

Oxidative Stress and Sleep Apnea-Hypopnea Syndrome

A. Barceló^a and F. Barbé^b^aServicio de Análisis Clínicos, Hospital Universitari Son Dureta, IUNICS, Palma de Mallorca, Islas Baleares, Spain.^bServicio de Neumología, Hospital Universitario Son Dureta, IUNICS, Palma de Mallorca, Islas Baleares, Spain.

Introduction

Sleep apnea-hypopnea syndrome (SAHS) is associated with an increased risk of cardiovascular disease.¹⁻⁴ The pathogenic bases for this association are unknown, but one of the possible mechanisms involved is oxidative stress.⁵ It has been suggested that the intermittent hypoxia and episodes of hypoxia/reoxygenation that accompany apneas may lead to increased vascular release of free radicals, favor the process of formation of atheromata, and increase the risk of cardiovascular disease in patients with SAHS.^{6,7} In this review we present some general concepts concerning the genesis of oxidative stress in the vascular wall and the existing evidence for the involvement of this process in the pathogenesis of cardiovascular disease in patients with SAHS.

Oxidative Stress

General Considerations

Although free radicals or reactive oxygen species (ROS) have well defined physiological functions (such as generating oxidative bursts in neutrophils or activating growth-related intracellular signal transduction pathways), they are highly reactive molecules that can damage cells.⁸ The unchecked production of ROS can be a source of disease through the alteration of macromolecules (lipids, proteins, carbohydrates, and nucleic acids) and diverse cellular processes (membrane function, enzyme production, or gene induction). ROS are produced during metabolic reactions when the cells of the organism transform food into energy, especially under conditions of hyperoxia, intense exercise, and

ischemia. They are also produced through exposure to certain external agents, such as ionizing radiations, ultraviolet light, or tobacco smoke. The most important inorganic ROS are molecular oxygen (O₂), superoxide anion radical (O₂⁻), hydroxyl radical (HO⁻), and its immediate precursor, hydrogen peroxide (H₂O₂); the most important organic ROS are peroxy radical (ROO⁻), organic hydroperoxide (ROOH), and lipid peroxides.⁸⁻¹⁰

The organism possesses antioxidant defense systems whose function is to eliminate free radicals immediately. In addition, there are certain ingested antioxidants that the organism cannot synthesize (Table). An antioxidant is defined as any substance which, when present at concentrations lower than those of an oxidizable substrate, significantly delays or prevents the oxidation of that substrate. The substrate contains organic and inorganic molecules found in living cells, such as proteins, lipids, carbohydrates, or DNA.

TABLE
Types of Antioxidants*

Internal	External
<i>Enzymatic</i>	
Superoxide dismutase	Carotenes and carotenoids (vitamin A)
Glutathione peroxidase	Tocopherols (vitamin E)
Catalase	Ascorbic acid (vitamin C)
Other enzymatic systems involved in redox reactions	Flavonoids
Glutathione reductase	
Methionine reductase	
DT diaphorase	
NADPH-dehydroascorbic acid reductase	
DNA repair enzymes	
<i>Non-enzymatic</i>	
Uric acid	
Albumin	
Bilirubin	
Ceruloplasmin	
Reduced glutathione	
Transferrin	
Ubiquinones	

The following agencies or foundations supported this study: ABEMAR, SEPAR, Fondo de Investigaciones Sanitarias (01/0786, 02/0334), and Red Respira (RTIC C03/11).

Correspondence: Dra. A. Barceló.
Andrea Doria, 55, 07014 Palma de Mallorca, Islas Baleares, España.
E-mail: abarcelo@hds.es

Manuscript received July 30, 2004. Accepted for publication September 22, 2004.

*NADPH indicates nicotinamide adenine dinucleotide phosphate.

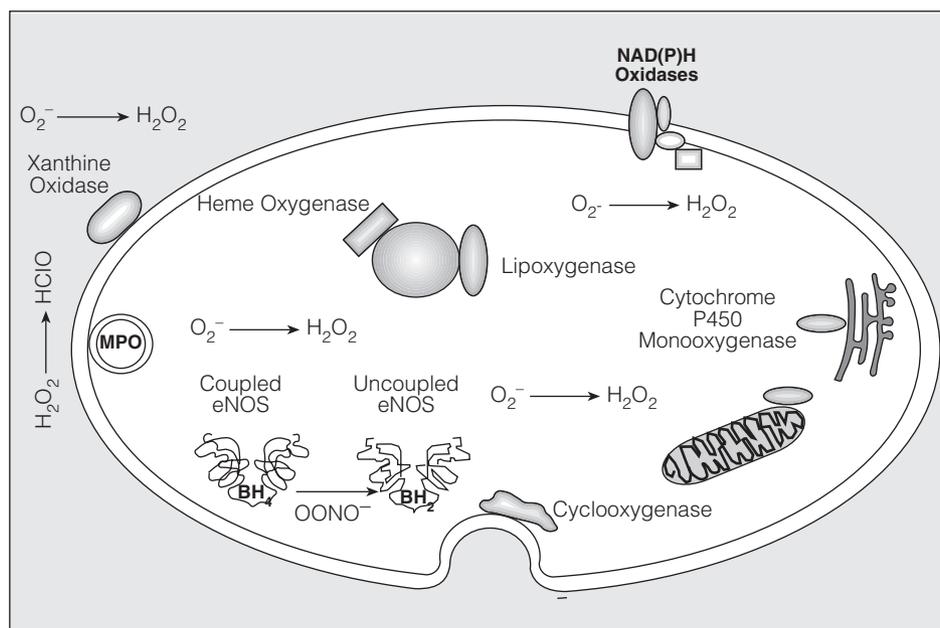


Figure. Potential sources of reactive oxygen species. Many enzymes—such as those that participate in mitochondrial electron transport, xanthine oxidase, cyclooxygenase, lipoxigenase, myeloperoxidase, cytochrome P450 monoxygenase, uncoupled epithelial nitric synthase (eNOS), heme oxygenase, peroxidase, nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate NAD(P)H oxidases—produce reactive oxygen species. Depending on their location in the cell, these reactive oxygen species are generated intracellularly, extracellularly, or in specific intracellular compartments. MPO indicates: myeloperoxidase; O_2^- , superoxide anion radical; H_2O_2 , hydrogen peroxide; $OONO^-$, peroxynitrite; BH_4 , tetrahydrobiopterin; BH_2 , dihydrobiopterin. (Adapted with permission from Griendling et al.¹²)

A shift in the balance between free radicals and antioxidant systems in favor of free radicals leads to what is known as oxidative stress, which plays an important role in numerous degenerative processes, such as aging, arteriosclerosis, or cancer.⁸⁻¹⁰ ROS are key players in the development of vascular diseases, including arteriosclerosis, hypertension, and postangioplasty restenosis.¹¹ It is now clear that many ROS are produced in the arterial wall, and that, alone or in combination, they contribute to the various abnormalities associated with vascular disease.^{12,13}

Reactive Oxygen Species and the Vascular Wall

There are various ROS that play major roles in vascular physiology and pathophysiology. The most important of these are nitric oxide (NO), O_2^- , H_2O_2 and peroxynitrite ($ONOO^-$).¹² ROS are involved in some of the basic functions of the arterial wall. NO is a crucial mediator in endothelium-dependent vasodilation, while O_2^- and H_2O_2 intervene in the growth, differentiation, and apoptosis of smooth muscle cells.^{14,15} Furthermore, $ONOO^-$ -induced lipid peroxidation and protein nitration are early atherogenic events.¹⁶ Each of the ROS is derived from specific chemical or enzymatic reactions (Figure). NO is produced in endothelial cells by the activation of the enzyme epithelial nitric oxide synthase (eNOS), but macrophages and smooth muscle cells can express inducible NO synthase and contribute to the production of NO. NO is a crucial mediator in endothelium-dependent vasodilation. It also participates in the process of platelet aggregation and in maintaining the balance between growth and differentiation of smooth muscle cells. The eNOS enzyme can be activated by diverse vasodilating hormones and physical

forces. The expression of inducible NO synthase in macrophages and smooth muscle cells causes an increase in cytokine concentrations that give rise to a local inflammatory response. Under certain conditions, eNOS is uncoupled due to a deficiency of tetrahydrobiopterin, an essential cofactor, and O_2^- is produced instead of NO. In other words, NO synthase enzymes are potential sources of NO and O_2^- , depending on environmental conditions.^{13,17,18}

All vascular cells produce O_2^- and H_2O_2 . O_2^- is the result of the single-electron reduction of oxygen by a variety of oxidases. When O_2^- is produced along with NO, they rapidly react to form the highly reactive molecule $ONOO^-$. $ONOO^-$ is an important mediator of lipid oxidation—such as the oxidation of low-density lipoproteins (LDL), which has significant proatherogenic effects.

In the absence of NO, O_2^- is rapidly dismutated to a more stable ROS, H_2O_2 , by superoxide dismutase and is then converted to H_2O by catalase or glutathione peroxidase.^{12,13} The effects of O_2^- and H_2O_2 on vascular function depend on the amounts produced. When they form intracellularly in small quantities, they can act as second messengers to modulate the function of biochemical mechanisms that participate in smooth muscle cell or fibroblast growth processes. High ROS production can damage DNA and cause cellular toxicity and apoptosis, as has been demonstrated in both endothelial and smooth muscle cells.¹² In addition to mitochondrial production of ROS, various enzymes—such as nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) oxidases—can synthesize O_2^- and H_2O_2 . Enzyme activity can be modified by various stimuli. Thus, angiotensin II, tumor necrosis factor- α , thrombin, and

platelet-derived growth factor all increase oxidase activity and raise concentrations of O_2^- and H_2O_2 in smooth muscle cells. Physical forces such as changes in blood flow are also potent activators of O_2^- production in endothelial cells.^{12,18} Macrophages are the principal vascular sources of O_2^- in the presence of disease. They oxidize LDL through the activation of various enzymes. Neutrophils and monocytes can also secrete myeloperoxidase, which is important in initiating lipid peroxidation.^{12,16}

Macrophages also release into the extracellular space ROS that can activate metalloproteinases. Once activated, metalloproteinases can degrade the extracellular matrix, weaken the fibrous layer of an atheromatous plaque and facilitate its rupture.¹⁶ In addition, more stable products of ROS may influence cellular function by acting upon specific mechanisms, or by acting as ligands for nuclear and membrane receptors. ROS can directly modify the affinity of certain transcription factors (nuclear factor κB or activating protein 1) for their DNA binding sites. ROS regulate diverse classes of genes, including adhesion molecules, chemotactic factors, antioxidant enzymes, and vasoactive factors. Some of these actions, such as the induction of superoxide dismutase and catalase enzymes by H_2O_2 , are clearly an adaptive response. The upregulation of adhesion and chemotactic molecules by oxidant-sensitive mechanisms is of particular relevance in vascular disease. These molecules promote the adhesion and migration of monocytes to the interior of the vessel wall. Conversely, NO inhibits the transcriptional induction of adhesion molecules by cytokines. These mechanisms combine to suppress the expression of adhesion molecules in the normal arterial wall and induce their expression in vascular disease.^{12,19}

The influence of genetic factors that might modulate the generation of ROS in vascular tissue cannot be ruled out. Thus, a polymorphism in the *CYBA* gene (*C242T*), which encodes the p22phox subunit of the enzyme NADPH oxidase, has been identified and associated with NADPH activity and the generation of O_2^- in vascular tissue.²⁰

Monitoring of Reactive Oxygen Species Formation

Techniques for measuring levels of ROS, which are unstable molecules, are complex and have undergone various changes. Traditional indices, such as the susceptibility of LDL to oxidation, have given way to various biomarkers of oxidative stress. The approach is to identify stable compounds derived from the action of ROS, such as products of lipid peroxidation (like isoprostanes), modified proteins (like nitrated fibrinogen), or indices of modified DNA (like 8-oxo-deoxyguanosine). Antibodies directed against epitopes present in oxidized LDL that are capable of measuring lipid peroxidation have also been developed. Mass spectrometry has also been used to detect amino acids that have undergone oxidative modification.

Excessive release of ROS can also be evaluated through the use of various indirect markers, such as detection of redox-sensitive gene activation, of reductions in the release of NO, of increases in homocysteine concentrations, or of decreases in levels of antioxidants such as enzymes and/or vitamins. Such methods are continually being perfected and new markers will probably emerge that will allow us to measure the action of ROS more precisely.²¹⁻²⁴

Studies in Humans

The latest studies of markers of oxidative stress have detected such markers in patients with various cardiovascular risk factors and have shown oxidative stress to be a characteristic of many cardiovascular diseases. In patients with hypercholesterolemia, elevated levels of isoprostanes and antibodies against oxidized LDL have been found. Statin therapy seems to reduce concentrations of these markers.^{25,26} Elevated levels have also been identified in smokers, as has an increase in the oxidative modification of fibrinogen and elevated concentrations of 8-oxo-deoxyguanosine.^{27,28} Elevated concentrations of isoprostanes have also been associated with obesity. Concentrations decrease with weight loss.²⁹

Many experimental studies have demonstrated the beneficial effects of antioxidants. For example, vitamin C reduces the adhesion of monocytes to endothelial cells, inhibits LDL oxidation, and stimulates the activity of eNOS. Vitamin E also inhibits leukocyte adhesion and LDL oxidation in vitro.³⁰⁻³³ Numerous clinical trials have attempted to validate these observations in humans. Vitamin C therapy has been found to reduce concentrations of isoprostanes in smokers and the beneficial effect of this vitamin on endothelial dysfunction has been shown in patients with cardiovascular disease.^{28,34} The alleged benefits of vitamin E have been more difficult to prove, and there is no agreement on the effects of vitamin E supplementation.^{35,36} While some studies have shown improvement in endothelial function and a decrease in both lipid oxidation markers and cardiovascular episodes following administration of vitamin E, the results of other interventional studies do not support such conclusions on the efficacy of this vitamin.^{35,36}

Oxidative Stress and Sleep Apnea-Hypopnea Syndrome

The potential role that oxidative stress may play in the pathogenesis of cardiovascular disease in patients with SAHS is suggested by observations involving, on the one hand, the elevated production of free radicals during hypoxia-reoxygenation and, on the other hand, the predisposition of such patients to develop arteriosclerosis.^{6,37} This hypothesis is supported by data currently available on the detection of direct markers of

oxidative stress, such as elevated production of ROS in the leukocytes of patients with SAHS, or the detection of indirect markers, such as molecules derived from the activation of redox-sensitive genes.⁵

Increase in the Production of Reactive Oxygen Species in Leukocytes

Leukocytes are activated in situations of hypoxia or through exposure to cytokines or other factors, giving rise to an elevated production of ROS. In 2 studies, an increase in the production of ROS in isolated leukocytes in patients with SAHS was observed, both following stimulation and under basal conditions, indicating chronic production of ROS in such patients.^{38,39} Continuous positive airway pressure (CPAP) therapy was associated with a decrease in the release of ROS in both studies.

Lipoprotein Oxidation

Several lines of evidence support the hypothesis that oxidative modification of LDL plays a crucial role in the pathogenesis of atherosclerosis. Oxidized LDL alter vasomotor response and induce inflammation, as they enhance the expression of adhesion molecules in the endothelium and the release of cytokines and growth factors. Oxidized LDLs are rapidly captured by macrophages, which are transformed into foamy cells. They are simultaneously a consequence of and mediators of oxidative stress. This leads to the perpetuation of the inflammatory response and oxidative damage. It is believed that one of the principal benefits of statin therapy, independent of its capacity to reduce lipid concentrations in plasma, is that it disrupts the inflammation-oxidation cycle.^{16,17,40} One study found no difference in the susceptibility to oxidation of isolated plasma LDL in patients with SAHS compared to healthy subjects,⁴¹ but 3 others showed an increase in LDL oxidation in patients with SAHS and a beneficial effect of CPAP therapy on LDL oxidation.⁴²⁻⁴⁴ These results are indicative of the importance of this pathogenic mechanism in connection with the increased risk of atherogenesis associated with SAHS.

Homocysteine

Homocysteine is a highly reactive sulfurated amino acid that causes endothelial dysfunction through various mechanisms, such as increased production of ROS, decreased release of NO, and alterations in the expression of several genes in endothelial cells.⁴⁵ An association between elevated homocysteine levels and frequency of cardiovascular disease has been shown.^{45,46} A beneficial effect on endothelial dysfunction when homocysteine levels are lowered through treatment with folic acid and vitamins B₆ and B₁₂ has also been reported.^{45,46} However,

the possible effects of such treatments on the reduction of mortality from cardiovascular disease are unknown, and randomized trials are currently being carried out to determine the extent of their usefulness.

In a study carried out by Lavie et al⁴⁷ elevated homocysteine concentrations were found in patients with SAHS and cardiovascular disease compared to patients with SARS and hypertension and with SAHS but without cardiovascular disease. This difference was also detected in a group of patients with cardiovascular disease but without SAHS. No differences related to homocysteine metabolism were found for other risk factors or for concentrations of vitamins B₆, B₁₂, and/or folic acid. These results suggest that homocysteine might be involved in the pathogenesis of cardiovascular disease in patients with SAHS.

Nitric Oxide

The NO produced by the enzyme eNOS in the endothelium regulates vascular flow and has important antiatherogenic effects on platelets, smooth muscle cells, and endothelial cells. In humans, NO-mediated endothelial function is deficient in preatherosclerotic states and several studies have shown endothelial dysfunction to be an independent predictor of future cardiovascular episodes.¹³ There are many mechanisms by which availability of NO may be reduced. The production of ROS rapidly reacts with NO and contributes to NO deficiency. In addition, ONOO⁻, which forms with the reaction of NO and superoxide, has a toxic effect on the cell and a negative effect on vascular function through the oxidation of proteins and lipids. Also, eNOS can generate superoxide instead of NO under certain circumstances, as in cases of tetrahydrobiopterin deficiency, or in response to atherogenic stimuli such as hyperglycemia or hypercholesterolemia.^{13,17} Several studies have shown an improvement in endothelial function following tetrahydrobiopterin supplementation.^{48,49}

In several studies, in which levels of stable derivatives of NO in patients with SAHS were measured, decreased circulating NO concentrations were found. These concentrations increased significantly following CPAP therapy.^{50,51} High levels of asymmetric dimethylarginine, a NO synthase inhibitor, have also been found in such patients, suggesting the existence of several mechanisms that might contribute to a decrease in NO availability and a greater susceptibility to endothelial dysfunction.⁵²

Xanthine Oxidase

Xanthine oxidase catalyzes the degradation of hypoxanthine to uric acid and stimulates the release of ROS. Furthermore, xanthine oxidase bound to endothelial cells can use NADH and together with NADPH oxidase is responsible for endothelial

production of production of O_2^- . The main evidence for the involvement of xanthine oxidase in the pathogenesis of endothelial dysfunction comes from studies in which improvements in endothelium-dependent vasodilation were observed following administration of oxypurinol and allopurinol.

Sahebjami⁵³ observed that in patients with SAHS urinary excretion of uric acid correlated significantly with the apnea-hypopnea index and decreased to levels similar to those of the control group after CPAP therapy. In other studies, an increase in purine degradation products was also detected in such patients.⁵⁴⁻⁵⁶ These results suggest that the release of ROS that occurs during the metabolism of all these molecules probably contributes to the oxidative damage associated with SAHS.

Expression of Redox-Sensitive Genes

The expression of genes sensitive to an increase in ROS is accompanied by the activation of transcription factors and signaling pathways related to proatherogenic events. These transcription factors are influenced by redox state and availability of oxygen.¹⁹ Hypoxia-inducible factor-1 regulates the transcription of genes that code for proteins that participate in adaptive responses to hypoxia, while nuclear factor κ B and activator protein 1 participate in the regulation of genes related to the expression of the cytokines, growth factors, and adhesion molecules involved in inflammatory responses and in the progression of arteriosclerosis.⁵⁷⁻⁵⁹

The studies that point to the activation of these transcription factors in patients with SAHS are indirect ones and the conclusions are based on the detection in plasma of elevated concentrations of proteins coded by such genes, such as vascular endothelial growth factor, endothelin 1, erythropoietin, tumor necrosis factor- α , interleukin 1 and interleukin 6, intracellular adhesion molecule 1, vascular cell adhesion molecule 1, L-selectin, and E-selectin.⁶⁰⁻⁶⁹ The production and release of cytokines such as tumor necrosis factor- α and interleukin 6 might contribute to a systemic inflammatory response which, in turn, seems to be associated with other cardiovascular risk factors, such as obesity, that are quite prevalent in such patients.⁷⁰⁻⁷³ There is also evidence of an increase in the expression of adhesion molecules (CD15 and CD11) in isolated monocytes of patients with SAHS.³⁹ This lends support to the hypothesis of the functional importance of this process in the pathogenesis of the cardiovascular complications of SAHS and highlights the need for further study for a better understanding of the molecular mechanisms involved in such alterations.

Conclusions

Various lines of study support the hypothesis that oxidative stress is an important pathophysiologic

mechanism in cardiovascular disease associated with SAHS. However, at present the main cellular sources that contribute to an increase in ROS in SAHS remain unknown. A better understanding of these mechanisms will lead to more effective therapeutic intervention and to the prevention of the cardiovascular risk attributable to oxidative stress in such patients.

REFERENCES

- Hung J, Whitford EG, Parsons RW, Hillman DR. Association of sleep apnea with myocardial infarction in men. *Lancet*. 1990;336:261-4.
- Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto J, et al. Sleep-disordered breathing and cardiovascular disease. *Am J Respir Crit Care Med*. 2001;163:19-25.
- Peppard P, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med*. 2000;342:1378-84.
- Young T, Peppard P. Sleep-disordered breathing and cardiovascular disease: epidemiological evidence for a relationship. *Sleep*. 2000;23 Suppl 4:122-6.
- Lavie L. Obstructive sleep apnoea 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;16:15-22.
- Hedner JA, Wilcox I, Sullivan CE. Speculations on the interaction between vascular disease and obstructive sleep apnea. *Sleep and breathing*. New York: Marcel Dekker, Inc.; 1994.
- Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human diseases: an overview. *Methods Enzymol*. 1990;186:1-88.
- Elejalde JI. Estrés oxidativo, enfermedades y tratamientos antioxidantes. *An Med Interna*. 2001;18:326-35.
- Ballester M. Antioxidantes, radicales libres y salud. Un enfoque químico-orgánico-físico. *Med Clin (Barc)*. 1996;107:509-15.
- Griendling KK, Fitzgerald GA. Oxidative stress and cardiovascular injury. Part II: animal and human studies. *Circulation*. 2003;108:2034-40.
- Griendling KK, Fitzgerald GA. Oxidative stress and cardiovascular injury. Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation*. 2003;108:1912-6.
- Channon KM, Guzik TJ. Mechanisms of superoxide production in human blood vessels: relationship to endothelial dysfunction, clinical and genetic risk factors. *J Physiol Pharmacol*. 2002;53:515-24.
- Umans JG, Levi R. Nitric oxide in the regulation of blood flow and arterial pressure. *Annu Rev Physiol*. 1995;57:771-90.
- Irani K. Oxidant signaling in vascular cell growth, death, and survival: a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ Res*. 2000;87:179-83.
- Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol*. 2003;91 Suppl:12A-6A.
- Fenster BE, Tsao PS, Rockson SG. Endothelial dysfunction: clinical strategies for treating oxidant stress. *Am Heart J*. 2003;146:218-26.
- Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol*. 2003;91 Suppl:7A-11A.
- Kunsch C, Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. *Circ Res*. 1999;85:753-66.
- Guzik TJ, West NEJ, Black E, McDonald D, Ratnatunga C, Pillai R, et al. Functional effect of the C242T polymorphism in the NADPH oxidase p22phox subunit on vascular superoxide production in atherosclerosis. *Circulation*. 2000;102:1744-7.
- Lawson JA, Rokach J, Fitzgerald GA. Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. *J Biol Chem*. 1999;274:24441-4.

22. Lorch S, Lightfoot R, Ohshima H, Virag L, Chen Q, Hertkom C, et al. Detection of peroxynitrite-induced protein and DNA modifications. *Methods Mol Biol.* 2002;196:247-75.
23. Roberts LJ, Morrow JD. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell Mol Life Sci.* 2002;59:808-20.
24. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. *Circulation.* 2001;103:1930-2.
25. Davi G, Alessandrini P, Mezzetti A, Minotti G, Bucciarelli T, Costantini F, et al. In vivo formation of 8-epiprostaglandin F2 alpha is increased in hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 1997;17:3230-5.
26. Reilly MP, Pratico D, Delanty N, DiMinno G, Tremoli E, Rader D, et al. Increased formation of distinct F2 isoprostanes in hypercholesterolemia. *Circulation.* 1998;98:2822-8.
27. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers: smoking as a cause of oxidative damage. *N Engl J Med.* 1995;332:1198-203.
28. Reilly M, Delanty N, Lawson JA, Fitzgerald GA. Modulation of oxidant stress in vivo in cigarette smokers. *Circulation.* 1996;94:19-25.
29. Davi G, Guagnano MT, Ciabattone G, Basili S, Falco A, Marinopicolli M, et al. Platelet activation in obese women: role of inflammation and oxidant stress. *JAMA.* 2002;288:2008-14.
30. Pratico D, Tangirala RK, Rader DJ. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat Med.* 1998;4:1189-92.
31. Nunes GL, Robinson K, Kalynych A, King SB, Sgoutas DS, Berk BC. Vitamins C and E inhibit O₂⁻ production in the pig coronary artery. *Circulation.* 1997;96:3593-601.
32. Freyschuss A, Stiko-Rahm A, Swedenborg J, Henrikson P, Bjorkhem I, Berglund L, et al. Antioxidant treatment inhibits the development of intimal thickening after balloon injury of the aorta in hypercholesterolemic rabbits. *J Clin Invest.* 1993;91:1282-8.
33. Weber C, Erl W, Weber K, Weber PC. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. *Circulation.* 1996;93:1488-92.
34. Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Greager MA. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest.* 1996;97:22-8.
35. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet.* 1996;347:781-6.
36. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study investigators. *N Engl J Med.* 2000;342:154-60.
37. Prabhakar NR. Sleep apneas. An oxidative stress? *Am J Respir Crit Care Med.* 2002;165:859-60.
38. Schulz R, Mahmoudi S, Hattar K, Sibelius U, Olschewski H, Mayer K, et al. 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;162:566-70.
39. 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;165:934-9.
40. Steinberg D, Witztum JL. Lipoproteins and atherogenesis. *JAMA.* 1990;264:3047-52.
41. Wali SO, Bahammam AS, Massaeli H, Pierce GN, Iliskovic N, Singal PK, et al. Susceptibility of LDL to oxidative stress in obstructive sleep apnea. *Sleep.* 1998;21:290-6.
42. Barceló A, Miralles C, Barbe F, Vila M, Pons S, Agustí AGN. Abnormal lipid peroxidation in patients with sleep apnea. *Eur Respir J.* 2000;16:644-7.
43. Lavie L, Vishnevsky A, Lavie P. Evidence for lipid peroxidation in obstructive sleep apnea. *Sleep.* 2004;27:123-8.
44. Saarelainen S, Lehtimäki T, Jaakkola O, Poussa T, Nikkilä M, Solakivi T, et al. Autoantibodies against oxidised low-density lipoprotein in patients with obstructive sleep apnoea. *Clin Chem Lab Med.* 1999;37:517-20.
45. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: current evidence and future prospects. *Am J Med.* 2002;112:556-65.
46. Clarke R, Stansbie D. Assessment of homocysteine as a cardiovascular risk factor in clinical practice. *Ann Clin Biochem.* 2001;38:624-32.
47. Lavie L, Perelman A, Lavie P. Plasma homocysteine levels in obstructive sleep apnea. association with cardiovascular morbidity. *Chest.* 2001;120:900-8.
48. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, et al. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest.* 1997;99:41-6.
49. Heitzer T, Brockhoff C, Mayer B, Wamholtz A, Mollnan H, Henne S, et al. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. *Circ Res.* 2000;86:E36-E41.
50. Ip MSM, Lam B, Chan L-Y, Zheng L, Tsang KWT, Fung PCW, et al. 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;162:2166-71.
51. Schulz R, Schmidt D, Blum A, Lopes-Ribeiro X, Lücke C, Mayer K, et al. Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnoea: response to CPAP therapy. *Thorax.* 2000;55:1046-51.
52. Carlson J, Hedner J, Patterson A. Increased plasma concentration of ADMA, a naturally occurring nitric oxide synthesis inhibitor in OSA patients. *Am J Respir Crit Care Med.* 1997;155:A869.
53. Sahebajami H. Changes in urinary uric acid excretion in obstructive sleep apnea before and after therapy with nasal continuous positive airway pressure. *Chest.* 1998;113:1604-8.
54. Findley LJ, Boykin M, Fallon T, Belardinelli L. Plasma adenosine and hypoxaemia in patients with sleep apnea. *J Appl Physiol.* 1988;64:556-61.
55. Hasday JD, Grum CM. Nocturnal increase of urinary uric acid: creatinine ratio. A biochemical correlate of sleep-associated hypoxaemia. *Am Rev Respir Dis.* 1987;135:534-8.
56. Braghiroli A, Sacco C, Erbetta M, Ruga V, Donner CF. Overnight urinary uric acid: creatinine ratio for detection of sleep hypoxaemia. Validation study in chronic obstructive pulmonary disease and obstructive sleep apnea before and after treatment with nasal continuous positive airway pressure. *Am Rev Respir Dis.* 1993;148:173-8.
57. Semenza GL. Oxygen regulated transcription factors and their role in pulmonary disease. *Respir Res.* 2000;1:159-62.
58. Piacentini L, Karliner JS. Altered gene expression during hypoxia and reoxygenation of the heart. *Pharmacol Ther.* 1999;83:21-37.
59. Neubauer J. Physiological and genomic consequences of intermittent hypoxia. Invited review: physiological and pathophysiological responses to intermittent hypoxia. *J Appl Physiol.* 2001;90:1593-9.
60. Lavie L, Kraiczi H, Hefetz A, Ghandour H, Perelman A, Hedner J, et al. Plasma vascular endothelial growth factor in sleep apnea syndrome. Effects of nasal continuous positive air pressure treatment. *Am J Respir Crit Care Med.* 2002;165:1624-8.
61. Gozal D, Lipton AJ, Jones KL. Circulating vascular endothelial growth factor levels in patients with obstructive sleep apnea. *Sleep.* 2002;25:59-65.
62. Imagawa S, Yamaguchi Y, Higuchi M, Neichi T, Hasegawa Y, Mukai HY, et al. Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea-hypopnea syndrome. *Blood.* 2001;98:1255-7.
63. Schulz R, Hummel C, Heinemann S, Seeger W, Grimminger F. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. *Am J Respir Crit Care Med.* 2002;165:67-70.
64. Saarelainen S, Hasan J. Circulating endothelin-1 and obstructive sleep apnoea. *Eur Respir J.* 2000;16:794-5.
65. Vgontzas AN, Papanicolaou DA, Bixler EO, Hopper K, Lotsikas A, Lin HM. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance and hypercytokinemia. *J Clin Endocrinol Metab.* 2000;85:1151-8.
66. Entzian P, Linnemann k, Schlaak M, Zabel P. Obstructive sleep apnea syndrome and circadian rhythms of hormones and cytokines. *Am J Respir Crit Care Med.* 1996;153:1080-6.
67. Chin K, Nakamura T, Shimizu K, Mishima M, Nakamura T, Miyasaka M, et al. Effects of nasal continuous positive airway pressure on soluble cell adhesion molecules in patients with obstructive sleep apnea syndrome. *Am J Med.* 2000;109:562-7.

68. Ohga E, Nagase T, Tomita T, Teramoto S, Matsuse T, Katayama H, et al. Increased levels of circulating ICAM-I, VCAM-I, and L-selectin in obstructive sleep apnea syndrome. *J Appl Physiol.* 1999;87:10-4.
69. 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;121:1541-7.
70. Yokoe T, Minoguchi K, Matsuo H, Oda N, Minoguchi H, Yoshino G, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation.* 2003;107:1129-34.
71. Shamsuzzaman ASM, Winnicki M, Lanfranchi P, Wolk R, Kara T, Accurso V, et al. Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation.* 2002;105:2462-4.
72. Barceló A, Barbé F, Llompert E, Mayoralas LR, Lalaria A, Bosch M, et al. Effects of obesity on C-reactive protein level and metabolic disturbances in male patients with obstructive sleep apnea. *Am J Med.* 2004;117:118-21.
73. Barceló A, Barbé F. Las citocinas en la patogenia del síndrome de apneas/hipopneas del sueño. *Trastornos respiratorios del sueño. Monografía de la Sociedad Madrileña de Neumología y Cirugía Torácica.* Madrid: Ergón; 2004. p. 149-16.