
85. Prabhakar NR, Fields RD, Baker T, Fletcher EC. Intermittent hypoxia: cell to system. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L524–L528.
86. Fletcher EC. Invited review: physiological consequences of intermittent hypoxia: systemic blood pressure. *J Appl Physiol* 2001;90:1600–1605.
87. Tagaito Y, Polotsky VY, Campen MJ, Wilson JA, Balbir A, Smith PL, Schwartz AR, O'Donnell CP. A model of sleep-disordered breathing in the C57BL/6J mouse. *J Appl Physiol* 2001;91:2758–2766.
88. Fletcher EC. Effect of episodic hypoxia on sympathetic activity and blood pressure. *Respir Physiol* 2000;119:189–197.
89. Tahawi Z, Orolinova N, Joshua IG, Bader M, Fletcher EC. Altered vascular reactivity in arterioles of chronic intermittent hypoxic rats. *J Appl Physiol* 2001;90:2007–2013.
90. Lattimore JD, Wilcox I, Nakhla S, Langenfeld M, Jessup W, Celermajer DS. Repetitive hypoxia increases lipid loading in human macrophages: a potentially atherogenic effect. *Atherosclerosis* 2005;179:255–259.
91. Li J, Thorne LN, Punjabi NM, Sun CK, Schwartz AR, Smith PL, Marino RL, Rodriguez A, Hubbard WC, O'Donnell CP, et al. Intermittent hypoxia induces hyperlipidemia in lean mice. *Circ Res* 2005;97:698–706.
92. Li J, Grigoryev DN, Ye SQ, Thorne L, Schwartz AR, Smith PL, O'Donnell CP, Polotsky VY. Chronic intermittent hypoxia upregulates genes of lipid biosynthesis in obese mice. *J Appl Physiol* 2005;99:1643–1648.
93. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation* 2005;112:2660–2667.
94. Greenberg H, Ye X, Wilson D, Htoo AK, Hendersen T, Liu SF. Chronic intermittent hypoxia activates nuclear factor-kappaB in cardiovascular tissues in vivo. *Biochem Biophys Res Commun* 2006;343:591–596.
95. Li RC, Row BW, Kheirandish L, Brittian KR, Gozal E, Guo SZ, Sachleben LR Jr, Gozal D. Nitric oxide synthase and intermittent hypoxia-induced spatial learning deficits in the rat. *Neurobiol Dis* 2004;17:44–53.
96. Zhan G, Fenik P, Pratico D, Veasey SC. Inducible nitric oxide synthase in long-term intermittent hypoxia: hypersomnolence and brain injury. *Am J Respir Crit Care Med* 2005;171:1414–1420.
97. Yuan G, Nanduri J, Bhaskar CR, Semenza GL, Prabhakar NR. Ca²⁺/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J Biol Chem* 2005;280:4321–4328.
98. Li J, Bosch-Marce M, Nanayakkara A, Savransky V, Fried SK, Semenza GL, Polotsky VY. Altered metabolic responses to intermittent hypoxia in mice with partial deficiency of hypoxia-inducible factor-1alpha. *Physiol Genomics* 2006;25:450–457.
99. Haddad JJ, Harb HL. L-gamma-glutamyl-L-cysteinyl-glycine (glutathione; GSH) and GSH-related enzymes in the regulation of pro- and anti-inflammatory cytokines: a signaling transcriptional scenario for redox(y) immunologic sensor(s)? *Mol Immunol* 2005;42:987–1014.
100. Prabhakar NR, Kumar GK. Oxidative stress in the systemic and cellular responses to intermittent hypoxia. *Biol Chem* 2004;385:217–221.
101. Kumar GK, Rai V, Sharma SD, Ramakrishnan DP, Peng YJ, Souvannakitti D, Prabhakar NR. Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla via oxidative stress. *J Physiol* 2006;575:229–239.
102. Chen L, Einbinder E, Zhang Q, Hasday J, Balke CW, Scharf SM. Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats. *Am J Respir Crit Care Med* 2005;172:915–920.
103. Row BW, Liu R, Xu W, Kheirandish L, Gozal D. Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *Am J Respir Crit Care Med* 2003;167:1548–1553.
104. Xu W, Chi L, Row BW, Xu R, Ke Y, Xu B, Luo C, Kheirandish L, Gozal D, Liu R. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. *Neuroscience* 2004;126:313–323.
105. Zhan G, Serrano F, Fenik P, Hsu R, Kong L, Pratico D, Klann E, Veasey SC. NADPH oxidase mediates hypersomnolence and brain oxidative injury in a murine model of sleep apnea. *Am J Respir Crit Care Med* 2005;172:921–929.
106. Veasey SC, Davis CW, Fenik P, Zhan G, Hsu YJ, Pratico D, Gow A. Long-term intermittent hypoxia in mice: protracted hypersomnolence with oxidative injury to sleep-wake brain regions. *Sleep* 2004;27:194–201.
107. Yuan G, Adhikary G, McCormick AA, Holcroft JJ, Kumar GK, Prabhakar NR. Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. *J Physiol* 2004;557:773–783.
108. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* 2000;86:494–501.
109. Ray R, Shah AM. NADPH oxidase and endothelial cell function. *Clin Sci (Lond)* 2005;109:217–226.
110. Stocker R, Keaney JF Jr. New insights on oxidative stress in the artery wall. *J Thromb Haemost* 2005;3:1825–1834.
111. Lassegue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R277–R297.
112. Boulden BM, Widder JD, Allen JC, Smith DA, Al-Baldawi RN, Harrison DG, Dikalov SI, Jo H, Dudley SC Jr. Early determinants of H(2)O(2)-induced endothelial dysfunction. *Free Radic Biol Med* 2006;41:810–817.
113. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: part I: basic mechanisms and in vivo monitoring of ROS. *Circulation* 2003;108:1912–1916.
114. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: part II: animal and human studies. *Circulation* 2003;108:2034–2040.
115. Jacobi J, Sela S, Cohen HI, Chezard J, Kristal B. Priming of polymorphonuclear leukocytes: a culprit in the initiation of endothelial cell injury. *Am J Physiol Heart Circ Physiol* 2006;290:H2051–H2058.
116. Fortuno A, San Jose G, Moreno MU, Belouqui O, Diez J, Zalba G. Phagocytic NADPH oxidase overactivity underlies oxidative stress in metabolic syndrome. *Diabetes* 2006;55:209–215.