Eur Respir J 2009; 33: 1467–1484
DOI: 10.1183/09031936.00086608

SERIES "THE GENETIC AND CARDIOVASCULAR ASPECTS OF OBSTRUCTIVE SLEEP APNOEA/HYPOPNOEA SYNDROME"
Edited by R.L. Riha and W.T. McNicholas
Number 5 in this Series

Molecular mechanisms of cardiovascular disease in OSAHS: the oxidative stress link

L. Lavie and P. Lavie

ABSTRACT: **Obstructive sleep apnoea/hypopnoea syndrome (OSAHS) is a highly prevalent breathing disorder in sleep that is an independent risk factor for cardiovascular morbidity and mortality. A large body of evidence, including clinical studies and cell culture and animal models utilising intermittent hypoxia, delineates the central role of oxidative stress in OSAHS as well as in conditions and comorbidities that aggregate with it. Intermittent hypoxia, the hallmark of OSAHS, is implicated in promoting the formation of reactive oxygen species (ROS) and inducing oxidative stress. The ramifications of increased ROS formation are pivotal. ROS can damage biomolecules, alter cellular functions and function as signalling molecules in physiological as well as in pathophysiological conditions. Consequently, they promote inflammation, endothelial dysfunction and cardiovascular morbidity. Oxidative stress is also a crucial component in obesity, sympathetic activation and metabolic disorders such as hypertension, dyslipidaemia and type 2 diabetes/insulin resistance, which aggregate with OSAHS. These conditions and comorbidities could result directly from the oxidative stress that is characteristic of OSAHS or could develop independently. Hence, oxidative stress represents the common underlying link in OSAHS and the conditions and comorbidities that aggregate with it.**

KEYWORDS: **Cardiovascular morbidity, inflammation, metabolic dysregulation, obesity, obstructive sleep apnoea/hypopnoea, oxidative stress**

In recent years, obstructive sleep apnoea/hypopnoea syndrome (OSAHS) has become a major public health problem, as predicted by PHILLIPSON [1] some 15 yrs ago in an editorial accompanying the seminal paper by YOUNG *et al*. [2] on the prevalence of symptomatic sleep apnoea in the general population. The close association between OSAHS and cardiovascular morbidity has been a crucial factor in the process of making OSAHS a public health problem. Since the early days, when OSAHS patients were diagnosed in sleep laboratories, it has become evident that a substantial number exhibit cardiovascular risk factors and overt cardiovascular diseases [3–5]. These initial observations, relying mostly on case series of patients studied in sleep clinics and cross-sectional studies, have been confirmed and expanded in recent years by well designed large-scale epidemiological, treatment and prospective studies, and by animal models of OSAHS. These demonstrated close association between OSAHS and hypertension, ischaemic heart disease, strokes, arrhythmias, chronic heart failure and cardiovascular mortality. Extensive reviews on cardiovascular morbidity and mortality in OSAHS and the association of OSAHS with components of the metabolic syndrome have been recently published [6–8]. Moreover, patients with OSAHS without overt cardiovascular diseases show subclinical signs of atherosclerosis that are related both to the structure of the vasculature, such as increased intima-media thickness, arterial plaque formation and calcified artery atheromas [9–12], and to its function, such as endothelial

AFFILIATIONS
Lloyd Rigler Sleep Apnea Research Laboratory, Unit of Anatomy and Cell Biology, The Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel.

CORRESPONDENCE
L. Lavie
Unit of Anatomy and Cell Biology
The Ruth and Bruce Rappaport Faculty of Medicine
Technion
POB 9649
31096
Haifa
Israel
Fax: 972 48343934 or 972 48295403
E-mail: lenal@tx.technion.ac.il

Received:
June 07 2008
Accepted after revision:
February 25 2009

SUPPORT STATEMENT
This study was supported in part by a grant from the United States–Israel Binational Science Foundation (Jerusalem, Israel), grant no. 1006695.

STATEMENT OF INTEREST
A statement of interest for P. Lavie can be found at www.erj.ersjournals.com/misc/statements.dtl

Previous articles in this series: No. 1: MacLeod AK, Liewald DCM, McGilchrist MM, *et al*. Some principles and practices of genetic biobanking studies. *Eur Respir J* 2009; 33: 419–425. **No. 2:** Riha RL, Gislasson T, Diefenbach K. The phenotype and genotype of adult obstructive sleep apnoea/hypopnoea syndrome. *Eur Respir J* 2009; 33: 646–655. **No. 3:** Jennum P, Riha RL. Epidemiology of sleep apnoea/hypopnoea syndrome and sleep-disordered breathing. *Eur Respir J* 2009; 33: 907–914. **No. 4:** Garvey JF, Taylor CT, McNicholas WT. Cardiovascular disease in obstructive sleep apnoea syndrome: the role of intermittent hypoxia and inflammation. *Eur Respir J* 2009; 33: 1195–1205.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

dysfunction and higher pulse wave velocity [13–16]. Some of these subclinical conditions were improved by treatments with nasal continuous positive airway pressure (nCPAP) or a dental device [17–19]. Thus, today, there is a better understanding of the pathophysiology of cardiovascular diseases in OSAHS and of its natural evolution.

In the present review we will examine the role of oxidative stress in initiating cardiovascular consequences in OSAHS. We will argue that oxidative stress is not only a characteristic of OSAHS but is also an important component in the associated conditions and comorbidities that aggregate with it, such as sympathetic activation, obesity, hypertension, hyperlipidaemia and diabetes mellitus, of which some may precede the appearance of OSAHS, or develop independently. A discussion is dedicated to the potential synergistic effects of oxidative stress associated with these conditions and comorbidities and with sleep apnoea itself, which may greatly enhance the impact of oxidative stress and the resultant inflammatory/immune cell activation on the cardiovascular system. Wherever relevant, animal models treated with intermittent hypoxia (IH) mimicking OSAHS will be described to reinforce detailed mechanisms that cannot be directly investigated in OSAHS patients. A simplified scheme of the central role of oxidative stress in OSAHS and the associated risk factors leading to cardiovascular morbidity is illustrated in figure 1.

PATHOPHYSIOLOGY OF CARDIOVASCULAR MORBIDITY IN OSAHS

The pathophysiological mechanisms of cardiovascular morbidity in OSAHS are complex and involve neural, humoral, mechanical and haemodynamic components that may be modified by genetic makeup, nutrition and lifestyle-related variables [20, 21]. OSAHS is associated with elevated sympathetic discharge during sleep and waking that has been linked with increased systemic blood pressure and could be ameliorated by nCPAP treatment [22–25]. Moreover, there is evidence that the sympathetic responses to hypoxic chemoreflex stimulation are enhanced in OSAHS [26], which could be mimicked by exposing healthy subjects to IH [27]. Also, OSAHS patients have impaired glucose tolerance, leptin resistance and increased incidence of the metabolic syndrome [28]. Higher fasting blood glucose levels in OSAHS increased the risk of diabetes mellitus. Mechanical and haemodynamic changes, which arise from the repeated negative intrathoracic pressure swings occurring because of the upper airway obstructions, result in altering stroke volume and cardiac output [29]. Treatment with nCPAP was reported to decrease blood pressure, improve insulin sensitivity and decrease left ventricular wall thickness in OSAHS patients [30]. Recent studies have shown that OSAHS, as well as an animal model of chronic IH, is also associated with hypercholesterolaemia independent of adiposity [31]. Treatment with nCPAP decreased total and low-density lipoprotein (LDL) cholesterol without any change in body weight. Accumulated data from our laboratory and others have shown, however, that OSAHS is also strongly associated with oxidative stress, which, as will be demonstrated later in this review, is a major component in the chain of events leading to atherogenesis and cardiovascular morbidity. In the following sections we will review the evidence supporting the involvement of oxidative stress in OSAHS as well as in conditions and comorbidities that aggregate with it.

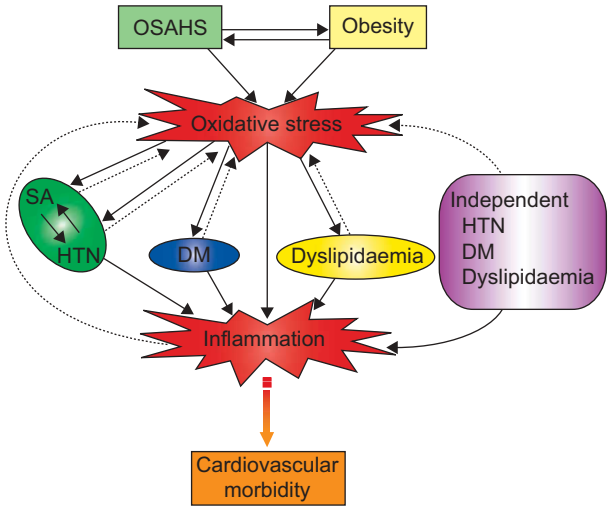

FIGURE 1. A schematic illustration suggestive of the central role played by oxidative stress and inflammation in obstructive sleep apnoea/hypopnoea syndrome (OSAHS) and the development of associated conditions and comorbidities. Associated conditions and comorbidities can be induced by oxidative stress or develop independently. Once these conditions and comorbidities develop, regardless of the initiating factors, they elicit a series of intricate interactions with various transduction pathways, promoting oxidative stress and inflammation. The enhanced oxidative stress exacerbates inflammation, which in turn further exacerbates oxidative stress, generating a vicious cycle, eventually leading to cardiovascular morbidity. SA: sympathetic activation; HTN: hypertension; DM: type 2 diabetes.

REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS IN HEALTH AND DISEASE

Reactive oxygen species: seminal discoveries and sources

Reactive oxygen species (ROS) or reactive nitrogen species (RNS) are atoms or molecules possessing one or more unpaired electrons in the outer orbit and, therefore, are prone to react chemically [32]. The predominant ROS molecule is the superoxide radical ($O_2^{\cdot-}$), which is generated by univalent reduction of molecular oxygen, mainly during mitochondrial respiration but also by several enzymatic systems, such as xanthine oxidase, ''uncoupled'' endothelial nitric oxide synthase (eNOS) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase from primed leukocytes and endothelial cells [33, 34]. The superoxide is a relatively weak radical, but reacting with other molecules can yield additional more potent ROS molecules and oxidants as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and lipid peroxides. Additional toxic radicals, such as peroxynitrite ($OONO^{-}$), which is formed by the reaction of superoxide with the primary vasodilator nitric oxide (NO), also contribute to oxidative/nitrosative stress. This reaction results in diminished NO availability and severely affects endothelial function [35].

While ROS are produced in normal aerobic metabolism, in order to keep oxidation–reduction under tight control, antioxidant defence systems have evolved to help maintain the tightly regulated balance termed oxidative homeostasis [32]. Upon disruption of this balance, oxidative stress ensues. Thus, oxidative stress results in an imbalance between oxidant-producing systems and antioxidant defence mechanisms,

resulting in excessive formation of ROS. This excess ROS/RNS can damage cellular components and various biomolecules, such as lipids, proteins, DNA and carbohydrates, and thus alter their biological functions.

The first to suggest that superoxide radicals were involved in pathologies such as atherosclerosis and cancer as well as ageing was HARMAN [36, 37]. However, three major discoveries later established the roles of superoxide radicals in the biology and pathophysiology of medicine. In 1969, MCCORD and FRIDOVICH [38, 39] discovered the superoxide dismutase (SOD) enzyme, which catalyses the dismutation of the superoxide radical to H_2O_2 and molecular oxygen. This discovery had far-reaching implications, since it indicated that the superoxide radical was indeed produced in the physiological setting, as suggested over a decade earlier by HARMAN. The second discovery, by BABIOR *et al.* in 1973 [40], established the importance of the superoxide radical that is released by blood leukocytes as a protective mechanism against invading microorganisms, later shown to be the product of activated NADPH oxidase [41]. The third and most relevant discovery to OSAHS was that by GRANGER *et al.* [42], which established the involvement of superoxide radicals in ischaemia/reperfusion injury. Specifically, the relevance of ischaemia/reperfusion injury was shown in conditions such as ischaemic heart disease, stroke, surgery and organ transplantation. The main sources of ROS for these pathologies were identified as xanthine oxidase [43], inflammatory cells and damaged mitochondria [44]. Since then, more than 80 pathologies have been associated with ROS and oxidative stress, including all inflammatory diseases (such as arthritis, glomerulonephritis and adult respiratory distress syndrome), cancer, ischaemic heart diseases and stroke, diabetes, hypertension, atherosclerosis and neurodegenerative diseases (such as Alzheimer's and Parkinson's diseases) [45] and, more recently, OSAHS. The fact that a great number of diverse pathologies share such a common feature (overproduction of ROS) is likely to result from altered oxidative metabolism of injured cells, activated inflammatory cells or altered oxygen supply. However, a fundamental question arises: are ROS the cause or a consequence in each of these conditions? Apparently in many of the diseases this is an unresolved issue. However, we will confine the current discussion primarily to OSAHS.

ROS as signalling molecules

Although for many years ROS were considered merely as toxic and unavoidable by-products of normal respiratory oxidative metabolism, in the last decade they were consistently described as regulators of signal transduction and as second messengers in many signalling pathways in all cells [46]. Since ROS are abundant molecules that react with many molecules and atoms, their specificity, which is a prerequisite for signalling, was questioned [47]. However, their importance in the maintenance of strict cellular redox homeostasis is now well established [32, 47]. The evidence that implicates ROS in the activation of a plethora of signalling pathways is rising. To date, pathways involved in initiation of inflammatory responses, ultimately leading to atherosclerosis, were described in an array of pathologies, such as hypertension, hyperlipidaemia, diabetes mellitus and obesity [48]. Specifically, ROS were implicated in mitogen-activated protein kinase (MAPK) pathways, which induce activation of various nuclear transcription factors, such as nuclear factor (NF)-κB, activator protein (AP)-1, hypoxia-inducible factor (HIF)-1α, sterol regulatory element binding proteins (SREBPs) and GATA-4 [32, 49, 50]. NF-κB is of particular significance to the pathology of OSAHS since it initiates inflammatory pathways and orchestrates the production of adhesion molecules, inflammatory cytokines and adipokines. Moreover, NF-κB participates in obesity and the metabolic syndrome and, similarly to OSAHS, it induces inflammatory and atherosclerotic consequences. However, these mechanisms are not yet fully elucidated [51]. HIF-1α is another transcription factor with potentially important implications to the pathology of OSAHS and in the augmentation of sympathetic nerve activity [52, 53], and associated comorbidities such as hypertension [54, 55] and hyperlipidaemia [56], arising from IH. Additional transcription factors that are redox sensitive and could possibly be implicated in OSAHS pathology include NF-interleukin (IL)-6, early growth response-1, specificity protein-1 and NF-(erythroid-derived 2)-related factor (Nrf)2-antioxidant responsive element (ARE), which regulates antioxidant genes. Additional findings on transcription factors in OSAHS are described in following sections of this review.

Evidence for oxidative stress in OSAHS and IH models

The evidence implicating oxidative stress as a fundamental component of OSAHS pathophysiology has been consistently rising in the last decade. It was shown in cells [57, 58], plasma [59–61], urine [62] and exhaled air [63], and could be moderated by various treatments, such as nCPAP or dental device [19, 59, 60, 63]. The chain of events that promotes oxidative stress is most likely initiated by repeated breathing cessation, accompanied by drastic changes in oxygen tension, and is considered analogous to hypoxia and reoxygenation injury [33, 43, 64, 65]. This process of hypoxia/reoxygenation affects cells and cellular components, resulting in increased ROS production. One of the most prominent features of hypoxia/reoxygenation is mitochondrial dysfunction, which induces increased production of ROS, particularly through complex I of the respiratory chain [33, 66]. Leukocytes are affected as well and thus largely contribute to increased ROS production in OSAHS patients *via* the NADPH oxidase system, while treatment with nCPAP attenuates ROS production [57, 58, 67]. Xanthine oxidase is another enzyme affected by OSAHS [33, 68]. Additional ROS sources in OSAHS include endothelial cells and uncoupled eNOS [69]. Accordingly, oxidation of various macromolecules further attests to enhanced oxidative stress in OSAHS. Of these, lipids are the most prone to oxidation. Increased lipid peroxidation in OSAHS that was attenuated by the use of nCPAP was demonstrated in several studies [59, 60, 70]. Moreover, the reported increased lipid peroxidation was specifically found to be apnoea/hypopnoea index (AHI; the number of apnoeas plus hypopnoeas divided by hours of sleep)-severity dependent. Also, it was less affected by comorbidities such as hypertension and cardiovascular diseases, or by age and body mass index (BMI) [59]. The fact that lipid peroxidation, which is a surrogate marker of atherosclerosis and cardiovascular morbidity, was found to be AHI-severity dependent emphasises the possible involvement of ROS in amplifying atherosclerotic processes in patients with OSAHS. Notably, a single night of

treatment with nCPAP was sufficient to attenuate lipid peroxidation (L. Lavie, A. Vishnevsky and P. Lavie, Technion Institute of Technology, Haifa, Israel; personal communication). In addition, DNA oxidation was increased in patients with OSAHS [62]. Similarly, oxidation of proteins, nucleic acids and lipids were shown in several studies using animal models exposed to chronic IH [71, 72]. Furthermore, in rats exposed to chronic IH, lipid peroxidation was increased while antioxidant activity was decreased. These findings were correlated with blood pressure and left ventricular myocardial dysfunction [73].

Disruption of the tightly regulated cellular oxidation–reduction (redox) state, maintained between oxidant-producing systems and antioxidant defence mechanisms, could also be affected by decreased antioxidant capacities. As noted earlier, in patients with OSAHS this balance was shown to be perturbed by excess ROS formation. Thus, decreased antioxidant capacity was also shown to contribute to oxidative stress [61, 74]. Additionally, the antioxidant enzyme paraoxonase-1, which is located exclusively on high-density lipoprotein (HDL) and protects both LDL and HDL from oxidative modification, was shown to decrease in patients with OSAHS. Moreover, the decreased paraoxonase-1 activity was more pronounced in patients who already had cardiovascular comorbidities [59]. Paraoxonase-1 activity was also significantly negatively correlated with AHI but not with BMI or age (L. Lavie, A. Vishnevsky and P. Lavie; personal communication). In the clinical setting, paraoxonase-1 activity was shown to decrease in patients with acute myocardial infarction, hypercholesterolaemia, diabetes mellitus and hyperleptinaemia [75, 76]. This is in agreement with the observation that HDL of OSAHS patients was shown to be dysfunctional [77].

The impact of oxidative stress on endothelial dysfunction is a likely possibility due to the decline in NO bioavailability noted in the circulation of patients with OSAHS [78–80], as discussed previously [33, 65, 81]. A recent study conducted on freshly harvested venous endothelial cells from patients with OSAHS provided the first direct evidence at the cellular and molecular levels on the significance of oxidative stress to endothelial cell function [69]. In that study, the eNOS and its phosphorylated active form were attenuated in patients with OSAHS, whereas the oxidative stress marker nitrotyrosine, which is indicative of NO inactivation by oxidative stress, was elevated. Treatment with nCPAP reversed these values and improved endothelial function as determined by flow-mediated dilation [69]. Furthermore, data have demonstrated that treating OSAHS patients with allopurinol, a xanthine oxidase inhibitor, or the antioxidant vitamin C improved endothelial function [68, 82]. Additionally, the potential importance of antioxidant treatment for attenuating oxidative stress and inflammation resulting from chronic IH is exemplified in a recent animal model treated orally with antioxidant green tea polyphenols (GTP) [83]. While IH induced cognitive decline, increased brain lipid peroxidation *via* NADPH oxidase activation, and elevated the levels of the inflammatory prostaglandin E2, treatment with GTP improved these measures [83].

Taken together, the wealth of data accumulated thus far, in OSAHS and in animal models treated with IH, clearly attests to the presence of oxidative stress. This was shown by increased production of ROS, decreased antioxidant capacity, diminished NO bioavailability and reversal by treatment with antioxidants, nCPAP or dental device.

Activation of transcription factors in OSAHS and in animal models of IH

NF-κB and AP-1

Activation of inflammatory pathways by upregulation of NF-κB was demonstrated in neutrophils and monocytes of patients with OSAHS [84–86], and in an *in vitro* model of HeLa cells treated with IH [87]. Consequently, upregulation of adhesion molecules and inflammatory cytokines and adipocytokines, the gene products of NF-κB, were also noted, further supporting activation of NF-κB in OSAHS [57, 67, 88, 89], as well as in associated conditions and comorbidities [90]. Upregulated activity of AP-1 and tyrosine hydroxylase mRNA, which is an AP-1-regulated downstream gene, was demonstrated in tissue culture in PC12 cells exposed to IH [91]. Since AP-1 upregulation, similarly to NF-κB, involves upregulated expression of adhesion molecules and inflammatory cytokines, the involvement of AP-1 is also implicated in the pathogenesis of OSAHS [33, 81]. Interestingly, IH *in vitro* was shown to activate the NF-κB in an IκB kinase-dependent manner *via* activation of p38 MAPK [92]. Thus, the data on the involvement of inflammation in OSAHS are well established, but the pathways of activation need to be elucidated.

HIF-1α

Induction of the master regulator HIF-1α, which is essential for oxygen homeostasis and adaptive response to hypoxia, was documented primarily in several experimental models of IH in tissue culture, and in rodents exposed to chronic IH [93]. By delineating the transduction signals that activate HIF-1α under IH conditions, it was shown that IH in PC12 cells induced HIF-1α transcriptional activity *via* a novel signalling pathway involving Ca^{2+}/calmodulin-dependent kinase [94]. In yet another study conducted on endothelial cells *in vitro*, four cycles of repeated hypoxia/reoxygenation induced a modification in HIF-1α phosphorylation patterns *via* p42/p44 activation [95]. By contrast, RYAN *et al.* [92] did not find increased HIF-1α activation in bovine aortic cells exposed to IH *in vitro*. Such contradictory findings may result from different cell types or from the different IH patterns employed. In animal models exposed to chronic IH, HIF-1α was implicated in hypertension [54, 55] and in components of the metabolic syndrome [56]. In wild-type mice treated with chronic IH, hypercholesterolaemia and hypertriglyceridaemia were evident after 5 days of treatment, whereas, in mice with partial deficiency for HIF-1α, the development of hypertriglyceridaemia was inhibited [56]. Thus far, HIF-1α activation has not directly been demonstrated in patients with OSAHS. However, upregulation of some of its gene products, including erythropoietin, vascular endothelial growth factor and heat shock proteins, supports this notion [33, 96, 97], although erythropoietin was not reported by all [87]. Combined with the data from animal studies described above, the role of HIF-1α in OSAHS pathophysiology is waiting to be unveiled.

SREBPs

The SREBPs are another group of transcription factors with possible implications in the pathology of OSAHS. The SREBPs

that activate genes regulating lipid metabolism [98, 99] were shown to be upregulated in experimental models of IH [100, 101]. Furthermore, SREBPs were also shown to be affected by redox imbalance and oxidative stress [102, 103]. To date, all studies describing the possible involvement of SREBPs in OSAHS were derived from rodents exposed to chronic IH. In this model, the development of atherosclerosis was demonstrated in response to chronic IH and both lipid peroxidation and atherosclerosis were dependent on the severity of the chronic IH [104]. Interestingly, although the hyperlipidaemia observed was mediated by the SREBP-1 pathway [31, 105], HIF-1α was also implicated in the upregulation of serum triglyceride levels through post-translational regulation of SREBP-1 [56].

Given that OSAHS is associated with hyperlipidaemia independent of obesity, as manifested by various studies [106–110], it is a likely possibility that the hyperlipidaemia observed in OSAHS is mediated *via* upregulated SREBP-1 pathway and HIF-1α involvement, as demonstrated by the animal models previously cited. Furthermore, while oxidative stress was implicated in the upregulation of SREBP, antioxidants were inhibitory [102, 103]. Thus, IH and oxidative stress in humans, like in experimental models, may upregulate SREBP-1 leading to the hyperlipidaemia associated with OSAHS. Yet the possible complex interactions with HIF-1α remain elusive.

GATA

The GATA transcription factors owe their name to their ability to bind the consensus DNA sequence (A/T) and (A/G) through two highly conserved zinc finger domains. Based on animal models of chronic IH, the GATA family is emerging as yet another set of redox-sensitive transcription factors with far-reaching implications for cardiovascular morbidity in OSAHS. The GATA-4 and GATA-6 members of this family were implicated in the regulation of cardiac development and growth as well as in heart failure. However, an altered balance of GATA-4 may cause cardiac hypertrophy or promote cardioprotection [111]. Moreover, recent data show that GATA-4 might be an important mediator of cardiac myocyte survival *via* endothelin-1 and hepatocyte growth factor, to prevent cardiomyocyte death by oxidative stress-induced apoptosis [112].

By treating a mouse model with IH, GATA-4 was shown to exert preconditioning-like cardioprotective effects, by protecting cardiomyocytes from apoptosis. While treatment with acute IH exerted cardioprotective effects, treating mice with a prolonged period of IH induced increased susceptibility of the heart, which was mediated by increased oxidative stress. Additional treatment with prolonged IH reversed the added myocardial damage [113, 114]. It is therefore of utmost importance to delineate the complex effects of IH on the GATA family of transcription factors in the human heart. This could help in identifying patients with OSAHS at risk of developing cardiovascular morbidity or alternatively those who may develop cardioprotection.

Nuclear transcription factors act in concert and, apart from being activated by oxidative stress, are also activated by a variety of other signals, such as hormones, growth factors and cytokines, to regulate gene expression. Thus, investigating the mechanisms of their upregulation and the transduction pathways that are activated in OSAHS may prove difficult, mainly due to various inter-individual differences in the levels of signals other than IH. Employing tissue culture models, knockouts and transgenic mice exposed to IH may prove useful in delineating such mechanisms.

INFLAMMATORY PATHWAY ACTIVATION AND INTERACTIONS WITH ENDOTHELIAL CELLS IN OSAHS

As noted earlier, ROS molecules and a state of oxidative stress are considered potent activators of a cascade of inflammatory pathways that induce overexpression of adhesion molecules and pro-inflammatory cytokines. These adhesion molecules facilitate the recruitment and accumulation of leukocytes, platelets and possibly red blood cells (RBCs) on the endothelium lining the vasculature. Such cellular interactions between blood cells and endothelial cells may promote endothelial cell injury [33].

Blood cell activation and expression of adhesion molecules

Normally, circulating leukocytes are free flowing in the circulation and resist interactions with endothelial cells. Upon encounter with a variety of stimuli or insults, including inflammation, infections, hypercholesterolaemia, cytokines, hypoxia/reoxygenation and sleep apnoea, adhesion molecules and cytokines are upregulated in blood cells as well as in endothelial cells. Expression of adhesion molecules is a highly regulated and orderly process that facilitates these interactions between blood cells and endothelial cells. Such cellular interactions promote adherence and injury to the vascular endothelium. The selectins (L-selectins in leukocytes, E-selectins in endothelial cells and P-selectins in platelets and endothelial cells) facilitate weak binding between leukocytes and endothelial cells. A firm binding is mediated by the integrins, which also mediate transmigration into the interstitial layer through the endothelial cell layer [48, 115]. In OSAHS, several studies have described the expression of adhesion molecules on various leukocyte subpopulations and the interactions with endothelial cells [57, 67, 88, 89, 116, 117].

Polymorphonuclear leukocytes

The polymorphonuclear leukocytes (PMNs) are the most abundant of the leukocyte subpopulations, representing approximately 60% of all circulating leukocytes. They are short lived (up to 24 h in the bloodstream) terminally differentiated cells that continuously undergo cell death by apoptosis. PMNs actively participate in inflammatory responses in order to protect from invading microorganisms, foreign particles or cellular debris. While recruited to inflammatory sites or in conditions characterised by ischaemia and reperfusion, they express various injurious molecules, such as ROS, inflammatory cytokines, adhesion molecules and cell surface receptors [118]. Interestingly, PMNs were shown to infiltrate eroded or ruptured plaques obtained from patients with acute coronary syndromes [119, 120], and to participate in the pathogenesis of lethal myocardial reperfusion [121]. Notably, depleting PMNs resulted in reduced myocardial infarct size and a protected myocardium [122, 123].

In patients with OSAHS, increased expression of selectins CD62 and CD15 was noted in a severity-dependent manner. Treatment with nCPAP effectively lowered the expression of CD15 [67]. However, the expression of the β-subunits of the

integrins CD11b (O. Golan-Shany, P. Lavie and L. Lavie, Technion Institute of Technology, Haifa, Israel; personal communication) or CD11c, and adhesion to endothelial cells, were unaffected [57]. Since the selectins but not integrins of OSAHS patients' PMNs were upregulated, this implies that only the interactions involving binding and tethering with endothelial cells are increased while those for firm adhesion are not necessarily affected. In addition, PMN apoptosis, a fundamental injury-limiting mechanism and a key event in the control of inflammation, was suppressed in OSAHS PMNs. Suppressed apoptosis and increased expression of selectins in OSAHS PMNs could suggest increased PMN/endothelial cell interactions and, therefore, amplification of their destructive potential towards the endothelium [67].

Monocytes

Like PMNs, monocytes act as professional phagocytes, but unlike PMNs, they are long lived and their initiation, participation in progression and persistence of atherosclerosis are well established [48, 124]. Upon activation by various stimuli and inflammatory conditions they too express higher amounts of adhesion molecules, ROS molecules and inflammatory cytokines. In OSAHS, monocytes were shown to be activated and expressed higher amounts of CD15 and CD11c, ROS and cytokines [57, 125]. Furthermore, CD15 expression was shown to depend on the severity of OSAHS [89] and in healthy individuals hypoxia *in vitro* resulted in upregulated CD15 expression [57]. Unlike in PMNs, the CD11c integrin of OSAHS monocytes was also elevated, while treatment with nCPAP attenuated the levels of both CD15 and CD11c. Accordingly, increased adhesion of OSAHS monocytes to endothelial cells of venous origin (human umbilical vein endothelial cells) or arterial origin (human coronary artery endothelial cells) was noted. Treatment with antibodies neutralising selectins (anti-CD62) and integrins (anti-CD54) abrogated the adhesion of monocytes to endothelial cells [57]. The involvement of monocytes in atherogenesis in OSAHS was further implicated by the observation that lipid uptake was increased in human macrophages that were treated with experimental IH *in vitro* [126].

T-lymphocytes

Numerous subpopulations of lymphocytes have mainly been implicated in various atherogenic processes *via* cytokine secretion and antibody production. Also, lymphocytes have been shown to be prevalent in atherosclerotic lesions and to modulate atherosclerotic responses [127, 128]. Natural killer (NK) lymphocytes, CD8+, CD4+ and γδ T-cells were all implicated in atherosclerotic sequelae, further adding to the complexity of atherosclerosis. In patients with OSAHS, most T-cell subpopulations were meticulously investigated in our laboratory. In fact, all T-cells investigated (CD8+, CD4+ and γδ T-cells) expressed an activated and a cytotoxic phenotype.

Assessment of γδ T-cell phenotype and function revealed that expression of CD62L selectins was increased in OSAHS compared with controls. Also, adhesion to endothelial cells and cytotoxicity towards endothelial cells were higher in OSAHS. The higher avidity and cytotoxicity of OSAHS γδ T-cells were mainly attributed to the pro-inflammatory cytokine tumour necrosis factor (TNF)-α. Treatment with antibodies that neutralise TNF-α abolished the cytotoxicity of γδ T-cells against endothelial cells [117].

Unlike in γδ T-cells, adhesion of CD4+ and CD8+ T-cells to endothelial cells was unaffected by OSAHS, but their cytotoxic capacities against endothelial cells were increased. Moreover, the killing capacities of CD8+ T-lymphocytes were also found to be AHI-severity dependent. However, each subpopulation employed different killing mechanisms to damage endothelial cells [88, 116, 117]. Unlike in $\gamma\delta^+$ T-lymphocytes, which primarily utilised TNF-α for endothelial cell killing, CD8+ T-cells expressed higher amounts of the CD56 NK receptors, higher perforin levels and more than three-fold higher CD40 ligand, accounting for their higher cytotoxicity [116]. In CD4+ T-cells of OSAHS patients, the cytotoxic CD4+/CD28-null subpopulation was increased three-fold. All in all, the most potent cytotoxicity against endothelial cells was expressed by OSAHS γδ T-cells; CD8+ cytotoxicity was somewhat lower and that of CD4+ T-cells was the lowest.

Platelets

Platelets maintain vascular homeostasis by clot formation and wound healing. Under physiological conditions, platelets circulate in a quiescent state protected from activation by inhibitory mediators released from intact endothelial cells, including NO. When encountering vascular damage or under oxidative stress, or in response to endothelial dysfunction, platelets rapidly undergo activation. This is followed by increased interactions with monocytes and PMNs and increased adhesion and aggregation in the vessel wall, which implicates their involvement in atherosclerosis [129]. Also, in conditions such as hypoxia/reoxygenation, platelets have been shown to acquire an activated and a pro-thrombotic phenotype [130]. In patients with OSAHS, platelets have been shown to express increased activation and aggregability *in vitro*. The expression of P-selectins (CD62P) was increased [131, 132], mainly in the severe group of patients [133], and was lowered by treatment with nCPAP [134]. In addition, increased levels of haematocrit, blood viscosity and fibrinogen in patients with OSAHS could further affect hypercoagulability and contribute to the increased incidence of cardiovascular events in OSAHS [135–137].

Jointly, increased expression of adhesion molecules on leukocytes and platelets followed by increased avidity to endothelial cells and higher cytotoxicity of T-cells towards endothelial cells, delayed PMN apoptosis, higher ROS generation by leukocytes and the higher aggregability of platelets, are all markers of activation of these cells and indicative of the possible ongoing processes that may affect endothelial function and elicit atherogenesis in patients with OSAHS. Such cellular interactions of leukocytes and endothelial cells were also demonstrated by intravital microscopy in a rat model of IH [138].

RBCs

The RBCs comprise the major cell type in the circulation. Their main function is oxygen delivery to all tissues and organs. Under normal blood flow their adherence to endothelial cells is nonsignificant and their deformability facilitates tissue perfusion. Under hypoxic/ischaemic conditions, RBCs are capable of inducing and participating in inflammatory responses, most likely through ROS molecules and redox-sensitive transcription factors [139]. Moreover, adhesiveness and aggregation of

RBCs were shown to be elevated in hypertension [140], atherosclerosis [141] and obesity [142]. Increased RBC aggregation/adhesion was also associated with OSAHS, and correlated with increased C-reactive protein (CRP) levels [143]. Being the major component in the circulation, the effects of sleep apnoea and IH on RBC functions and adhesive properties should be considered for investigation.

Endothelial cells

Endothelial cells line the vasculature and form the endothelial cell layer, which provides a permeability barrier for the vasculature, regulates vessel tone and maintains an anti-inflammatory and anti-thrombotic phenotype. In their non-activated state, endothelial cells resist adhesion to leukocytes, platelets and RBCs. However, activation or injury by various factors, such as hypercholesterolaemia, obesity, hypertension and hypoxia/reoxygenation, triggers the expression of adhesion molecules, which mediate these interactions [130, 144]. Recently, JELIC *et al.* [69] demonstrated *in vivo* activation of endothelial cells in patients with OSAHS. Additionally, both oxidative stress and inflammation were induced by OSAHS while NO bioavailability and the repair capacity of endothelial cells were attenuated [69]. Earlier studies indirectly corroborating these findings include identification of soluble variants of endothelial cell adhesion molecules in the circulation of OSAHS patients, such as E- and P-selectin, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [110, 145–148]. Circulating adhesion molecules released from the endothelium are considered as markers of active atherosclerotic diseases and as predictors of future cardiovascular disease [81]. Numerous recent studies also investigated apoptosis of endothelial cells and endothelial progenitor cells (EPCs) in OSAHS. Results, however, were inconclusive. While the numbers of circulating apoptotic endothelial cells in patients with OSAHS were lowered after nCPAP in one study [149], no changes were noted in another [150]. Conflicting results were also reported on the amounts of EPCs, which at low numbers are indicative of impaired vascular function. While one study described a reduced number of circulating EPCs in OSAHS [151], in another study no differences were noted between patients with OSAHS and controls [150]. Such conflicting results can stem from the very small numbers of apoptotic cells or EPCs in the circulation. Thus, more rigorous measures are needed to investigate the involvement of endothelial cell apoptosis or EPC function in OSAHS.

Inflammatory mediators in OSAHS

Cytokines

Similarly to adhesion molecules, pro-inflammatory cytokines are also induced by the redox state of the cells in which they are synthesised. Cytokines actively participate and modulate inflammatory responses by complex interactions with various transcription factors. These multipurpose molecules are synthesised and released by many cell types and regulate both the innate and adaptive immune system. These include regulation of macrophage activation, modulation of smooth muscle cell proliferation, NO production and activation of endothelial cells. Many of these functions are involved in the progression of atherosclerosis.

TNF-α is a pro-inflammatory cytokine involved with the initiation and progression of cardiovascular pathology [152, 153]. It is synthesised by a variety of cells including inflammatory leukocytes and adipocytes. TNF-α induces oxidative stress on the endothelium, upregulates endothelial cell adhesion molecules and stimulates cytokine production *via* NF-κB-dependent pathways. TNF-α is also one of the most studied cytokines in OSAHS. In many of these studies, TNF-α of OSAHS patients was increased in plasma or serum [154–156]. Similarly, IL-6, IL-8 and the anti-inflammatory cytokine IL-10 were shown to be affected by OSAHS [9, 155, 157, 158]. As IH initiates the inflammatory response, these pro-inflammatory cytokines can in turn activate NF-κB and thus can further exacerbate inflammation. Interestingly, TNF-α was also shown to induce HIF-1α activation *via* NF-κB-dependent pathways in normoxic conditions [159].

Apart from circulating levels of cytokines, elevated cytokine levels were described in monocytes and in various cytotoxic T-lymphocytes [88, 116, 117, 125]. Specifically, in $\gamma\delta^+$ T-lymphocytes the pro-inflammatory TNF-α was increased and the anti-inflammatory IL-10 was decreased compared with control values. Also, the expression of IL-8, a pro-inflammatory cytokine with strong chemoattractant and activating properties for PMNs, was shown to increase in γδ T-cells of patients with OSAHS [117]. This clearly attests to a pro-inflammatory state of these cells. In CD8+ T-cells, both TNF-α and IL-10 were increased compared with controls. However, TNF-α was increased four-fold whereas IL-10 was increased only 1.3-fold. By contrast, in CD4+ T-cells the percentage of cells expressing TNF-α was unaffected by OSAHS, but the expression of IL-10 was increased 4.9-fold compared with control values [88, 160]. Such altered cytokine balance can result in activated T-cells and can lead to their differentiation into effector cells with a tissue-damaging potential or alternatively with the capacity to moderate inflammation depending on these cytokine ratios [124].

Adipokines

In recent years the adipose tissue has gained recognition as a highly active endocrine organ and as a rich source for cytokine production. These cytokines are termed adipocytokines or adipokines due to their source. The key adipokines include TNF-α, IL-6, CRP, leptin, resistin and angiotensinogen [161]. Some are also synthesised in other cells and tissues as aforementioned. The blood levels of some of these adipokines are elevated in the obese as well as in patients with OSAHS, resulting in low-grade inflammation in both instances [162]. Since the fat tissue represents a major source for cytokines/adipokines, the cytokines released by adipocytes, when measured in the circulation, can pose a problem in identifying their source: whether they are synthesised in obesity *per se* and/or because of OSAHS. Thus, when obese OSAHS patients are investigated, the contribution of obesity should be separated from that of OSAHS. Such data were clearly demonstrated in obese and overweight patients undergoing surgical treatment [155]. In the majority of studies, cytokine/adipokine levels were determined in serum or plasma of OSAHS patients and thus represent the overall pool of cytokines/adipokines released from various inflammatory cells, adipocytes, the liver and other tissues. Therefore, such

data cannot provide information on specific inflammatory/anti-inflammatory responses, or on an ongoing process in specific inflammatory cells, as each cell has a unique cytokine profile, as already discussed [160]. Adipokines specific for the fat tissue, such as leptin and adiponectin will be discussed in the section on obesity.

CRP

CRP is another molecule potentially linking OSAHS to oxidative stress, inflammation and atherosclerosis, which is secreted primarily by the liver but also by other cell types. It is an acute-phase reactant induced by IL-6, a marker of inflammation and a strong predictor of coronary heart disease and of future cardiovascular events [163, 164]. As inflammation and atherogenesis are closely associated with oxidative stress and altered redox balance, analysis was conducted on coronary arteries of patients undergoing atherectomy procedures. Immunohistochemical analysis clearly demonstrated that CRP protein, as well as its mRNA, were co-expressed with NADPH oxidase in vascular smooth muscle cells and macrophages obtained from vulnerable plaques. Moreover, when added to cultured coronary artery smooth muscle cells, CRP had pro-oxidant effects. This suggests that CRP could play a crucial role in plaque instability and acute coronary syndrome *via* its pro-oxidant effects [165]. Another function of CRP that is oxidative stress dependent is its ability to induce tissue factor expression by vascular smooth muscle cells, which implicates it in the pathogenesis of arterial thrombosis [166]. In addition, CRP levels were shown to affect endothelial cells and induce their expression of adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 *in vitro* [167], and to sensitise endothelial cells to killing by T-cells [168]. CRP was also shown to inhibit endothelium-dependent NO-mediated dilation in retinal arterioles by initiating superoxide production from NADPH oxidase. This effect was abolished using the antioxidant tempol [169]. These diverse effects of CRP on endothelial cells and vascular smooth muscle cells can promote endothelial dysfunction and atherothrombosis. Thus, it is obvious that CRP is not merely an inflammatory marker but rather a modulator of functions that may contribute to the development of inflammatory/atherosclerotic processes *via* oxidative stress [167].

In OSAHS, the question of whether CRP levels are elevated is controversial. Earlier, CRP was reported to be elevated in a severity-dependent manner [170] and to decrease with nCPAP [157]. More recently, however, obesity rather than OSAHS was suggested as a risk factor for elevated CRP [171–175]. Current data from the large Wisconsin Sleep Cohort Study support the mediation of obesity in the elevated CRP levels observed in OSAHS [173]. However, the fact that CRP levels are also affected by sleep duration [176] makes it difficult to separate the independent contribution of each of the factors affecting CRP in OSAHS. It is likely that all these components contribute to varying degrees. Thus, a patient with OSAHS also having high CRP levels should be considered at a high risk for developing cardiovascular complications regardless of its cause.

OXIDATIVE STRESS IN CONDITIONS AND COMORBIDITIES THAT AGGREGATE WITH OSAHS

Thus far, we have examined the role of oxidative stress in sleep apnoea within the context of IH and its more pronounced consequences such as inflammation [50]. However, as will be discussed next, oxidative stress is associated with a variety of conditions such as obesity and sympathetic activation and comorbidities such as hypertension, hyperlipidaemia and diabetes, which aggregate with OSAHS. Hence, in OSAHS there could be a confluence of different potentially independent sources of ROS that could greatly amplify its effects.

Obesity

Obesity is strongly associated with OSAHS. Notably, between 60% and 90% of OSAHS patients are obese [177]. Although the nature of this association is not clear, gaining weight aggravates the severity of OSAHS, while drastic weight reduction by controlled diet or by surgical means improves it [178]. Obesity, particularly visceral obesity, is also a cardiovascular risk factor, as demonstrated in cross-sectional, clinic-based and population-based studies [76]. Similarly to OSAHS, obesity is associated with the male sex, post-menopausal status in females, cardiovascular morbidity, hypertension, stroke, insulin resistance and type 2 diabetes [179]. Obesity is also associated with oxidative stress. For instance, in the community-based cohort of the Framingham Heart Study, KEANEY *et al.* [180] demonstrated that smoking, diabetes and BMI were significantly and independently associated with systemic oxidative stress markers. FURUKAWA *et al.* [181] demonstrated that, in nondiabetic subjects, fat accumulation was closely correlated with markers of systemic oxidative stress and plasma adiponectin levels were inversely correlated with oxidative stress. Moreover, a drastic weight reduction by surgical intervention attenuated oxidative stress markers, free fatty acids and cholesterol, in the obese [182]. A recent review summarising the presence of various oxidative stress markers in plasma, serum, urine and erythrocytes in humans concluded that oxidative stress levels are elevated in human obesity [76]. Notably, as OSAHS has not been taken into consideration in studies investigating oxidative stress in obese individuals (this can apply to at least 60% of the obese), the contribution of OSAHS to oxidative stress in the obese cannot be excluded. However, reproducing these results in several mouse models of obesity suggests that accumulated fat contributes to oxidative stress independently of OSAHS. Furthermore, animal models displaying oxidative stress in accumulated fat indicate that it is mediated by the obesity-associated development of metabolic syndrome *via* dysregulated production of adipokines [181]. The mechanisms involved with increased oxidative stress in this animal model include upregulated expression of NADPH oxidase and concomitant decreases in the expression of antioxidant enzymes in adipocytes. Additional suggested sources of oxidative stress in obese individuals include uncoupled mitochondria, free fatty acid-associated protein and lipid peroxidation, decreased antioxidant defence, and leukocytes and endothelial cells [76]. Also, other sources of ROS are derived from conditions and comorbidities associated with obesity, such as hypertension, insulin resistance, hyperglycaemia and inflammation [76].

Exposure of adipocytes to hypoxia also elicits dysregulated production of adipokines such as TNF-α, leptin, resistin and adiponectin, which result in low-grade inflammation [183]. Similar findings on dysregulated adipokine production and inflammation were also reported in OSAHS [90]. TRAYHURN and WOOD [184] suggested that the inflammation in adipose

tissue, in the obese, is a response to hypoxia of enlarged adipocytes distant from the vasculature. Thus, obesity renders adipocytes hypoxic and predisposes the obese to sustained hypoxia [184]. Collectively, many of the sources of oxidative stress in the obese are parallel to those in OSAHS. Yet, the lower vascularisation characteristic of fat tissue, which renders it partially hypoxic in a sustained manner, should be considered as well in the obese with OSAHS.

Hyperleptinaemia

Normal leptin production and action through its receptors are essential for regulating body weight and appetite by inhibiting food intake and maintaining energy balance, thus influencing energy expenditure [185]. Leptin was also shown to exert angiogenic properties *via* induction of the redox-sensitive HIF-1α under hypoxic conditions [32, 186]. In several studies leptin was shown to increase ROS and the potent vasoconstrictor endothelin (ET)-1, to activate protein kinase C and to promote the secretion of atherogenic compounds [187, 188]. The increase in oxidative stress and ET-1 levels by leptin can elicit increased expression of adhesion molecules and recruitment of leukocytes directly damaging endothelial cells and vascular smooth muscle cells and possibly increase blood pressure [76]. Also, hyperleptinaemia lowers the antioxidant paraoxonase-1 activity, as previously shown also for OSAHS patients [59, 187]. Jointly with the increases in atherogenic factors in the circulation, leptin may further contribute to endothelial dysfunction and the development of atherosclerosis.

In patients with OSAHS, leptin levels were reported to be higher in the majority of studies. Large differences were particularly noted in studies investigating non-obese or at most overweight subjects [108, 189, 190]. However, by comparing obese patients with obese controls (BMI >30 kg·m^{-2}), the differences disappeared [189]. The association between hyperleptinaemia and OSAHS is supported by observations that long-term nCPAP treatment lowered leptin levels [106, 189, 191], and by data from experimentally induced chronic IH in animal models [192]. Notably, the disrupted leptin metabolism due to IH also affected insulin resistance. Thus, it was suggested that elevated leptin levels induced by OSAHS may represent an important compensatory mechanism to minimise metabolic dysfunction [192]. However, given the angiogenic properties of leptin, its levels may also rise to compensate for the decrease in oxygen supply due to OSAHS.

Adiponectin

Adiponectin is the most abundant of all circulating adipokines, and is exclusively produced by adipose tissue. It decreases in the obese and in patients with type 2 diabetes, and was shown to correlate with insulin sensitivity [193, 194]. Notably, it acts as an anti-diabetogenic and anti-inflammatory cytokine with cardiovascular protective effects. In contrast to leptin, adiponectin attenuates endothelial cell adhesion molecules and the levels of pro-inflammatory cytokines such as TNF-α, IL-8 and IL-6, while increasing the levels of the anti-inflammatory cytokine IL-10. In a rat model of angiotensin (Ang)II-induced hypertension, oxidative stress was increased while attenuating mRNA levels of adiponectin. However, the antioxidant tempol abolished the hypertension, decreased the oxidative stress and increased mRNA adiponectin levels [195]. In addition, this has been directly demonstrated in adipose tissue of obese mice and humans. Adipocytes treated with hypoxia *in vitro* were also shown to have altered adiponectin levels [196]. Thus, both hypoxia and oxidative stress greatly attenuate adiponectin levels.

In OSAHS, most studies have reported that adiponectin levels were mainly unaffected by the syndrome. This is particularly evident in studies selecting non-obese, comorbidity-free patients [67, 108, 197]. However, adiponectin levels were also shown to increase in obese OSAHS [198], or to decrease in severe patients [199]. Notably, decreased adiponectin levels in OSAHS were attributed to obesity [197]. In line with the data reporting on unchanged adiponectin levels in OSAHS, long-term nCPAP treatment was also shown to be ineffective [200, 201]. As noted earlier, adiponectin is primarily released by adipose tissue and decreases in the obese and yet is affected by oxidative stress. However, the majority of studies show that it is not affected by OSAHS. This may imply that, regarding adiponectin, obesity and increased tissue-specific oxidative stress override the influence of OSAHS.

Sympathetic activation and hypertension

Sympathetic activation is a prominent feature of OSAHS [22–25] and has been linked with hypertension. There is evidence, however, mostly from animal studies, that ROS and oxidative stress participate in cardiac-autonomic signalling [202]. Oxidative stress may induce sympathetic hyperactivation and, *vice versa*, sympathetic activation may increase oxidative stress, thus creating a vicious cycle that greatly affects the cardiovascular system. It was shown that oxidative stress mediates increased expression of inducible nitric oxide synthase (iNOS) in the rostral ventrolateral medulla in rats and thus causes sympathetic activation and blood pressure elevation. Administration of tempol, an antioxidant inhibiting superoxide production, significantly inhibited the pressor response induced by iNOS [203]. Likewise, tempol significantly attenuated sympathetic nerve activity in a dose-dependent manner without alterations in mean arterial pressure and heart rate in spontaneously hypertensive rats [204]. Increased superoxide production that was mediated by ET-1 was also shown in sympathetic ganglia of hypertensive rats. These data indicate that a change in the redox environment of sympathetic ganglionic neurons may activate sympathetic neurons and result in vasoconstriction and hypertension [205]. In fact, a great number of animal studies have demonstrated an association between hypertension and increased formation of ROS and impaired endogenous antioxidant defence mechanisms [206]. Both AngIIb and ET-1 were implicated in ROS formation accompanied by vasoconstriction and hypertension in rat vasculature. Furthermore, treatment with SOD, which scavenges superoxide, reduced blood pressure [206]. ROS formation was increased *via* activation of vascular NADPH oxidase and xanthine oxidase, and resulted in increased oxidative stress markers in heart vessels and various other tissues [206, 207]. It should be noted that AngII also induced insulin resistance in skeletal muscle that was mediated by NF-κB activation *via* NADPH oxidase [208]. Similarly, ET-1, another potent vasoconstrictive and mitogenic peptide with blood pressure-elevating properties, was also shown to induce hypertension *via* ROS formation. The source

of ROS was also shown to be NADPH oxidase in the vasculature [209].

Two recent studies in animal models implicate oxidative stress in the development of hypertension by exposure to chronic IH. In one study, ROS formation directly increased the production of ET-1 and thus also increased hypertension. Treatment with the antioxidant tempol prevented the increase in blood pressure and lowered oxidative stress and plasma ET-1 [210]. In another study, PENG *et al.* [55] demonstrated that arterial blood pressure, plasma noradrenalin and oxidative stress markers were increased in a mouse model of chronic IH. Treatment with a potent superoxide scavenger lowered blood pressure and attenuated noradrenalin and oxidative stress. Conversely, chronic IH treatment of a mouse model partially deficient in HIF-1α did not increase mean blood pressure, adrenalin or oxidative stress. This study not only implicates free radicals in the development of hypertension due to IH but also implicates HIF-1α activation and the complex interactions between oxidative stress and HIF-1α in affecting blood pressure due to IH.

Jointly, the results from animal studies suggest that, in OSAHS, oxidative stress could be one of the mediating factors between IH, AngII, ET-1 and hypertension. Data from patients with OSAHS are mostly in agreement with the animal studies. AngII and ET-1 were reported to be elevated in OSAHS and were correlated with blood pressure, while long-term nCPAP attenuated blood pressure. The decrease in blood pressure was correlated with lower plasma renin and AngII levels [211]. However, delineating the complex interactions between sympathetic activation, AngII, ET-1, HIF-1α and oxidative stress in humans may prove difficult.

Dyslipidaemia

Dyslipidaemia is also a prevalent finding among sleep apnoea patients. Increased total serum cholesterol and triglyceride levels and decreased HDL independent of age and BMI were shown in epidemiological studies and in matched case–control studies of sleep apnoea patients [107, 108]. Additionally, dysfunctional HDL [77] and lower antioxidant activity of paraoxonase-1 bound to HDL were detected in patients with OSAHS, particularly in those who also had cardiovascular comorbidities [59]. Effective amelioration of the apnoeas with nCPAP treatment lowered serum total cholesterol levels [109]. Animal models treated with chronic IH also demonstrated increased hypercholesterolaemia and accelerated atherosclerosis, which are directly attributed to the IH *via* activation of SREBP [100, 104]. Hypercholesterolaemia has a profound effect on endothelial function. In relatively short periods (within days), hypercholesterolaemia can alter the phenotype of endothelial cells from anti-inflammatory/anti-thrombotic to pro-inflammatory/pro-thrombotic phenotype. The altered vascular phenotype in hypercholesterolaemia is largely attributed to increased oxidative stress, which also results in a decreased bioavailability or bioactivity of NO, increased expression of adhesion molecules and increased leukocytes/platelets/endothelial cell interactions [212, 213]. Notably, hypercholesterolaemia, even in an acute state, induces oxidative stress *via* activation of NADPH oxidase or xanthine oxidase, yet the initiating factors remain elusive [213]. Hence, IH in sleep apnoea could be one possible initiating factor.

Insulin resistance and type 2 diabetes

Oxidative stress was also suggested to be one of the major causes of hyperglycaemia and diabetes, since it was shown to impair glucose uptake in muscle and fat and to decrease insulin secretion from pancreatic β-cells [214–216]. Conversely, hyperglycaemia was also shown to trigger increased formation of ROS *via* glucose auto-oxidation. Accordingly, consumption of a high free-glucose diet promoted the development of oxidative stress [217]. Increased production of free radicals in diabetic patients was also shown by protein glycation and the formation of glycosylation end-products [218, 219]. In the insulin-resistant obese Zucker rat, acute pro-oxidant challenge *in vivo* exacerbated insulin resistance, impaired glucose tolerance and promoted the onset of type 2 diabetes [220]. Activation of NF-κB [221] and increased NADPH oxidase activity were suggested as likely mechanisms in this sequence [208]. Furthermore, in both type 1 and type 2 diabetes, the late

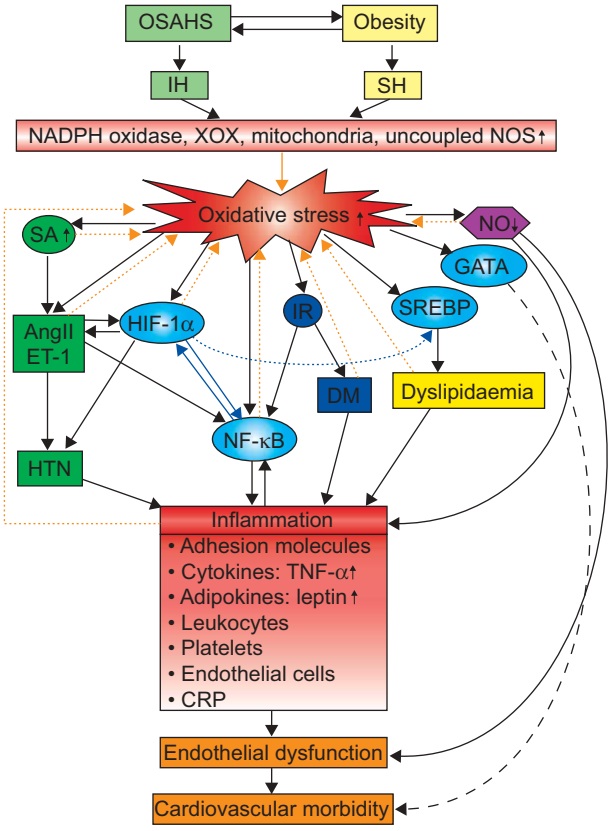

FIGURE 2. A tentative model suggestive of oxidative stress as a unifying paradigm in obstructive sleep apnoea/hypopnoea syndrome (OSAHS) and the development of conditions and comorbidities that aggregate with OSAHS. These include obesity, hypertension, inflammation, sympathetic activation, type 2 diabetes and dyslipidaemia, all of which have an oxidative stress component. IH: intermittent hypoxia; SH: sustained hypoxia; NADPH: reduced nicotinamide adenine dinucleotide phosphate; XOX: xanthine oxidase; NOS: nitric oxide synthase; SA: sympathetic activation; NO: nitric oxide; GATA: GATA transcription factor; Ang: angiotensin; ET: endothelin; HIF: hypoxia-inducible factor; IR: insulin resistance; SREBP: sterol regulatory element binding protein; DM: type 2 diabetes; NF: nuclear factor; HTN: hypertension; TNF: tumour necrosis factor; CRP: C-reactive protein. Orange dotted arrows: oxidative stress induced by the various conditions and comorbidities, further augmenting oxidative stress and, consequently, inflammation.

diabetic complications in neurons, vascular endothelium and kidney arise from common stress-activated signalling pathways such as NF-κB and p38 MAPK [222]. The facts that elevated insulin levels generate free radicals by NAD(P)H-dependent mechanisms [223], and that plasma concentrations of inflammatory mediators, such as TNF-α and IL-6, are increased in the insulin-resistant states of obesity and type 2 diabetes, support the involvement of oxidative stress in atherogenesis and cardiovascular sequelae in diabetes [224].

Although it is not clear if OSAHS causes diabetes, insulin resistance was shown to be independently associated with OSAHS in severity-dependent measures such as AHI and minimum oxygen saturation. However, obesity is also a major determinant in insulin resistance [225]. PUNJABI *et al.* [226] have shown that sleep-disordered breathing is a prevalent finding in mildly obese males and is independently associated with glucose intolerance and insulin resistance. This association was verified in large-scale epidemiological studies of the Wisconsin Sleep Cohort and the Sleep Heart Health Study [227, 228]. Additionally, treatment with nCPAP immediately restored blood glucose levels, mainly in non-obese OSAHS patients [229], although improvement was also noted in diabetic OSAHS patients [230]. This, however, was not shown in all studies [201].

SUMMARY

The accumulated evidence presented in this review is illustrated by the tentative model presented in figure 2, which introduces oxidative stress as the unifying link between OSAHS and the conditions and comorbidities that aggregate with OSAHS.

Obesity can develop independently, due to genetic, behavioural or lifestyle-related variables, but it is suggested that it may also induce or exacerbate OSAHS. Conversely, OSAHS may also induce or exacerbate obesity. Thus, obesity and OSAHS may exacerbate each other, yet both promote oxidative stress. OSAHS *via* IH, and obesity through sustained hypoxia, can activate enzymes such as NADPH oxidase, xanthine oxidase, complex I in mitochondria and uncoupled eNOS, to produce ROS.

Once oxidative stress is initiated it affects multiple systems. By reaction of ROS with NO, oxidative stress is increased while NO is diminished, thus promoting inflammation and endothelial dysfunction. Oxidative stress can also induce sympathetic activation and increases in AngII and ET-1 and, therefore, may promote hypertension. At the same time, oxidative stress can induce the upregulation of numerous redox-sensitive transcription factors, such as HIF-1α, NF-κB, SREBPs and GATA. Also, insulin resistance is affected by oxidative stress and, when combined with upregulated NF-κB activity, may promote type 2 diabetes. Dyslipidaemia may develop *via* upregulation of SREBP. Upregulated HIF-1α activity may be involved in the development of hypertension and induction of hypertriglyceridaemia *via* SREBP activation. HIF-1α is also upregulated in obesity. Diabetes, dyslipidaemia, obesity and hypertension, as well as OSAHS, are involved with NF-κB activation and inflammation. Inflammatory pathway activation is characterised by increased expression of adhesion molecules, cytokines, adipokines, CRP, activated blood cells and endothelial cells.

Many of the inflammatory pathways activated by NF-κB, such as TNF-α, further induce oxidative stress through activation of NADPH oxidase. In many of the pathways, the conditions and comorbidities that develop can further induce oxidative stress, thus creating a vicious cycle of oxidative stress and inflammation. The possible interactions described in this review are much more complex than could be depicted in figure 2. For instance, AngII, through NF-κB activation, may induce NADPH oxidase activation and insulin resistance. ET-1 may activate GATA-4, which could prevent cardiomyocyte apoptosis by oxidative stress [112]. The involvement of HIF-1α in upregulation of leptin and the effects of hyperleptinaemia on oxidative stress and inflammation, as well as additional interactions between obesity and oxidative stress, have been illustrated previously [76]. Details of possible intricate interactions between NF-κB and HIF-1α have been described elsewhere [159].

CONCLUSION

In recent years a large body of evidence has implicated oxidative stress, inflammation, sympathetic activation, obesity and hyperlipidaemia as fundamental components in the pathophysiology of cardiovascular morbidity in OSAHS. The data presented in this review demonstrate that ROS play a significant role in a great number of pathologies and conditions that aggregate with OSAHS (fig. 1). Although the specific pathways and sites (tissues) at which ROS are generated and induce damage may vary from one condition to another, their involvement in signalling pathways, particularly those activating inflammatory/immune sequences, is common to all conditions. Thus, obesity, hypertension, hyperlipidaemia, hyperleptinaemia and insulin resistance all share with sleep apnoea ROS-dependent pathway activation and inflammatory responses, which ultimately lead to endothelial dysfunction and atherosclerosis. In some of these conditions and comorbidities it is not always clear what the initiating factor is. These comorbidities could develop independently of OSAHS due to genetic, hormonal, nutritional or lifestyle-related variables, or be a direct consequence of OSAHS. Thus, metabolic dysregulations and obesity that aggregate with OSAHS could be in many instances a consequence of sleep apnoea. That is, the apnoeas related to IH and the ensued oxidative stress, followed by the chain of events described in figure 2, may orchestrate the simultaneous or sequenced development of sympathetic nerve activity, hypertension, hyperlipidaemia, insulin resistance and diabetes. However, obesity could be a likely candidate for an initiating factor. Regardless, however, of who preceded whom, whether sleep apnoea or metabolic dysregulation, it is obvious that once sleep apnoea develops and aggregates with the aforementioned conditions, the oxidative stress it initiates night after night becomes a central factor in eliciting the cascade of events that eventually result in cardiovascular morbidities. Also, it is not always clear which comes first, oxidative stress or inflammation, as both are fundamental components in each of the conditions and comorbidities that aggregate with sleep apnoea. Furthermore, as most OSAHS patients are obese, sustained hypoxia, which is characteristic of

adipose tissue, may also contribute to oxidative stress in addition to the IH, and may activate different ROS-dependent signalling pathways as well. Thus, clarifying various pathways of activation in the obese apnoeic patient would prove difficult, or incorrect, for understanding sleep apnoea-related mechanisms. This also could explain some of the contradictory results in the literature regarding oxidative/inflammatory markers in OSAHS.

In order to clarify basic mechanisms imposed by intermittent hypoxia, more *in vitro* studies at the cellular level and animal studies should be conducted. Using specific cells, such as endothelial cells, cardiomyocytes, adipocytes, hepatocytes and blood cells, under intermittent and sustained hypoxic conditions, could help to delineate cell-specific mechanisms. Complementary studies, employing various transgenic and knockout mice for specific genes, could further expand our understanding of the basic mechanisms that are governed by intermittent hypoxia.

REFERENCES

1 Phillipson EA. Sleep apnea – a major public health problem. *N Engl J Med* 1993; 328: 1271–1273.
2 Young T, Palta M, Dempsey J, *et al.* The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993; 328: 1230–1235.
3 Malhotra A, White DP. Obstructive sleep apnoea. *Lancet* 2002; 360: 237–245.
4 Deegan PC, McNicholas WT. Pathophysiology of obstructive sleep apnoea. *Eur Respir J* 1995; 8: 1161–1178.
5 Shamsuzzaman AS, Gersh BJ, Somers VK. Obstructive sleep apnea: implications for cardiac and vascular disease. *JAMA* 2003; 290: 1906–1914.
6 Lopez-Jimenez F, Sert Kuniyoshi FH, Gami A, *et al.* Obstructive sleep apnea: implications for cardiac and vascular disease. *Chest* 2008; 133: 793–804.
7 Somers VK, White DP, Amin R, *et al.* Sleep apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. *J Am Coll Cardiol* 2008; 52: 686–717.
8 Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. *Lancet* 2009; 373: 82–93.
9 Minoguchi K, Yokoe T, Tazaki T, *et al.* Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005; 172: 625–630.
10 Drager LF, Bortolotto LA, Lorenzi MC, *et al.* Early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med* 2005; 172: 613–618.
11 Friedlander AH, Yueh R, Littner MR. The prevalence of calcified carotid artery atheromas in patients with obstructive sleep apnea syndrome. *J Oral Maxillofac Surg* 1998; 56: 950–954.
12 Jelic S, Bartels MN, Mateika JH, *et al.* Arterial stiffness increases during obstructive sleep apneas. *Sleep* 2002; 25: 850–855.
13 Carlson JT, Rangemark C, Hedner JA. Attenuated endothelium-dependent vascular relaxation in patients with sleep apnoea. *J Hypertens* 1996; 14: 577–584.
14 Kato M, Roberts-Thomson P, Phillips BG, *et al.* Impairment of endothelium-dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation* 2000; 102: 2607–2610.
15 Itzhaki S, Lavie L, Pillar G, *et al.* Endothelial dysfunction in obstructive sleep apnea measured by peripheral arterial tone response in the finger to reactive hyperemia. *Sleep* 2005; 28: 594–600.
16 Nagahama H, Soejima M, Uenomachi H, *et al.* Pulse wave velocity as an indicator of atherosclerosis in obstructive sleep apnea syndrome patients. *Intern Med* 2004; 43: 184–188.
17 Drager LF, Bortolotto LA, Figueiredo AC, *et al.* 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.
18 Ip MS, Tse HF, Lam B, *et al.* Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med* 2004; 169: 348–353.
19 Itzhaki S, Dorchin H, Clark G, *et al.* 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.
20 Riha RL, Diefenbach K, Jennum P, *et al.* Genetic aspects of hypertension and metabolic disease in the obstructive sleep apnoea-hypopnoea syndrome. *Sleep Med Rev* 2008; 12: 49–63.
21 Goldbart AD, Row BW, Kheirandish-Gozal L, *et al.* High fat/refined carbohydrate diet enhances the susceptibility to spatial learning deficits in rats exposed to intermittent hypoxia. *Brain Res* 2006; 1090: 190–196.
22 Somers VK, Mark AL, Abboud FM. Sympathetic activation by hypoxia and hypercapnia – implications for sleep apnea. *Clin Exp Hypertens A* 1988; 10: Suppl. 1, 413–422.
23 Hedner J, Darpo B, Ejnell H, *et al.* Reduction in sympathetic activity after long-term CPAP treatment in sleep apnoea: cardiovascular implications. *Eur Respir J* 1995; 8: 222–229.
24 Carlson JT, Hedner J, Elam M, *et al.* Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* 1993; 103: 1763–1768.
25 Narkiewicz K, Somers VK. Obstructive sleep apnea as a cause of neurogenic hypertension. *Curr Hypertens Rep* 1999; 1: 268–273.
26 Narkiewicz K, van de Borne PJ, Pesek CA, *et al.* Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation* 1999; 99: 1183–1189.
27 Leuenberger UA, Hogeman CS, Quraishi S, *et al.* Short-term intermittent hypoxia enhances sympathetic responses to continuous hypoxia in humans. *J Appl Physiol* 2007; 103: 835–842.
28 Vgontzas AN, Bixler EO, Chrousos GP. Sleep apnea is a manifestation of the metabolic syndrome. *Sleep Med Rev* 2005; 9: 211–224.
29 Escourrou P, Tessier O, Bureau A, *et al.* Non-invasive measurement of haemodynamics during sleep apnoea. *J Sleep Res* 1995; 4: 78–82.

30 McNicholas WT. Cardiovascular outcomes of CPAP therapy in obstructive sleep apnea syndrome. *Am J Physiol Regul Integr Comp Physiol* 2007; 293: R1666–R1670.
31 Li J, Nanayakkara A, Jun J, *et al.* Effect of deficiency in SREBP cleavage-activating protein on lipid metabolism during intermittent hypoxia. *Physiol Genomics* 2007; 31: 273–280.
32 Valko M, Leibfritz D, Moncol J, *et al.* Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39: 44–84.
33 Lavie L. Obstructive sleep apnoea syndrome – an oxidative stress disorder. *Sleep Med Rev* 2003; 7: 35–51.
34 Grisham MB, Granger DN, Lefer DJ. Modulation of leukocyte-endothelial interactions by reactive metabolites of oxygen and nitrogen: relevance to ischemic heart disease. *Free Radic Biol Med* 1998; 25: 404–433.
35 Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 1996; 271: C1424–C1437.
36 Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956; 11: 298–300.
37 Harman D. Atherosclerosis: a hypothesis concerning the initiating steps in pathogenesis. *J Gerontol* 1957; 12: 199–202.
38 McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969; 244: 6049–6055.
39 McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions. I. Radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J Biol Chem* 1969; 244: 6056–6063.
40 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.
41 Babior BM. The leukocyte NADPH oxidase. *Isr Med Assoc J* 2002; 4: 1023–1024.
42 Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. *Gastroenterology* 1981; 81: 22–29.
43 McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985; 312: 159–163.
44 McCord JM, Edeas MA. SOD, oxidative stress and human pathologies: a brief history and a future vision. *Biomed Pharmacother* 2005; 59: 139–142.
45 McCord JM. The evolution of free radicals and oxidative stress. *Am J Med* 2000; 108: 652–659.
46 Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 1997; 22: 269–285.
47 D'Autreaux B, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 2007; 8: 813–824.
48 Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420: 868–874.
49 Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; 82: 47–95.
50 Lavie L. Intermittent hypoxia: the culprit of oxidative stress, vascular inflammation and dyslipidemia in obstructive sleep apnea. *Expert Rev Respir Med* 2008; 2: 75–84.
51 Singer G, Granger DN. Inflammatory responses underlying the microvascular dysfunction associated with obesity and insulin resistance. *Microcirculation* 2007; 14: 375–387.
52 Kumar GK, Rai V, Sharma SD, *et al.* Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla *via* oxidative stress. *J Physiol* 2006; 575: 229–239.
53 Prabhakar NR, Dick TE, Nanduri J, *et al.* Systemic, cellular and molecular analysis of chemoreflex-mediated sympathoexcitation by chronic intermittent hypoxia. *Exp Physiol* 2007; 92: 39–44.
54 Semenza GL. Oxygen-regulated transcription factors and their role in pulmonary disease. *Respir Res* 2000; 1: 159–162.
55 Peng YJ, Yuan G, Ramakrishnan D, *et al.* Heterozygous HIF-1α deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol* 2006; 577: 705–716.
56 Li J, Bosch-Marce M, Nanayakkara A, *et al.* Altered metabolic responses to intermittent hypoxia in mice with partial deficiency of hypoxia-inducible factor-1α. *Physiol Genomics* 2006; 25: 450–457.
57 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.
58 Schulz R, Mahmoudi S, Hattar 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: 566–570.
59 Lavie L, Vishnevsky A, Lavie P. Evidence for lipid peroxidation in obstructive sleep apnea. *Sleep* 2004; 27: 123–128.
60 Barcelo A, Miralles C, Barbe F, *et al.* Abnormal lipid peroxidation in patients with sleep apnoea. *Eur Respir J* 2000; 16: 644–647.
61 Christou K, Moulas AN, Pastaka C, *et al.* Antioxidant capacity in obstructive sleep apnea patients. *Sleep Med* 2003; 4: 225–228.
62 Yamauchi M, Nakano H, Maekawa J, *et al.* Oxidative stress in obstructive sleep apnea. *Chest* 2005; 127: 1674–1679.
63 Carpagnano GE, Kharitonov SA, Resta O, *et al.* 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.
64 Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during obstructive sleep apnea. *Sleep* 1993; 16: S15–S21.
65 Suzuki YJ, Jain V, Park AM, *et al.* Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radic Biol Med* 2006; 40: 1683–1692.
66 Peng Y, Yuan G, Overholt JL, *et al.* Systemic and cellular responses to intermittent hypoxia: evidence for oxidative stress and mitochondrial dysfunction. *Adv Exp Med Biol* 2003; 536: 559–564.

67 Dyugovskaya L, Polyakov A, Lavie P, *et al.* Delayed neutrophil apoptosis in patients with sleep apnea. *Am J Respir Crit Care Med* 2008; 177: 544–554.

68 El Solh AA, Saliba R, Bosinski T, *et al.* Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. *Eur Respir J* 2006; 27: 997–1002.

69 Jelic S, Padeletti M, Kawut SM, *et al.* Inflammation, oxidative stress, and repair capacity of the vascular endothelium in obstructive sleep apnea. *Circulation* 2008; 117: 2270–2278.

70 Minoguchi K, Yokoe T, Tanaka A, *et al.* Association between lipid peroxidation and inflammation in obstructive sleep apnoea. *Eur Respir J* 2006; 28: 378–385.

71 Xu W, Chi L, Row BW, *et al.* 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.

72 Zhan G, Serrano F, Fenik P, *et al.* 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.

73 Chen L, Einbinder E, Zhang Q, *et al.* Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats. *Am J Respir Crit Care Med* 2005; 172: 915–920.

74 Barcelo A, Barbe F, de la Pena M, *et al.* Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. *Eur Respir J* 2006; 27: 756–760.

75 Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001; 21: 473–480.

76 Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes (Lond)* 2006; 30: 400–418.

77 Tan KC, Chow WS, Lam JC, *et al.* HDL dysfunction in obstructive sleep apnea. *Atherosclerosis* 2006; 184: 377–382.

78 Lavie L, Hefetz A, Luboshitzky R, *et al.* Plasma levels of nitric oxide and L-arginine in sleep apnea patients: effects of nCPAP treatment. *J Mol Neurosci* 2003; 21: 57–63.

79 Schulz R, Schmidt D, Blum A, *et al.* Decreased plasma levels of nitric oxide derivatives in obstructive sleep apnoea: response to CPAP therapy. *Thorax* 2000; 55: 1046–1051.

80 Ip MS, Lam B, Chan LY, *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: 2166–2171.

81 Lavie L. Sleep-disordered breathing and cerebrovascular disease: a mechanistic approach. *Neurol Clin* 2005; 23: 1059–1075.

82 Grebe M, Eisele HJ, Weissmann N, *et al.* Antioxidant vitamin C improves endothelial function in obstructive sleep apnea. *Am J Respir Crit Care Med* 2006; 173: 897–901.

83 Burckhardt IC, Gozal D, Dayyat E, *et al.* Green tea catechin polyphenols attenuate behavioral and oxidative responses to intermittent hypoxia. *Am J Respir Crit Care Med* 2008; 177: 1135–1141.

84 Htoo AK, Greenberg H, Tongia S, *et al.* Activation of nuclear factor κB in obstructive sleep apnea: a pathway leading to systemic inflammation. *Sleep Breath* 2006; 10: 43–50.

85 Greenberg H, Ye X, Wilson D, *et al.* Chronic intermittent hypoxia activates nuclear factor-κB in cardiovascular tissues *in vivo*. *Biochem Biophys Res Commun* 2006; 343: 591–596.

86 Yamauchi M, Tamaki S, Tomoda K, *et al.* Evidence for activation of nuclear factor κB in obstructive sleep apnea. *Sleep Breath* 2006; 10: 189–193.

87 Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation* 2005; 112: 2660–2667.

88 Dyugovskaya L, Lavie P, Lavie L. Lymphocyte activation as a possible measure of atherosclerotic risk in patients with sleep apnea. *Ann NY Acad Sci* 2005; 1051: 340–350.

89 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.

90 Alam I, Lewis K, Stephens JW, *et al.* Obesity, metabolic syndrome and sleep apnoea: all pro-inflammatory states. *Obes Rev* 2007; 8: 119–127.

91 Yuan G, Adhikary G, McCormick AA, *et al.* Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. *J Physiol* 2004; 557: 773–783.

92 Ryan S, McNicholas WT, Taylor CT. A critical role for p38 MAP kinase in NF-κB signaling during intermittent hypoxia/reoxygenation. *Biochem Biophys Res Commun* 2007; 355: 728–733.

93 Semenza GL, Prabhakar NR. HIF-1-dependent respiratory, cardiovascular, and redox responses to chronic intermittent hypoxia. *Antioxid Redox Signal* 2007; 9: 1391–1396.

94 Yuan G, Nanduri J, Bhasker CR, *et al.* Ca^{2+}/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J Biol Chem* 2005; 280: 4321–4328.

95 Toffoli S, Feron O, Raes M, *et al.* Intermittent hypoxia changes HIF-1α phosphorylation pattern in endothelial cells: unravelling of a new PKA-dependent regulation of HIF-1α. *Biochim Biophys Acta* 2007; 1773: 1558–1571.

96 Winnicki M, Shamsuzzaman A, Lanfranchi P, *et al.* Erythropoietin and obstructive sleep apnea. *Am J Hypertens* 2004; 17: 783–786.

97 Lavie L, Kraiczi H, Hefetz A, *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: 1624–1628.

98 Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; 109: 1125–1131.

99 Brown MS, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci USA* 1999; 96: 11041–11048.

100 Li J, Grigoryev DN, Ye SQ, *et al.* Chronic intermittent hypoxia upregulates genes of lipid biosynthesis in obese mice. *J Appl Physiol* 2005; 99: 1643–1648.

101 Li J, Thorne LN, Punjabi NM, *et al.* Intermittent hypoxia induces hyperlipidemia in lean mice. *Circ Res* 2005; 97: 698–706.

102 Waris G, Felmlee DJ, Negro F, *et al.* Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation *via* oxidative stress. *J Virol* 2007; 81: 8122–8130.

103 Lin CC, Yin MC. Effects of cysteine-containing compounds on biosynthesis of triacylglycerol and cholesterol and anti-oxidative protection in liver from mice consuming a high-fat diet. *Br J Nutr* 2008; 99: 37–43.

104 Savransky V, Nanayakkara A, Li J, *et al.* Chronic intermittent hypoxia induces atherosclerosis. *Am J Respir Crit Care Med* 2007; 175: 1290–1297.

105 Li J, Savransky V, Nanayakkara A, *et al.* Hyperlipidemia and lipid peroxidation are dependent on the severity of chronic intermittent hypoxia. *J Appl Physiol* 2007; 102: 557–563.

106 Chin K, Shimizu K, Nakamura T, *et al.* Changes in intra-abdominal visceral fat and serum leptin levels in patients with obstructive sleep apnea syndrome following nasal continuous positive airway pressure therapy. *Circulation* 1999; 100: 706–712.

107 Newman AB, Nieto FJ, Guidry U, *et al.* Relation of sleep-disordered breathing to cardiovascular disease risk factors: the Sleep Heart Health Study. *Am J Epidemiol* 2001; 154: 50–59.

108 McArdle N, Hillman D, Beilin L, *et al.* Metabolic risk factors for vascular disease in obstructive sleep apnea: a matched controlled study. *Am J Respir Crit Care Med* 2007; 175: 190–195.

109 Robinson GV, Pepperell JC, Segal HC, *et al.* Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials. *Thorax* 2004; 59: 777–782.

110 Chin K, Nakamura T, Shimizu K, *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: 562–567.

111 Pikkarainen S, Tokola H, Kerkela R, *et al.* GATA transcription factors in the developing and adult heart. *Cardiovasc Res* 2004; 63: 196–207.

112 Suzuki YJ. Growth factor signaling for cardioprotection against oxidative stress-induced apoptosis. *Antioxid Redox Signal* 2003; 5: 741–749.

113 Park AM, Nagase H, Vinod Kumar S, *et al.* Acute intermittent hypoxia activates myocardial cell survival signaling. *Am J Physiol Heart Circ Physiol* 2007; 292: H751–H757.

114 Park AM, Nagase H, Kumar SV, *et al.* Effects of intermittent hypoxia on the heart. *Antioxid Redox Signal* 2007; 9: 723–729.

115 Panes J, Granger DN. Leukocyte-endothelial cell interactions: molecular mechanisms and implications in gastrointestinal disease. *Gastroenterology* 1998; 114: 1066–1090.

116 Dyugovskaya L, Lavie P, Hirsh M, *et al.* Activated CD8+ T-lymphocytes in obstructive sleep apnoea. *Eur Respir J* 2005; 25: 820–828.

117 Dyugovskaya L, Lavie P, Lavie L. Phenotypic and functional characterization of blood γδ T cells in sleep apnea. *Am J Respir Crit Care Med* 2003; 168: 242–249.

118 McDonald PP. Transcriptional regulation in neutrophils: teaching old cells new tricks. *Adv Immunol* 2004; 82: 1–48.

119 Naruko T, Ueda M, Haze K, *et al.* Neutrophil infiltration of culprit lesions in acute coronary syndromes. *Circulation* 2002; 106: 2894–2900.

120 Zidar N, Jeruc J, Balazic J, *et al.* Neutrophils in human myocardial infarction with rupture of the free wall. *Cardiovasc Pathol* 2005; 14: 247–250.

121 Vinten-Johansen J. Involvement of neutrophils in the pathogenesis of lethal myocardial reperfusion injury. *Cardiovasc Res* 2004; 61: 481–497.

122 Jolly SR, Kane WJ, Hook BG, *et al.* Reduction of myocardial infarct size by neutrophil depletion: effect of duration of occlusion. *Am Heart J* 1986; 112: 682–690.

123 Kin H, Wang NP, Halkos ME, *et al.* Neutrophil depletion reduces myocardial apoptosis and attenuates NF-κB activation/TNF-α release after ischemia and reperfusion. *J Surg Res* 2006; 135: 170–178.

124 Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev* 2007; 65: S140–S146.

125 Minoguchi K, Tazaki T, Yokoe T, *et al.* Elevated production of tumor necrosis factor-α by monocytes in patients with obstructive sleep apnea syndrome. *Chest* 2004; 126: 1473–1479.

126 Lattimore JD, Wilcox I, Nakhla S, *et al.* Repetitive hypoxia increases lipid loading in human macrophages – a potentially atherogenic effect. *Atherosclerosis* 2005; 179: 255–259.

127 Vanderlaan PA, Reardon CA. Thematic review series: the immune system and atherogenesis. The unusual suspects: an overview of the minor leukocyte populations in atherosclerosis. *J Lipid Res* 2005; 46: 829–838.

128 Song L, Leung C, Schindler C. Lymphocytes are important in early atherosclerosis. *J Clin Invest* 2001; 108: 251–259.

129 Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet–neutrophil interactions: linking hemostasis and inflammation. *Blood Rev* 2007; 21: 99–111.

130 Gavins F, Yilmaz G, Granger DN. The evolving paradigm for blood cell–endothelial cell interactions in the cerebral microcirculation. *Microcirculation* 2007; 14: 667–681.

131 Bokinsky G, Miller M, Ault K, *et al.* Spontaneous platelet activation and aggregation during obstructive sleep apnea and its response to therapy with nasal continuous positive airway pressure. A preliminary investigation. *Chest* 1995; 108: 625–630.

132 Geiser T, Buck F, Meyer BJ, *et al. In vivo* platelet activation is increased during sleep in patients with obstructive sleep apnea syndrome. *Respiration* 2002; 69: 229–234.

133 Eisensehr I, Ehrenberg BL, Noachtar S, *et al.* Platelet activation, epinephrine, and blood pressure in obstructive sleep apnea syndrome. *Neurology* 1998; 51: 188–195.

134 Hui DS, Ko FW, Fok JP, *et al.* The effects of nasal continuous positive airway pressure on platelet activation in obstructive sleep apnea syndrome. *Chest* 2004; 125: 1768–1775.

135 Nobili L, Schiavi G, Bozano E, *et al.* Morning increase of whole blood viscosity in obstructive sleep apnea syndrome. *Clin Hemorheol Microcirc* 2000; 22: 21–27.

136 Guardiola JJ, Matheson PJ, Clavijo LC, *et al.* Hypercoagulability in patients with obstructive sleep apnea. *Sleep Med* 2001; 2: 517–523.

137 Reinhart WH, Oswald J, Walter R, *et al.* Blood viscosity and platelet function in patients with obstructive sleep apnea syndrome treated with nasal continuous positive airway pressure. *Clin Hemorheol Microcirc* 2002; 27: 201–207.

138 Nacher M, Serrano-Mollar A, Farre R, *et al.* Recurrent obstructive apneas trigger early systemic inflammation in a rat model of sleep apnea. *Respir Physiol Neurobiol* 2007; 155: 93–96.

139 Madjdpour C, Jewell UR, Kneller S, *et al.* Decreased alveolar oxygen induces lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L360–L367.

140 Kesler A, Yatziv Y, Shapira I, *et al.* Increased red blood cell aggregation in patients with idiopathic intracranial hypertension. A hitherto unexplored pathophysiological pathway. *Thromb Haemost* 2006; 96: 483–487.

141 Rotstein R, Landau T, Twig A, *et al.* The erythrocyte adhesiveness/aggregation test (EAAT). A new biomarker to reveal the presence of low grade subclinical smoldering inflammation in individuals with atherosclerotic risk factors. *Atherosclerosis* 2002; 165: 343–351.

142 Samocha-Bonet D, Ben-Ami R, Shapira I, *et al.* Flow-resistant red blood cell aggregation in morbid obesity. *Int J Obes Relat Metab Disord* 2004; 28: 1528–1534.

143 Peled N, Kassirer M, Kramer MR, *et al.* Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome. *Thromb Res* 2008; 121: 631–636.

144 Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin Chem* 2008; 54: 24–38.

145 Zamarron-Sanz C, Ricoy-Galbaldon J, Gude-Sampedro F, *et al.* Plasma levels of vascular endothelial markers in obstructive sleep apnea. *Arch Med Res* 2006; 37: 552–555.

146 Minoguchi K, Yokoe T, Tazaki T, *et al.* Silent brain infarction and platelet activation in obstructive sleep apnea. *Am J Respir Crit Care Med* 2007; 175: 612–617.

147 Ohga E, Nagase T, Tomita T, *et al.* Increased levels of circulating ICAM-1, VCAM-1, and L-selectin in obstructive sleep apnea syndrome. *J Appl Physiol* 1999; 87: 10–14.

148 Ursavas A, Karadag M, Rodoplu E, *et al.* Circulating ICAM-1 and VCAM-1 levels in patients with obstructive sleep apnea syndrome. *Respiration* 2007; 74: 525–532.

149 El Solh AA, Akinnusi ME, Berim IG, *et al.* Hemostatic implications of endothelial cell apoptosis in obstructive sleep apnea. *Sleep Breath* 2008; 12: 331–337.

150 Martin K, Stanchina M, Kouttab N, *et al.* Circulating endothelial cells and endothelial progenitor cells in obstructive sleep apnea. *Lung* 2008; 186: 145–150.

151 de la Pena M, Barcelo A, Barbe F, *et al.* Endothelial function and circulating endothelial progenitor cells in patients with sleep apnea syndrome. *Respiration* 2008; 76: 28–32.

152 von der Thusen JH, Kuiper J, van Berkel TJ, *et al.* Interleukins in atherosclerosis: molecular pathways and therapeutic potential. *Pharmacol Rev* 2003; 55: 133–166.

153 Ridker PM, Rifai N, Pfeffer M, *et al.* Elevation of tumor necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction. *Circulation* 2000; 101: 2149–2153.

154 Vgontzas AN, Papanicolaou DA, Bixler EO, *et al.* Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab* 2000; 85: 1151–1158.

155 Constantinidis J, Ereliadis S, Angouridakis N, *et al.* Cytokine changes after surgical treatment of obstructive sleep apnoea syndrome. *Eur Arch Otorhinolaryngol* 2008; 265: 1275–1279.

156 Ryan S, Taylor CT, McNicholas WT. Predictors of elevated nuclear factor-κB-dependent genes in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2006; 174: 824–830.

157 Yokoe T, Minoguchi K, Matsuo H, *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: 1129–1134.

158 Ohga E, Tomita T, Wada H, *et al.* Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1. *J Appl Physiol* 2003; 94: 179–184.

159 Jung Y, Isaacs JS, Lee S, *et al.* Hypoxia-inducible factor induction by tumour necrosis factor in normoxic cells requires receptor-interacting protein-dependent nuclear factor κB activation. *Biochem J* 2003; 370: 1011–1017.

160 Lavie L, Dyugovskaya L, Polyakov A. Biology of peripheral blood cells in obstructive sleep apnea – the tip of the iceberg. *Arch Physiol Biochem* 2008; 114: 244–254.

161 Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* 2003; 144: 2195–2200.

162 Lee YH, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. *Curr Diab Rep* 2005; 5: 70–75.

163 Libby P, Willerson JT, Braunwald E. C-reactive protein and coronary heart disease. *N Engl J Med* 2004; 351: 295–298.

164 Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004; 109: Suppl. 1, II2–II10.

165 Kobayashi S, Inoue N, Ohashi Y, *et al.* Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler Thromb Vasc Biol* 2003; 23: 1398–1404.

166 Wu J, Stevenson MJ, Brown JM, *et al.* C-reactive protein enhances tissue factor expression by vascular smooth muscle cells: mechanisms and *in vivo* significance. *Arterioscler Thromb Vasc Biol* 2008; 28: 698–704.

167 Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; 102: 2165–2168.

168 Nakajima T, Schulte S, Warrington KJ, *et al.* T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation* 2002; 105: 570–575.

169 Nagaoka T, Kuo L, Ren Y, *et al.* C-reactive protein inhibits endothelium-dependent nitric oxide-mediated dilation of retinal arterioles *via* enhanced superoxide production. *Invest Ophthalmol Vis Sci* 2008; 49: 2053–2060.

170 Shamsuzzaman AS, Winnicki M, Lanfranchi P, *et al.* Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation* 2002; 105: 2462–2464.

171 Ryan S, Nolan GM, Hannigan E, *et al.* Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. *Thorax* 2007; 62: 509–514.

172 Guilleminault C, Kirisoglu C, Ohayon MM. C-reactive protein and sleep-disordered breathing. *Sleep* 2004; 27: 1507–1511.

173 Taheri S, Austin D, Lin L, *et al.* Correlates of serum C-reactive protein (CRP) – no association with sleep duration or sleep disordered breathing. *Sleep* 2007; 30: 991–996.

174 Sharma SK, Mishra HK, Sharma H, *et al.* Obesity, and not obstructive sleep apnea, is responsible for increased serum hs-CRP levels in patients with sleep-disordered breathing in Delhi. *Sleep Med* 2008; 9: 149–156.

175 Lavie L, Vishnevsky A, Lavie P. Oxidative stress and systemic inflammation in patients with sleep apnea: role of obesity. *Sleep Biol Rhythm* 2007; 5: 100–110.

176 Meier-Ewert HK, Ridker PM, Rifai N, *et al.* Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Cardiol* 2004; 43: 678–683.

177 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.

178 Loube DI, Loube AA, Mitler MM. Weight loss for obstructive sleep apnea: the optimal therapy for obese patients. *J Am Diet Assoc* 1994; 94: 1291–1295.

179 Kopelman PG. Obesity as a medical problem. *Nature* 2000; 404: 635–643.

180 Keaney JF Jr, Larson MG, Vasan RS, *et al.* Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; 23: 434–439.

181 Furukawa S, Fujita T, Shimabukuro M, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; 114: 1752–1761.

182 Kisakol G, Guney E, Bayraktar F, *et al.* Effect of surgical weight loss on free radical and antioxidant balance: a preliminary report. *Obes Surg* 2002; 12: 795–800.

183 Hosogai N, Fukuhara A, Oshima K, *et al.* Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 2007; 56: 901–911.

184 Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* 2004; 92: 347–355.

185 Anubhuti, Arora S., Leptin and its metabolic interactions: an update. *Diabetes Obes Metab* 2008; 10: 973–993.

186 Ambrosini G, Nath AK, Sierra-Honigmann MR, *et al.* Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem* 2002; 277: 34601–34609.

187 Beltowski J, Wojcicka G, Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis* 2003; 170: 21–29.

188 Maingrette F, Renier G. Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C. *Diabetes* 2003; 52: 2121–2128.

189 Barcelo A, Barbe F, Llompart E, *et al.* Neuropeptide Y and leptin in patients with obstructive sleep apnea syndrome: role of obesity. *Am J Respir Crit Care Med* 2005; 171: 183–187.

190 Tatsumi K, Kasahara Y, Kurosu K, *et al.* Sleep oxygen desaturation and circulating leptin in obstructive sleep apnea-hypopnea syndrome. *Chest* 2005; 127: 716–721.

191 Harsch IA, Konturek PC, Koebnick C, *et al.* Leptin and ghrelin levels in patients with obstructive sleep apnoea: effect of CPAP treatment. *Eur Respir J* 2003; 22: 251–257.

192 Polotsky VY, Li J, Punjabi NM, *et al.* Intermittent hypoxia increases insulin resistance in genetically obese mice. *J Physiol* 2003; 552: 253–264.

193 Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005; 115: 911–919.

194 Chandran M, Phillips SA, Ciaraldi T, *et al.* Adiponectin: more than just another fat cell hormone? *Diabetes Care* 2003; 26: 2442–2450.

195 Hattori Y, Akimoto K, Gross SS, *et al.* Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia* 2005; 48: 1066–1074.

196 Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br J Nutr* 2008; 100: 227–235.

197 Sharma SK, Kumpawat S, Goel A, *et al.* Obesity, and not obstructive sleep apnea, is responsible for metabolic abnormalities in a cohort with sleep-disordered breathing. *Sleep Med* 2007; 8: 12–17.

198 Wolk R, Svatikova A, Nelson CA, *et al.* Plasma levels of adiponectin, a novel adipocyte-derived hormone, in sleep apnea. *Obes Res* 2005; 13: 186–190.

199 Nakagawa Y, Kishida K, Kihara S, *et al.* Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome. *Am J Physiol Endocrinol Metab* 2008; 294: E778–E784.

200 Harsch IA, Wallaschofski H, Koebnick C, *et al.* Adiponectin in patients with obstructive sleep apnea syndrome: course and physiological relevance. *Respiration* 2004; 71: 580–586.

201 West SD, Nicoll DJ, Wallace TM, *et al.* Effect of CPAP on insulin resistance and HbA1c in men with obstructive sleep apnoea and type 2 diabetes. *Thorax* 2007; 62: 969–974.

202 Danson EJ, Paterson DJ. Reactive oxygen species and autonomic regulation of cardiac excitability. *J Cardiovasc Electrophysiol* 2006; 17: Suppl. 1, S104–S112.

203 Kimura Y, Hirooka Y, Sagara Y, *et al.* Overexpression of inducible nitric oxide synthase in rostral ventrolateral medulla causes hypertension and sympathoexcitation *via* an increase in oxidative stress. *Circ Res* 2005; 96: 252–260.

204 Shokoji T, Nishiyama A, Fujisawa Y, *et al.* Renal sympathetic nerve responses to tempol in spontaneously hypertensive rats. *Hypertension* 2003; 41: 266–273.

205 Dai X, Galligan JJ, Watts SW, *et al.* Increased $O2^{\cdot-}$ production and upregulation of ETB receptors by sympathetic neurons in DOCA-salt hypertensive rats. *Hypertension* 2004; 43: 1048–1054.

206 Lassegue B, Griendling KK. Reactive oxygen species in hypertension: an update. *Am J Hypertens* 2004; 17: 852–860.

207 Mervaala EM, Cheng ZJ, Tikkanen I, *et al.* Endothelial dysfunction and xanthine oxidoreductase activity in rats with human renin and angiotensinogen genes. *Hypertension* 2001; 37: 414–418.

208 Wei Y, Sowers JR, Clark SE, *et al.* Angiotensin II-induced skeletal muscle insulin resistance mediated by NF-κB activation *via* NADPH oxidase. *Am J Physiol Endocrinol Metab* 2008; 294: E345–E351.

209 Li L, Watts SW, Banes AK, *et al.* NADPH oxidase-derived superoxide augments endothelin-1-induced venoconstriction in mineralocorticoid hypertension. *Hypertension* 2003; 42: 316–321.

210 Troncoso Brindeiro CM, da Silva AQ, Allahdadi KJ, *et al.* Reactive oxygen species contribute to sleep apnea-induced hypertension in rats. *Am J Physiol Heart Circ Physiol* 2007; 293: H2971–H2976.

211 Moller DS, Lind P, Strunge B, *et al.* Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. *Am J Hypertens* 2003; 16: 274–280.

212 Stokes KY, Cooper D, Tailor A, *et al.* Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med* 2002; 33: 1026–1036.

213 Stokes KY, Granger DN. Hypercholesterolemia: its impact on ischemia-reperfusion injury. *Expert Rev Cardiovasc Ther* 2005; 3: 1061–1070.

214 Maddux BA, See W, Lawrence JC Jr, *et al.* Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α-lipoic acid. *Diabetes* 2001; 50: 404–410.

215 Rudich A, Tirosh A, Potashnik R, *et al.* Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes* 1998; 47: 1562–1569.

216 Matsuoka T, Kajimoto Y, Watada H, *et al.* Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. *J Clin Invest* 1997; 99: 144–150.

217 Folmer V, Soares JC, Rocha JB. Oxidative stress in mice is dependent on the free glucose content of the diet. *Int J Biochem Cell Biol* 2002; 34: 1279–1285.

218 Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem Biophys Res Commun* 1990; 173: 932–939.

219 Leoncini G, Signorello MG, Piana A, *et al.* Hyperactivity and increased hydrogen peroxide formation in platelets of NIDDM patients. *Thromb Res* 1997; 86: 153–160.

220 Laight DW, Desai KM, Gopaul NK, *et al.* Pro-oxidant challenge *in vivo* provokes the onset of NIDDM in the insulin resistant obese Zucker rat. *Br J Pharmacol* 1999; 128: 269–271.

221 Ogihara T, Asano T, Katagiri H, *et al.* Oxidative stress induces insulin resistance by activating the nuclear factor-κB pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. *Diabetologia* 2004; 47: 794–805.

222 Evans JL, Goldfine ID, Maddux BA, *et al.* Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; 23: 599–622.

223 Ceolotto G, Bevilacqua M, Papparella I, *et al.* Insulin generates free radicals by an NAD(P)H, phosphatidylinositol 3′-kinase-dependent mechanism in human skin fibroblasts *ex vivo*. *Diabetes* 2004; 53: 1344–1351.

224 Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; 25: 4–7.

225 Ip MS, Lam B, Ng MM, *et al.* Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med* 2002; 165: 670–676.

226 Punjabi NM, Sorkin JD, Katzel LI, *et al.* Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med* 2002; 165: 677–682.

227 Reichmuth KJ, Austin D, Skatrud JB, *et al.* Association of sleep apnea and type II diabetes: a population-based study. *Am J Respir Crit Care Med* 2005; 172: 1590–1595.

228 Punjabi NM, Shahar E, Redline S, *et al.* Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. *Am J Epidemiol* 2004; 160: 521–530.

229 Schahin SP, Nechanitzky T, Dittel C, *et al.* Long-term improvement of insulin sensitivity during CPAP therapy in the obstructive sleep apnoea syndrome. *Med Sci Monit* 2008; 14: CR117–CR121.

230 Hassaballa HA, Tulaimat A, Herdegen JJ, *et al.* The effect of continuous positive airway pressure on glucose control in diabetic patients with severe obstructive sleep apnea. *Sleep Breath* 2005; 9: 176–180.