SLEEP
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CLINICAL REVIEW

Obstructive sleep apnoea syndrome – an oxidative stress disorder

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Summary   Obstructive sleep apnoea syndrome (OSA) is associated with increased cardiovascular morbidity and mortality. However, the underlying mechanisms are not entirely understood. This review will summarize the evidence that substantiates the notion that the repeated apnoea-related hypoxic events in OSA, similarly to hypoxia/reperfusion injury, initiate oxidative stress. Thus, affecting energy metabolism, redox-sensitive gene expression, and expression of adhesion molecules. A limited number of studies substantiate this hypothesis directly by demonstrating increased free radical production in OSA leukocytes and increased plasma-lipid peroxidation. A great number of studies, however, support this hypothesis indirectly. Increase in circulating levels of adenosine and urinary uric acid in OSA are implicated with increased production of reactive oxygen species (ROS). Activation of redox-sensitive gene expression is suggested by the increase in some protein products of these genes, including VEGF, erythropoietin, endothelin-1, inflammatory cytokines and adhesion molecules. These implicate the participation of redox-sensitive transcription factors as HIF-1 AP-1 and NFκB. Finally, adhesion molecule-dependent increased avidity of OSA monocytes to endothelial cells, combined with diminished NO bioavailability, lead to exaggerated endothelial cell damage and dysfunction. Cumulatively, these processes may exacerbate atherogenic sequelae in OSA.   © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Recent years have seen a rapid accumulation of evidence pointing at links between sleep apnoea syndrome and cardiovascular morbidity and mortality. Patients with sleep apnoea have been found to have higher rates of cardiovascular diseases (CAD) most

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notably hypertension [1, 2], and studies of random samples obtained from the general population suggest that the existence of breathing disorder events during sleep constitute a significant risk for CAD independently of other known risk factors [3–6]. Similarly, investigating patients with documented CAD revealed higher rates of clinically significant disordered breathing events during sleep, which were higher than what would be expected in the general population [7–11]. Evidently, CAD are multifactorial, and are likely to be affected by the genetic makeup, lifestyle-related variables, nutrition, as well as a variety of additional risk factors. Thus, the fact that in some of the studies there was a dose–response relationship between the

severity of breathing disorders in sleep and some of the cardiovascular morbidities [1, 4], suggests that the link between CAD and sleep apnoea is specific, and is most probably related to some of the abnormalities which are unique to the syndrome. So far, the exact nature of the underlying pathophysiology of cardiovascular morbidity in sleep apnoea patients has not been fully elucidated. Attempts, however, have focused on the potential influence of two of the major consequences of disordered breathing in sleep: intermittent hypoxia and hypercapnia, and repeated sleep fragmentation. Both hypoxemia and hypercapnia have diverse cardiovascular effects by acting locally, humorally, or by neuronal reflex mechanisms [12]. Thus, hypoxia or sleep fragmentation-mediated sympathetic activation, and altered baroreceptor and chemoreceptor responses, have been proposed to play a role in the development of CAD in sleep apnoea [13–16]. Others postulated hypoxia-related alteration in vasculature reactivity to both vasodilators and vasoconstrictors [17, 18].

One of the relatively less studied underlying mechanisms that may be involved in the development of CAD in sleep apnoea syndrome, is the formation of hypoxia-related free radicals and increased oxidative stress due to the intermittent hypoxia [19–21]. Free radicals are highly chemically reactive molecules that react with nucleic acids, lipids and proteins, thereby hindering cellular metabolism resulting in cell injury. So far, their potential role in sleep apnoea has mainly been speculated based on theoretical considerations linking increased levels of free radicals in hypoxic conditions on the one hand, and their proven involvement in atherosclerotic processes on the other. In recent years, experimental data have provided support for an association between OSA and free radical formation, which will be reviewed and discussed in the present paper. I will start by a brief overview of oxygen free radicals and their potential sources in OSA, then, I will discuss the prevailing views concerning the role of oxygen free radicals and oxidative stress in CAD, and the evidence pointing at the existence of exacerbated oxidative stress in sleep apnoea which eventually leads to one of the early atherogenic events – endothelial dysfunction.

WHAT ARE FREE RADICALS?

Free radicals, or reactive oxygen species (ROS), are atoms or molecules possessing one or more unpaired electrons in the outer orbit and, therefore, are prone to react chemically. Two radicals reacting with each other yield a non-radical product. When a radical reacts with a non-radical molecule, the product is a new radical, thus propagating radical chain reactions. When electrons are transferred from one molecule to another, reduction–oxidation (redox) reactions occur. Since ROS are by-products of normal oxygen metabolism, that are generated during normal cellular respiration, enzymatic and non-enzymatic anti-oxidant systems have evolved to eliminate excess free radicals. When ROS generation exceeds the capacity of cellular antioxidant mechanisms to eliminate them, oxidative stress and damage to cells and tissues ensues. This significantly contributes to many seemingly unrelated pathologies and pathological conditions including inflammatory diseases and ischaemia/reperfusion [22, 23]. On the other hand, a wealth of data in recent years demonstrate that under physiological conditions, maintenance of balanced free radical production is tightly regulated in order to serve for signalling purposes, activating redox sensitive transcription factors, and thus regulating redox adaptive gene expression [23, 24].

Chemistry

Superoxide anion ($O_2^{\bullet-}$) the predominant radical, and the source of ROS is generated by univalent reduction of molecular oxygen. This occurs during respiration or by several enzymic systems (reaction 1).

$$O_2 + e^- \rightarrow O_2^{\bullet-} \tag{1}$$
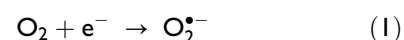
Once formed, $O_2^{\bullet-}$ can either spontaneously dismutate, or, it can be dismutated by the enzyme superoxide dismutase (SOD) yielding molecular oxygen and hydrogen peroxide (H_2O_2) (reaction 2).

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2 \tag{2}$$
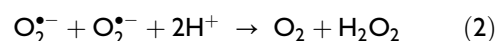
The reaction of superoxide with its product, H_2O_2, can give rise to hydroxyl radical ($OH^\bullet$). Formation of $OH^\bullet$, one of the most potent oxidants known, is facilitated by the presence of reduced transition metals as Fe^{2+} and Cu^+ [25] (reaction 3). Hydroxyl radical can initiate lipid peroxidation, cause DNA strand breaks and oxidize proteins, and other organic molecules.

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^\bullet + OH^\bullet \tag{3}$$
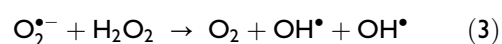
Collectively $O_2^{\bullet-}$, H_2O_2, and $OH^\bullet$, are termed reactive oxygen species or ROS.

Additional radicals include the toxic peroxynitrite ($OONO^\bullet$) which is formed by the reaction of $O_2^{\bullet-}$ with the primary vasodilator – nitric oxide (NO) (reaction 4), resulting in diminished NO availability [26]

$$O_2^{\bullet-} + NO \rightarrow OONO^\bullet \qquad (4)$$

POSSIBLE SOURCES OF OXYGEN FREE RADICALS IN OSA

Cells and tissues are continuously exposed to exogenous and endogenous sources of ROS. Here, I will focus on endogenous sources as they are relevant to the physiology and pathophysiology of OSA. Superoxide anions are primarily produced by: mitochondria and inflammatory leukocytes, by cardiac tissues and vascular cells as signalling molecules, due to hypoxia/reoxygenation insult, and by oxidation of small molecules as glucose and homocysteine. Additional sources include enzymes such as xanthine oxidase, cyclooxygenase, lipooxygenase, NO synthase and haem oxygenases, some of which will be discussed.

Mitochondria

The primary source of intracellular $O_2^{\bullet-}$ generation is free radical leakage from mitochondria during respiration (oxidative phosphorylation). Although carefully regulated, mitochondria are a major source of accidental free radical formation. It is estimated that under normal conditions about 5% of the oxygen consumed is transformed into ROS. Recent data suggest that ROS generated by mitochondria are utilized for signaling purposes. During hypoxia as PO_2 is lowered, ROS production is increased due to excessive mitochondrial reduction [27]. This can result from either inhibition of adenine nucleotide translocase, reduced activity of complex I or II of the respiratory chain, altered membrane fluidity, or due to calcium overload [28]. Accordingly, increased mitochondrial ROS production was reported for cultured endothelial cells (ECs) exposed to hypoxia [29]. It is therefore feasible that the excessive ROS generated during hypoxia, which serves to activate hypoxia adaptive genes [30], may leak from mitochondria, thereby injuring surrounding cellular components. However, this line of research was not explored so far in OSA.

ROS production by phagocytes

Phagocytes or leukocytes (neutrophils and monocytes) are the first line of defence of the immune system against invading pathogens. As such they destroy their prey by manufacturing a battery of reactive oxidizing agents. By utilizing NADPH oxidase, the primary enzyme for $O_2^{\bullet-}$ production, and additional enzymes as superoxide dismutase, myeloperoxidase and nitric oxide synthase, H_2O_2, hypochlorous acid (HClO) and $NO^\bullet$ are generated, respectively [22]. In addition, these ROS molecules react with each other non-enzymatically, and thus further augment ROS production yielding toxic molecules as $OH^\bullet$ and $OONO^\bullet$. Evidently, this arsenal of free radicals and oxidants, produced by phagocytes to kill invading pathogens, can also inflict damage to surrounding tissues.

Exposure of leukocytes to hypoxia, cytokines and other factors, leads to activation and thus to increased production of ROS that have far reaching implications to cardiovascular function. Thus far, two studies reported on increased ROS production by OSA leukocytes. Schulz et al. demonstrated increased superoxide production by neutrophils obtained from OSA patients after stimulation with the bacterial tripeptide fMLP and the calcium ionophor A23. Effective nCPAP therapy led to a rapid and long-lasting decrease of $O_2^{\bullet-}$ release by these neutrophils [31]. A recent study from our laboratory also demonstrated increased phorbol myristate acetate (PMA)-dependent ROS production by monocytes and granulocytes from OSA patients, expressing CD64 (receptors recognizing IgG and participating in inflammation). Similarly, ROS production was increased in PMA-elicited monocytes and granulocytes bearing CD11c receptors (counter receptors for intracellular adhesion molecule-1 i.e. ICAM-1) [32]. More importantly, we have also demonstrated increased ROS production by OSA monocytes under basal conditions, which probably contributes to a constant increase in oxidative metabolism and oxidative stress in these patients. Of note, this basal ROS production was attenuated as a result of effective nCPAP treatment. In this study, we showed for the first time, that the exacerbated leukocyte functions and increased oxidative stress in OSA patients were correlated with increased adhesiveness of monocytes to ECs. This increased

adhesiveness, which constitutes one of the initial steps in the atherogenic processeses, will be discussed later.

ROS production by the vasculature

NADPH oxidase isoforms, similar to the enzyme found in phagocytes, are present in a variety of non-phagocytic cells as well. These generate $O_2^{\bullet-}$ constitutively at a lower rate for physiological purposes [33, 34]. The NADPH oxidases expressed in vascular cells and cardiac tissue bear a great significance to cardiovascular function, as they were found to be essential in the physiological responses of vascular cells to growth, migration and modification of extracellular matrix, [35]. A variety of stimuli induce increased $O_2^{\bullet-}$ production by NADPH oxidases of the vasculature. These include growth factors, cytokines, angiotensin II, bradykinin, hormones, haemodynamic forces, and local metabolic products. In the vasculature, both $O_2^{\bullet-}$ and its metabolite H_2O_2 serve as second messengers to activate multiple intracellular signalling pathways. Redox-sensitive gene expression is most affected, resulting in adhesion molecules expression, chemotactic factors, antioxidant enzymes, and vasoactive substances. The growth of vascular smooth muscle cells and cardiac myocytes is affected by NADPH oxidase-derived $O_2^{\bullet-}$ as well. Angiotensin II, the principal product of the renin–angiotensin system and a potent vasoconstrictor, induces $O_2^{\bullet-}$ production by vascular cells, and stimulates a plethora of signalling pathways leading to cell growth and contraction. Moreover, it was implicated in the pathogenesis of hypertension and atherosclerosis [35]. Of note, angiotensin-converting enzyme (ACE) activity was shown to be higher in OSA patients (ACE, its main functions are to promote angiotensin II synthesis from angiotensin I and to degrade bradykinin) than in healthy controls, thus possibly implicating angiotensin II in the pathogenesis of hypertension and CAD in OSA [36]. Therefore, although there is no direct evidence demonstrating increased ROS production via NADPH oxidases of the vasculature in OSA patients, the close association between OSA and hypertension makes it an attractive hypothesis. It should be also noted that hypoxia *in vitro* was found to induce a considerable increase in NADPH-dependent $O_2^{\bullet-}$ production by pulmonary arteries and smooth muscle cells [37]. A progressive increase in blood pressure was noted in a rat model of OSA employing recurrent episodic hypoxia. This increase was mediated in part through renal sympathetic nerve activity that acts to increase renin–angiotensin system activity through angiotensin II type 1 receptors [38]. Moreover, blocking angiotensin II type 1 receptors blunted the increase in blood pressure response to episodic hypoxia. These could potentially explain the hypertension associated with OSA [39, 40]. Collectively, these data, possibly implicate the involvement of NADPH oxidases of the vasculature in the pathogenesis of CAD in OSA.

Hypoxia/reoxygenation injury (H/R)

Reperfusion–reoxygenation injury refers to the damage that occurs as a result of restoration of the blood circulation to an ischaemic or hypoxic tissue. Although several mechanisms inflict this damage, it is mainly attributed to free radical production during reoxygenation [22, 23, 41]. Hypoxia and possibly H/R induce complex metabolic and molecular changes. These include: (1) changes in energy metabolism, (2) alterations in gene expression, and (3) induction of cell surface molecules. All are intercalated with altered free radical flux, which affects NO bioavailability as well.

Sleep apnoea is accompanied by profound episodes of hypoxia, followed intermittently by a rapid reoxygenation of the blood as depicted in Fig. 1. This repeated ebb and flow in oxygen saturation could be considered analogous to repeated reperfusion injury as was eloquently put by Dean and Wilcox [19]. The injury due to ROS production of this well documented mechanism, especially during reperfusion following a period of hypoxia, was studied extensively in stroke and in certain surgical procedures, where it is clinically relevant to the outcome of myocardial infarcts. ROS were detected within minutes after restoration of the circulation to the ischaemic heart [42, 43]. Exposure of myocardium to ischaemia/reoxygenation resulted in myocardial tissue dysfunction, which was attributed to ROS. Exposure of myocardium to exogenous ROS resulted in myocardial tissue dysfunction similar to that elicited by ischaemia/reoxygenation. Hence, pretreatment with free radical scavengers such as SOD and catalase decreased formation of free radicals during reperfusion and reduced infarct size [44, 45]. However, more recent studies demonstrated also their participation in signalling pathways and communicating important information to the cell's genome in a carefully regulated manner. In fact, free radical regulation of gene expression by $O_2^{\bullet-}$ and other related oxidants is beginning to unravel as a fundamental mechanism in health and disease. Its implications to OSA will be discussed as well.
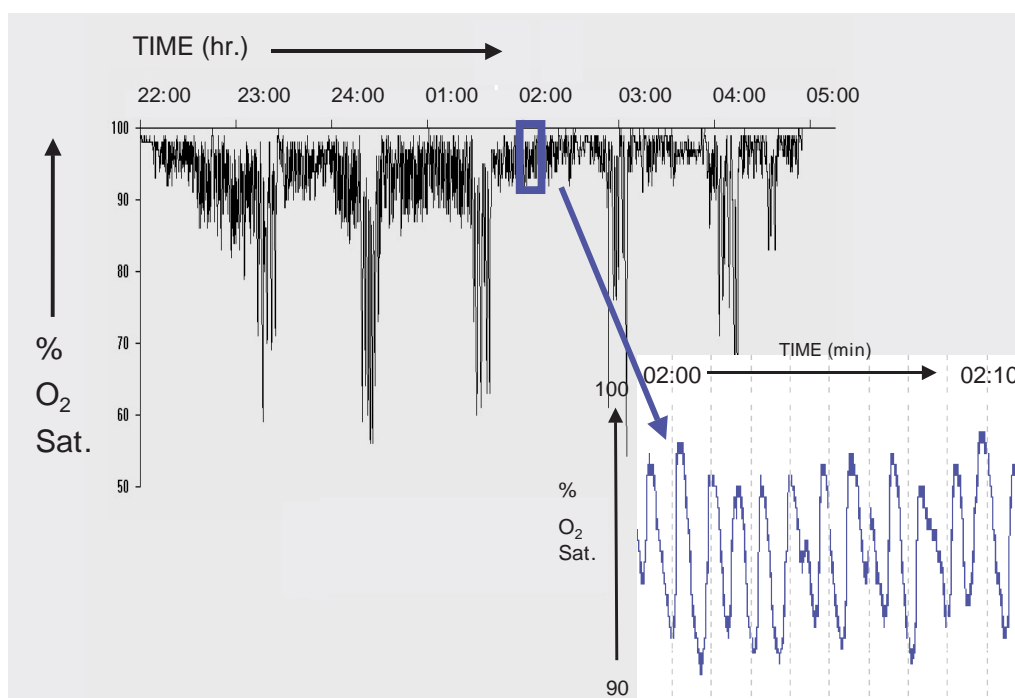
Figure 1 A condensed record of arterial O_2 desaturation of an entire night in an OSA patient. Two levels of intermittent hypoxia are clearly visible, moderate NONREM related desaturations (96–90%) on which profound REM-related desaturations (96–60%) are superimposed. Note that the persistent intermittent hypoxia is in fact a pattern of rapidly alternating hypoxia/reoxygenation.

Alterations in energy metabolism

In response to hypoxia the aerobic production of adenosine triphosphate (ATP) is impaired and degradation products as adenosine diphosphate (ADP), adenosine monophosphate (AMP) hypoxanthine and uric acid accumulate, indicating energy crisis. As a consequence, glycolysis is up-regulated. Concomitantly, due to hypoxia, circulating xanthine oxidase [46] and xanthine oxidase in ECs [47] are activated by action of proteases and Ca^{2+} which convert the xanthine dehydrogenase to xanthine oxidase. During the period of reoxygenation, the newly activated xanthine oxidases utilize the hypoxanthine and molecular oxygen to produce free radicals and oxidants as $O_2^{\bullet-}$, H_2O_2 and uric acid. When H/R ensues, transition metals such as Fe^{2+} and Cu^+ are released from the injured tissues. As a consequence, ROS production is amplified, namely due to the potent oxidant $OH^\bullet$ [25]. A schematic representation of this well established cascade of events is depicted in Fig. 2 (adapted from [41]).

Several independent studies that demonstrate increased accumulation of ATP degradation products in OSA patients are consistent with this mechanism. Findley et al. [48] found a three-fold increase in plasma adenosine levels of OSA patients with low O_2
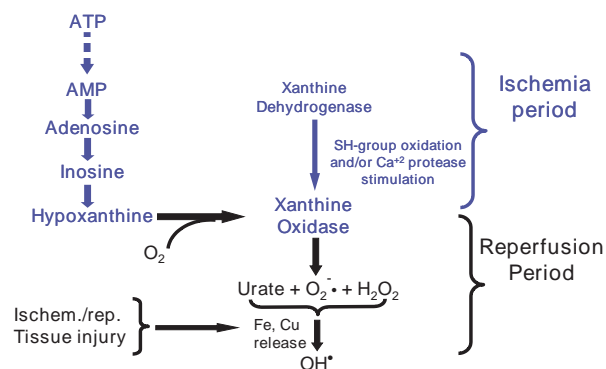
Figure 2 A mechanism for tissue injury upon reoxygenation of a hypoxic tissue. Modified from McCord [41].

saturation as compared to OSA patients with normal O_2 saturation. Effective nCPAP treatment and tracheostomy decreased plasma adenosine levels. Previous studies that demonstrated increased plasma adenosine in humans with episodic hypoxia and ischaemia support these findings [49, 50]. The significance of adenosine to sleep apnoea was further emphasized by its ability to depress respiration and induce apnoea in animal models [51, 52]. Uric acid, a catabolic end-product of ATP, was studied more extensively in OSA patients. Hasday and Grum [53] have demonstrated

increased urinary uric acid:creatinine (UA:CR) ratio, which clearly indicates that ATP degradation was increased due to hypoxaemia in OSA. Similarly, McKeon et al. [54] and Braghiroli et al. [55] confirmed these findings and further demonstrated that nCPAP treatment lowered UA:CR ratio. More recently, Sahebjani [56] confirmed these observations and established that uric acid excretion in OSA patients was significantly correlated with the apnoea–hypopnea index. This index is highly correlated with the degree of hypoxaemia. Furthermore, successful nCPAP treatment normalized UA:CR ratio. This ratio was suggested as a simple test for diagnosis and follow-up of OSA patients by this author [56]. In accord with this line of evidence, several studies demonstrated increased blood concentrations of 2,3, DPG, a glycolytic byproduct which decreases the affinity of oxygen to haemoglobin thus allowing a more effective oxygen delivery, indicating that glycolysis was indeed accelerated in OSA [54].

Collectively, these studies demonstrated that hypoxaemia during sleep leads to ATP depletion followed by an increase in purine catabolic products, namely adenosine and uric acid. Since the production of uric acid during reoxygenation is accompanied by free radical production, these findings indirectly suggest that increased oxidative stress does occur in OSA. Hence, emphasizing the possibility that the disorder is indeed one of H/R.

Alterations in gene expression

It is now well established that hypoxia-dependent ROS production is associated with a differential expression of specific genes, resulting in physiological and sometimes pathological consequences. The origin of ROS can be either from the main sources mentioned above, i.e. mitochondrial, from leukocytes or endothelial cells, or due to H/R. The expression of these genes relies on activation of redox-sensitive signaling pathways and transcription factors.

To date the most relevant redox activated transcription factors to OSA include: hypoxia-inducible factor-1 (HIF-1), nuclear factor kappa B (NFκB), activator protein-1 (AP-1), early growth response-1 (EGR-1), nuclear factor-interleukin-6 (NF-IL6) and Sp-1.

HIF-1

HIF-1 is a master regulator of an array of genes that is expressed in most, if not all, nucleated mammalian cells in response to hypoxia. It activates transcription of genes encoding proteins that mediate adaptive responses to reduced O_2 availability. These participate in glucose and energy metabolism, angiogenesis, vascular reactivity and remodeling, cell proliferation, and cell survival. Thus far, more than 30 protein products of the HIF-1 target genes were identified [57]. These include vascular endothelial growth factor (VEGF) and its receptor FLT-1, α_{1B}-adrenergic receptor, endothelin-1, haem oxygenase, TGF-β3, glycolytic enzymes such as aldolase A, aldolase C, enolase-1, glyceraldehydes-3-phosphate-dehydrogenase, hexokinases-1 and 2 and glucose transporters 1 and 3 – to compensate for energy loss from respiration, erythropoietin, inducible nitric oxide synthase, and many more [57, 58]. Although intermittent hypoxia was found to up-regulate HIF-1 expression in PC-12 cells in culture, implicating ROS activation in HIF-1 expression [59], no studies have demonstrated directly the up-regulation of HIF-1 in OSA. There is, however, an increasing number of reports demonstrating that some of the HIF-1 activated gene products are indeed increased in OSA. For instance, recent studies have shown that sleep apnoea patients had vastly elevated levels of *serum VEGF*, which were correlated with the severity of the syndrome as indexed by nocturnal hypoxia and apnoea–hypopnea index [60–62]. Similarly, a study from our own laboratory demonstrated that the levels of *plasma VEGF* were increased in OSA patients throughout the entire sleeping period, although moderately, as compared to VEGF concentrations in serum. Furthermore, VEGF concentrations were significantly higher in sleep apnoea patients than in age-similar snorers or in young adult healthy controls [63]. Thus, we showed that plasma VEGF concentrations were only moderately related to the severity of the syndrome as determined by AHI index, emphasizing the inter-individual heterogeneity in the hypoxic induction of VEGF [64]. Amelioration of the nocturnal hypoxia by nCPAP, significantly down-regulated morning VEGF concentrations [63]. It should be stressed that the relevance of VEGF values in serum was questioned as they reflect *ex-vivo* release from platelets and leukocytes [65, 66]. Therefore, although a correlation between plasma and serum was found, the absolute serum values vary considerably from 2–7-fold as compared to plasma [66]. Therefore serum VEGF values should be examined with great caution, as they depict extremely high levels, on the verge of pathology.

BIOCHEMICAL/MOLECULAR REVIEWS

Erythropoietin   EPO, the hormone that regulates red blood cell mass, studied extensively in sleep apnoea

syndrome. It is not conclusive whether it is increased in OSA; some studies claim that no differences were observed between OSA patients and controls [54, 67, 68], while others demonstrated a minor [60], or a substantial increase [69]. However, nCPAP treatment was shown to decrease EPO levels [70], and subjecting normal controls to normobaric hypoxia resulted in a 50% increase in EPO concentrations within 240 min of initiation of hypoxia [71]. These inconsistent and often conflicting results most likely reflect different levels of hypoxaemia these patients experienced and the individual variability in the response to the hypoxic stimulus, as we have demonstrated for VEGF [63, 64].

Endothelin-1 ET-1 is a potent vasoconstrictive and mitogenic peptide with blood pressure-elevating properties, produced by endothelial cells. The close association between OSA and systemic hypertension makes ET-1 an obvious candidate for investigation. In 1991 Ehlenz *et al.* [72] reported that renal secretion of ET-1 was decreased in OSA patients treated with nCPAP. Later on, several studies demonstrating enhanced levels of circulating endothelin-1 in OSA, and its decrease after nCPAP treatment, were reported [73, 74]. These studies were corroborated by findings in chronic obstructive pulmonary disease (COPD) patients and in a rat model of OSA utilizing intermittent hypoxia [75–77]. In contrast to these studies, one study reported that ET-1 levels were not elevated in OSA [78]. As with EPO, such conflicting findings probably reflect on one hand the actual levels of hypoxaemia patients experienced, and on the other the individual differences in the response to the hypoxic stimulus mounting to patients' char-acteristics [79]. Other molecules, which support HIF-1 upregulation in OSA, include possibly haem oxygenase-1 [80].

NFκB and AP-1
Both transcription factors are influenced by the cellular redox state. They have been implicated in the transcriptional regulation of a wide range of genes involved in inflammatory responses. Activation of NFκB is essential for the expression of a large number of cytokines as TNF-α and IL-1, chemokines, growth factors, and adhesion molecules, which are critical mediators of inflammatory responses and proliferative disorders of the vasculature including atherosclerosis [58]. Here again, although there is no direct evidence, which demonstrates NFκB- or AP-1 activation in OSA, several studies indirectly support the notion, by demonstrating the upregulation of several protein products of these genes. Some of

these gene products include cytokines as IL-6 and TNF-α [81–83] and adhesion molecules [32, 84–86].

Heat Shock Protein 70 family HSP70 is another set of molecules that are affected by ischaemia/hypoxia-reperfusion. At large, these proteins are synthesized in response to various stresses and protect cells by refolding of conformationally denatured proteins. The transcription of the human HSP70 is regulated by a complex array of redox sensitive transcription factors including AP-1 and Sp-1 [87]. In OSA patients, basal HSP-72 was increased at night-time as compared to controls. Moreover, a progressive decrease in HSP72 concentration was noted during sleep in the absence of nCPAP therapy [88]. We studied extensively morning HSP72 levels in plasma and in monocytes of OSA patients [89, 90]. In both, morning HSP72 levels were increased by at least 3-fold. Values of a representative experiment are summarized in Table 1. In nCPAP-treated OSA patients, HSP72 levels were similar to controls.

Induction of cell surface molecules
In the normal state, ECs that line the inner surface of blood vessels are essential for vascular homeostasis. They provide a permeability barrier for the vasculature, by maintaining a non-thrombotic surface, regulating vessel tone and inhibiting smooth muscle cell growth [91]. However, reperfusion–reoxygenation that follows the hypoxic period activates a variety of cells including ECs, leukocytes, and lymphocytes, and propagates inflammatory processes [92]. As these cells become activated, (whether via H/R or directly due to inflammatory cytokines as TNF-α, IL-1, IL-6 and IFN-γ), they express adhesion molecules which in turn

Table 1 Soluble HSP72 determined in plasma of OSA patients and controls

	Controls	OSA
N	19	40
Age	49.7 ± 8.7	44.8 ± 10.4
BMI	26.4 ± 3.9	29.9 ± 5.4
RDI	7.3 ± 1.9	31.4 ± 21.1
HTN (%)	0	0
IHD (%)	0	0
sHSP72 (ng/ml)	1.45 ± 1.65*	4.35 ± 3.99*

BMI – body mass index – Kg/m^2, RDI – respiratory disturbance index, HTN – hypertension, IHD – ischaemic heart disease, sHSP72 – soluble Heat Shock Protein 72.
* $P < 0.0002$ after adjustment for age and BMI.

result in increased ECs/leukocyte interactions and adherence to the vascular walls, initiating atherogenic processes [92, 93].

These EC/leukocyte interactions are tightly regulated in a sequential manner involving three independent stages; rolling, firm adhesion and transmigration [93]. Rolling is characterized by slowing down the flow of leukocytes in the blood stream, thus facilitating the initial binding to the endothelium. The slowing down of the leukocytes, which is mediated by selectins, allows interactions with inflammatory mediators released from ECs and, if leukocytes and ECs are activated, they firmly adhere to each other via adhesion molecules. The firm adhesion involves integrins (mainly of the CD18/CD11) on leukocytes and the counter receptors of the immunoglobulin superfamily – intracellular adhesion molecules and vascular cell adhesion molecules (ICAMs and VCAMs) on ECs. The firm adhesion between leukocytes and ECs is followed by migration of the leukocytes from post capillary venules to the interstitium where they release lytic enzymes and free radicals [93, 94].

From the several superfamilies of adhesion molecules, which participate in these processes: the selectins, the integrins, certain members of the immunoglobulin super family and cadherins, I will briefly describe the ones that we and others have studied with regard to sleep apnoea.

The selectins are a family of lectin-like adhesion glycoproteins that mediate rolling. The selectins are designated as L, P and E – named according to the cells in which they were originally discovered. L-selectin (CD62L) is constitutively expressed on leukocytes and binds to activated ECs. E-selectin (CD62E) is exclusively produced by cytokine activated ECs and binds to its counter-receptors on leukocytes. P-selectin (CD62P) is stored within specific granules in both platelets (α-granules) and ECs (Weibel Palade bodies), and is expressed only after cytokine activation. Thus, E and P selectin (CD62E, CD62P) are increased on the surface of activated endothelial cells. Both E-selectin and P-selectin bind to counter receptors on leukocytes – the L-selectins. All L-selectin ligands identified so far share common features, i.e. they are sialylated. These carbohydrates, the sialylated Lewis X (sLe$_x$-sCD15) and Lewis X (Le$_x$-CD15) on leukocytes, bind to the lectin domain on ECs, hence, directly mediating cell–cell contact of leukocyte/ECs. Upon activation, circulating leukocytes down-regulate or shed L-selectin within seconds to minutes to the plasma [95].

The β-integrines are heterodimeric proteins consisting of distinct α-subunits that are subdivided according to the β-subunit they possess. The leukocyte integrins are represented by three heterodimeric molecules. The α-subunits include CD11a, CD11b and CD11c and the β-subunit is CD18. Accordingly, designated CD11a/CD18, CD11b/CD18, and CD11c/CD18. β-integrines mediate firm adhesion of leukocytes to ECs by binding to ICAMs (members of the immunoglobulin super family that are expressed by ECs) [93–95].

The immunoglobulin superfamily the most important adhesion molecules serving as ligands for the integrins during leukocyte/ECs interactions are the ICAMs. ICAM-1 (also CD54) is strongly upregulated on ECs upon activation by hypoxia or by inflammatory mediators as TNF-α. As mentioned earlier ICAM-1 serves as a major endothelial ligand that mediates firm adhesion of polymorphonuclears, monocytes and lymphocytes via CD11b/CD18, CD11c/CD18 and CD11a/CD18, accordingly [93–95].

Evidence for up-regulation of adhesion molecules in OSA

We studied extensively the expression of adhesion molecules in monocytes of OSA patients. Our data demonstrated increased expression of CD15 (the counter-receptor for selectins on ECs) and CD11c (counter-receptor for ICAM-1 on ECs) in monocytes of OSA patients. This implicates exaggerated rolling and firm adhesion in the pathogenesis of vascular complications in OSA. The finding that monocytes of OSA patients adhered significantly more avidly to unstimulated ECs in culture further emphasized the functional significance of the increased expression of these molecules. Furthermore, CD15 upregulation was directly associated to the amount of hypoxia these patients experienced, as attested by RDI severity dependence (in preparation) and by the increase in CD15 expression obtained after exposing monocytes of control subjects to hypoxia *in vitro* [32]. More importantly, nCPAP treatment proportionally reduced the % of CD15 and CD11c expression, decreased basal ROS production of CD11c monocytes, and attenuated monocyte/ECs interactions in culture. By pretreating ECs with antibodies against E-selectin/P-selectin or ICAM-1, we were able to show the relative participation of these molecules in monocytes/ECs interactions in OSA and controls. This study clearly demonstrated increased monocyte activation in OSA by depicting

increased ROS production particularly at rest, which correlated with the increased expression of the adhesion molecules [32].

Increased expression of soluble adhesion molecules was reported as well [84, 85]. In these studies the authors demonstrated elevated circulating levels of the adhesion molecules ICAM-1, VCAM-1, L-selectin and E-selectin suggesting that the endothelium of these patients was activated as well. More recently, expression of soluble adhesion molecules (ICAM-1, VCAM-1, L-selectin E-selectin) was determined in coronary artery diseased patients with and without OSA in order to eliminate confounders as CAD and left ventricular dysfunction. All but L-selectin were increased in CAD patients with concomitant OSA in proportion to those without. These authors concluded that, in the setting of CAD, their findings suggest that OSA modulates proinflammtory mediators [86]. Of note, it remains unclear whether the circulating levels of adhesion molecules accurately reflect the actual numbers of adhesion molecules attached to the endothelium.

Nitric oxide

Another line of evidence, which supports damage and dysfunction to ECs of OSA patients, is the diminution in circulating NO levels. Indeed all the sources of exacerbated ROS production in OSA mentioned thus far invoke the possibility that NO availability may be compromised.

Nitric oxide, the most potent vascular relaxing factor and an intracellular signaling molecule, as well as a free radical, is affected by hypoxia and H/R as well. Nitric oxide is synthesized by a family of three enzymes – nitric oxide synthases (NOS). Nitric oxide of vascular endothelium is primarily catalyzed by eNOS (NOS3), while the other two isoforms: the inducible iNOS (NOS2) and neuronal nNOS (NOS1) may also be present in the vasculature and contribute to NO production [96, 97]. The NO generated by ECs accounts for a large part of the vasodilatory effects of these cells. Oxygen tension appears to be an important factor in regulating gene expression of both iNOS and eNOS (which is constitutively expressed and negatively regulated by cytokines). Activation of the iNOS gene includes binding sites for the transcription factors NFκB, AP-1, and HIF-1. This gene is induced by hypoxia, as shown in many *in-vitro* studies [58]. By contrast, the effects of hypoxia on eNOS activity are less clear and vary in both directions. In procaine aortic endothelial cells in culture, it appeared to be up-regulated via expression by redox sensitive AP-1 mediated transcriptional control [98]. On the other hand, a profound decrease in the transcript for eNOS and a corresponding fall in its protein levels were detected in human ECs exposed to low oxygen tension [99]. In accordance, NO levels were reported to increase with short-term ischaemia, or hypoxia, and to decrease with chronic exposure [100]. These inconsistencies most likely reflect differences in the nature of the hypoxic exposure as manifested by duration and severity.

In OSA patients, morning circulating NO levels were determined in plasma or serum, via measurement of nitrite and nitrate, NO's stable metabolic end-products [101, 102]. In these studies it was presumed that circulating nitrites/nitrates reflect NO of mainly endothelial origin (eNOS), although other sources for NO could not be excluded. In both studies NO levels were significantly increased after nCPAP treatment, supporting the hypothesis that the reduced endothelium-dependent vasodilatation in OSA (see later), at least partially, resulted from diminished NO levels. Yet, NO serum values were higher by 50% as compared to plasma values. We conducted a similar study to determine NO values in plasma of OSA patients. Our data confirm previous studies by demonstrating diminished NO levels throughout the entire night in severe OSA patients as compared to young adults and age-similar snorers. Moreover, we showed that both NO and arginine, the precursor of NO, were increased after effective nCPAP treatment (in preparation). It should be stressed that decreased NO levels also facilitate increased leukocyte binding to post-ischaemic endothelium, consistent with the increased expression of adhesion molecules [32, 92].

Since circulating levels of NO were measured, the mechanisms that underlie this attenuation in OSA can only be postulated. Possibly this reflects decreased NO bioavailability due to oxidative stress induced inactivation. However, direct effects on the enzymes synthesizing NO cannot be excluded. It could also be argued that hypoxaemia, which affects the appropriate transcription factors, suppresses NOS transcription or affects the stability of its mRNA. Decreased NO synthesis could also result from a lack of oxygen, the co-substrate for NOS or it can result from elevated endogenous levels in NOS inhibitors as asymmetric dimethyl arginine (AMDA), which were reported to increase in OSA [103]. Another possible explanation for the diminution of NO levels could be from a direct effect on the substrate arginine, as we have recently found. Although the absolute values of arginine

concentrations in plasma were within the normal range in OSA patients, they were increased by 16% after nCPAP treatment, indicating that nCPAP treatment increased substrate availability (in preparation). This decrease in plasma arginine levels, taken together with the increase observed in endogenous levels of NOS inhibitor – AMDA [103], indicate that NOS activity is compromised in OSA.

FREE RADICALS IN DISEASE PROCESSES RELEVANT TO OSA

Endothelial dysfunction, which is the first step in the development of atherosclerosis according to the response-to-injury hypothesis, was initially implicated with hypercholesterolaemia and increase in oxidized LDL (Ox-LDL) [104]. However, endothelial dysfunction could result from many seemingly unrelated pathological conditions such as increased plasma homocysteine levels, hypertension and diabetes mellitus [104]. Thus, since oxidative stress is a fundamental component in all these pathologies, they merit special attention with regard to OSA. I will focus on two; Ox-LDL and hyperhomocysteinaemia.

Oxidized LDL

A leading theory in the pathogenesis of atherosclerosis suggests that LDL when modified by oxidation (Ox-LDL) [105, 106] is a major cause of injury to endothelial cells and underlying smooth muscle cells [107–109]. A study by Wali *et al.* [110] measured whether LDL of hypoxic OSA patients was more susceptible to oxidative stress *in vitro* than LDL of non-hypoxic OSA and normal controls. They concluded that there was no difference in the susceptibility to oxidative stress *in vitro* between the three groups studied. This methodology, however, does not attest to the LDL oxidative potential *in vivo* due to increased oxidative stress in OSA. By contrast, Saarelainen *et al.* [111] found increased levels of autoantibodies against Ox-LDL in OSA patients as compared to controls. Since increased titre of autoantibodies against Ox-LDL was found to be an independent predictor for the progression of carotid atherosclerosis in humans [112], this could indicate that atherosclerosis in OSA is indeed exacerbated. In accordance, Barcelo *et al.* [113] demonstrated significantly higher susceptibility of LDL to oxidation in OSA patients. They assessed the oxidative potential of plasma derived from patients and

controls by the thiobarbituric acid assay and concluded that OSA was associated with abnormal lipid peroxidation, which was improved by nCPAP treatment. These results further support the existence of exacerbated oxidative stress in OSA.

Hyperhomocysteinaemia

In recent years, mildly elevated homocysteine (Hcy) concentration in plasma has emerged as an independent risk factor for the development and progression of CAD. Initially, homocysteine was described by McCully over 30 years ago in infants with inborn errors of metabolism as an atherogenic compound that accelerates atherosclerosis [114]. Many clinical and epidemiological studies since confirmed this observation that mild elevation in total plasma Hcy confers an increased risk for peripheral arterial occlusive disease, coronary artery disease, and cerebrovascular disease similar to other conventional risk factors such as hyperlipidaemia or smoking [115]. Moreover, a meta-analysis of 27 studies showed a graded risk for atherosclerosis such that an increment of 5 μmol/l in Hcy, elevated cardiovascular risk by as much as 60% in men and 80% in women [116]. Importantly, a clear correlation was shown between mortality and increased Hcy levels in patients with angiographically confirmed coronary artery disease [117]. Experimentally, diet-induced moderate hyperhomocysteinaemia was found to be associated with altered vascular function and impaired flow-mediated endothelium NO in monkeys and humans [118, 119]. Similarly, hyperhomocysteinaemia induced experimentally with methionine loading (the precursor for Hcy) in healthy subjects resulted in endothelial dysfunction [120]. This could be prevented by pretreatment with the antioxidant vitamin C [121]. In should be noted that dietary supplementation with folic acid and vitamin B12 and B6 (the vitamins and co-factors participating in methionine-Hcy metabolism) lowered plasma Hcy levels, and thus could possibly modify the risk for atherosclerosis [122]. Cumulative data *in vivo* and *in vitro* clearly demonstrate that Hcy affects multiple vascular functions such as promoting a prothrombotic phenotype of the endothelium by increasing platelet aggregation and activation, and stimulating vascular smooth muscle cell proliferation [123]. Currently, the leading mechanisms proposed for the adverse effects of Hcy on endothelial function implicate oxidative stress and depletion in NO bioavaliability [123]. Hcy, like other thiol-containing amino acids, undergoes auto-oxidation, forming in the process $O_2^{\bullet-}$ and

H_2O_2 [124]. The ability of $O_2^{\bullet-}$ to inactivate the vasodilator NO and form peroxynitrite, results in decreased NO bioavaliability [123]. Hence, the most convincing data that oxidative stress and subsequent loss of bioactive NO contribute to endothelial dysfunction in mild hyperhomocysteinaemia are provided by a study, which utilizes a hyperhomocysteinaemic mouse model [125].

The increased prevalence of hyperhomocysteinaemia, as an independent risk factor in cardiovascular morbidity and mortality on one hand and the mechanistic link between hyperhomocysteinaemia and increased oxidative stress and endothelial dysfunction on the other, led us to investigate plasma homocysteine levels in OSA male patients [126]. We studied a total of 345 subjects. The 237 OSA patients were subdivided into 3 groups; OSA without co-morbidities, hypertensive-OSA and OSA with ischaemic heart disease (IHD). The 108 controls consisted of 2 groups, controls without co-morbidities and patients suffering from IHD without OSA. We showed that OSA patients with IHD had significantly higher Hcy levels than all other groups including the non-OSA with IHD. Hypertensive OSA patients had comparable Hcy levels to IHD patients without OSA, while OSA patients without co-morbidities had comparable levels to normal controls. Also no differences in conventional risk factors or in B_{12}, B_6, or folate levels were found between groups. The fact that patients suffering from both IHD and OSA had exceptionally elevated Hcy levels raises the possibility that increased oxidative stress and endothelial dysfunction ensue in this group.

EVIDENCE FOR FUNCTIONAL ENDOTHELIAL DYSFUNCTION IN SLEEP APNEA

The accumulated data presented, thus far, lead to the conclusion that the nightly increase in oxidative stress should result in a state of functional endothelial dysfunction in OSA patients. The term endothelial dysfunction in this context is used to refer to an impairment of endothelium-dependent vasorelaxation caused by a loss of NO bioactivity in the vessel wall. Several human studies have shown that traditional risk factors for atherosclerosis such as hypertension, hypercholesterolaemia, congestive heart failure, and diabetes mellitus predispose to functional endothelial

dysfunction [91, 104]. Furthermore, impaired endothelium-dependent vasodilatation is thought to have prognostic implications in that it predicts future atherosclerotic processes. The most commonly employed test for discerning endothelial function is the response of the brachial artery to flow-mediated vasodilation. The ability of the brachial artery to dilate in response to reactive hyperaemia is often used as an index of endothelial cell integrity. Depressed brachial artery flow-mediated vasodilation is considered to be secondary to failure of shear stress-induced NO release [127]. Thus, endothelial dysfunction is considered to be a sub-clinical indicator of myocardial or vascular dysfunction before the emergence of clinical signs of overt cardiovascular disease [128].

There is evidence that sleep apnoea patients, who are free of any overt cardiovascular morbidity, actually suffer from endothelial dysfunction. Carlson et al. [17] reported that endothelial function in forearm resistance vessels was impaired in OSA patients in comparison with healthy subjects. Kato et al. [129] reported that patients with sleep apnoea had a blunted vasodilation response in the forearm to acetylcholine, a vasodilator that stimulates endothelial NO release, in comparison with carefully matched obese subjects free of breathing disorders in sleep. In contrast, OSA patients responded as non-apnoeics to sodium nitroprusside, which is an exogenous NO donor. Kraiczi et al. [130] reported on a significant association between sleep apnoea severity and reduced endothelium-dependent dilatory capacity of the brachial artery. It can be therefore concluded that the increased oxidative stress in sleep apnoea patients without any overt cardiovascular disease results in a sub-clinical condition of atherosclerosis, which may predispose sleep apnoea patients to cardiovascular morbidity.

PROPOSED CHAIN OF EVENTS

Figure 3 provides a schematic summary of the proposed sequence of events starting from episodic hypoxia in OSA and ending with endothelial dysfunction, which may eventually lead to CAD morbidity. The mechanisms proposed are based on the data accumulated in the field of ischaemia/reperfusion and oxidative stress in disease states and the results specifically pertained to sleep apnoea syndrome.

It is postulated that the episodic hypoxia in OSA leads to increased production of $O_2^{\bullet-}$ and other ROS molecules via several enzymic pathways. Activation of
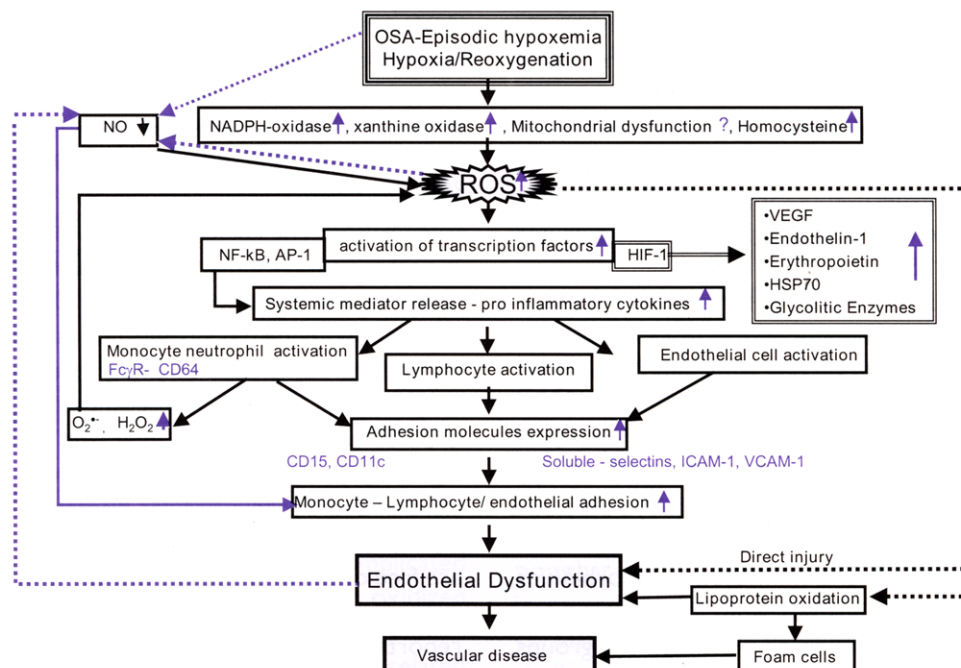
Figure 3   A schematic summary of the proposed sequence of events in OSA starting from episodic hypoxia and ending with endothelial dysfunction.

circulating and cellular xanthine oxidases and membrane bound NADPH oxidases from endothelial and leukocyte origin can lead to increase in oxidative stress. Increased free radical flux from dysfunctional mitochondria and increased concentrations of small molecules as homocysteine and glucose, which undergo auto-oxidation, can further contribute to increase ROS production. Activation of xanthine oxidases in OSA is indirectly supported by altered energy metabolism during H/R, depicted by increase in ATP degradation products, which are usually accompanied by increased ROS production (Fig. 2). However, direct evidence for increased oxidative stress in OSA stems from two studies that demonstrated NADPH-dependent increased ROS production by neutrophils and monocytes. Increase in ROS production is likely to activate redox-sensitive transcription factors as HIF-1, NFκB and AP-1. Upregulation of some of their gene products in OSA strongly supports this assumption. Thus, VEGF, ET-1 and erythropoietin upregulation supports HIF-1 participation, while upregulation of AP-1 and NFκB was implicated by increased production/release of systemic mediators as TNF-α and IL-6, which were shown to increase in OSA patients. Such inflammatory mediators elicit activation in a variety of cells including leukocytes, lymphocytes, and endothelial cells. Activation of monocytes and granulocytes was evident by the increased expression in adhesion molecules as

CD15 and CD11c and by the increased ROS production. Endothelial cell activation was suggested by the increased expression of soluble adhesion molecules as selectins, ICAM-1 and VCAM-1 (adhesion molecules expression can also be upregulated directly via activation of NFκB and AP-1). Consequently, this increased expression of adhesion molecules contributed to increased adhesiveness between leukocytes and endothelial cells leading to endothelial dysfunction and injury. Concomitantly, due to the intermittent hypoxia NO bioavailability was compromised, either because of decreased eNOS activity or/and via inactivation by ROS. This affects endothelial cell function and further facilitates increased leukocyte/ECs adhesion. Moreover, endothelial dysfunction could have also resulted from direct injury due to exacerbated ROS production (ECs are sensitive to H_2O_2) or via oxidized plasma lipids. Once started, a vicious cycle of an increased state of endothelial dysfunction, which leads to a further decrease in NO levels, in turn augments ECs/leukocyte interactions and endothelial dysfunction is exaggerated.

SUMMARY

Oxidative stress is a common denominator in all the processes, which eventually lead to endothelial

dysfunction. Whether from a direct injury due to excessive ROS production, or from decreased NO bioavaliability, increased adhesiveness of leukocytes, increased homocysteine concentrations, or via increase in oxidized LDL and foam cell formation. All these may eventually lead to increased CAD morbidity in OSA.

Research Agenda

Future research in OSA should focus on
1. Mitochondrial dysfunction.
2. Control of transcription factors activation.
3. Activation of NADPH oxidases of endothelial cells.
4. Elaborating the interactions between leukocytes and endothelial cells.
5. Investigating the mechanisms of nitric oxide diminution.
6. Effects of antioxidants on cardiovascular morbidity.

ACKNOWLEDGMENTS

REFERENCES

1. Lavie P, Herer P, Hoffstein V. Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study. *BMJ* 2000; **320**: 479–482.
2. Grote L, Hedner J, Peter JH. Sleep-related breathing disorder is an independent factor for uncontrolled hypertension. *J Hypertens* 2000; **18**: 679–685.
3. Shahar E, Whitney CW, Redline S *et al.*, for the Sleep Heart Health Study Research Group. Sleep-disordered breathing and cardiovascular disease cross-sectional results of the Sleep Heart Health Study. *Am J Respir Care Med* 2001; 163: 19–25.
4. Young T, Peppard PE, Palta M *et al.* Population-based study of sleep-disordered breathing as a risk factor for hypertension. *Arch Intern Med* 1997; **157**: 1746–1752.
5. Peppard P, Young, T, Palta, M, Skatrud, J. Prospective study of the association between sleep disordered breathing and hypertension. *N Engl J Med* 2000; **342**: 1378–1384.
6. Nieto FJ, Young TB, Lind BK *et al.* Association of sleep disordered breathing, sleep apnea and hypertension in a large community-based study. *JAMA* 2000; **283**: 1829–1836.
7. De Olazabal JR, Miller MJ, Cook WR, Mithoefer JC. Disordered breathing and hypoxia during sleep in coronary artery disease. *Chest* 1982; **82**: 548–552.
8. Hung J, Whitford EG, Parsons RW, Hillman DR. Association of sleep apnoea with myocardial infarction in men. *Lancet* 1990; **336**: 261–264.
9. Saito T, Yoshikawa T, Sakamoto Y, Tanaka K, Inoue T, Ogawa R. Sleep apnea in patients with acute myocardial infarction. *Am J Respir Crit Care Med* 1991; **19**: 938–941.
10. Mooe T, Rabben T, Wiklund U, Franklin KA, Eriksson P. Sleep-disordered breathing in men with coronary artery disease. *Chest* 1996; **109**: 659–663.
11. Bassetti C, Aldrich MS, Chervin RD, Quint D. Sleep apnea in patients with transient ischemic attack and stroke: a prospective study of 59 patients. *Neurology* 1996; **47**: 1167–1173.
12. Xie A, Skatrud JB, Crabtree DC, Puleo DS, Goodman BM, Morgan BJ. Neurocirculatory consequences of intermittent asphyxia in humans. *J Appl Physiol* 2000; **89**: 1333–1339.
13. Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C, Sinoway L. Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxaemia. *J Appl Physiol* 1995; 79: 581–588.
14. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 1995; **96**: 1897–1904.
15. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest* 1993; **103**: 1763–1768.
16. Narkiewicz K, van de Borne PJ, Pesek CA, Dyken ME, Montano N, Somers VK. Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation* 1999; **99**: 1183–1189.
17. Carlson JT, Rangemark C, Hedner JA. Attenuated endothelium-dependent vascular relaxation in patients with sleep apnoea. *J Hypertens* 1996; **14**: 577–584.
18. Imadojemu VA, Gleeson K, Gray KS, Sinoway LI, Leuenberger UA. Obstructive apnea during sleep is associated with peripheral vasoconstriction. *Am J Respir Crit Care Med* 2002; **165**: 61–66.
19. Dean RT, Wilcox I. Possible atherogenic effects of hypoxia during obstructive sleep apnea (Supplement: 2. Natural History of Sleep Apnea). *Sleep* 1993; **16**: S15–S22.
20. Prabhakar RN. Physiologic and genomic consequences of intermittent hypoxia. Invited review: Oxygen sensing during intermittent hypoxia: cellular and

molecular mechanisms. *J Appl Physiol* 2001; **90**: 1986–1994.

21. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; **82**: 47–95.

*22. Babior BM. Phagocytes and oxidative stress. *Am J Med* 2000; **109**: 33–44.

*23. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med* 2000; **108**: 652–659.

24. Sen CK. Cellular thiols and redox-regulated signal transduction. *Curr Top Cell Regul* 2000; **36**: 1–30.

25. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Meth Enzymol* 1990; **186**: 1–85.

26. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* 1996; **271**: C1421–C1437.

27. Dureanteau J, Chandel NS, Kulisz A et al. Intracellular signaling by Reactive Oxygen Species during hypoxia in Cardiomyocytes. *J Biol Chem* 1998; **273**: 11619–11624.

28. Bouaziz N, Redon M, Quere L, Remacle J, Michiels C. Mitocondrial respiratory chain as a new target for anti-ischemic molecules. *Eur J Pharmacol* 2002; **441**: 35–45.

29. Sanders SP, Zweier JL, Kuppusamy P et al. Hyperoxic sheep pulmonary microvascular endothelial cells generate free radicals via mitochondrial electron transport. *J Clin Invest* 1993; **91**: 46–52.

30. Chandel NS, Maltepe E, Goldwasser E et al. Mitochon-drial reactive oxygen species trigger hypoxia-induced transcription. *Procl Natl Acad Sci USA* 1998; **95**: 11715–11720.

31. Schulz R, Mahmoudi S, Katja Hattar et al. Enhanced release of superoxide form polymorphonuclear neu-trophils in obstructive sleep apnea. *Am J Respir Crit Care Med* 2000; **162**: 566–570.

*32. 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.

33. Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. *Am J Physiol* 1994; **266**: H2568–H2572.

34. Rajogopalan S, Kurz S, Munzel T et al. Angiotensin II mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. *J Clin Invest* 1996; **97**: 1916–1923.

35. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase. Role in cardiovascular biology and disease. *Circ Res* 2000; **86**: 494–501.

36. Barcelo A, Elorza MA, Barbe F et al. Angiotensin converting enzyme in patients with sleep apnoea syndrome: plasma activity and gene polymorphisms. *Eur Respir J* 2001; **17**: 728–732.

37. Marshall C, Mamary AJ, Verhoeven AJ, Marshall BE. Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. *Am J Resp Cell Mol Biol* 1996; **15**: 633–644.

38. Fletcher EC, Bao G, Li R. Renin activity and blood pressure in response to chronic episodic hypoxia. *Hypertension* 1999; **34**: 309–314.

39. Fletcher EC. Physiological and genomic consequences of intermittent hypoxia. Invited review: Physiological consequences of intermittent hypoxia: systemic blood pressure. *J Appl Physiol* 2001; **90**: 1600–1605.

40. Fletcher EC, Orolinova N, Bader M. Blood pressure response to chronic episodic hypoxia: the renin-angiotensin system. *J Appl Physiol* 2002; **92**: 627–633.

41. McCord MJ. Oxygen derived free radicals in post ischemic tissue injury. *New Engl J Med* 1985; **312**: 159–163.

42. Bolli R, Jeroudi MO, Patel BS et al. Direct evidence that oxygen-derived free radicals contribute to postis-chemic myocardial dysfunction in the intact dog. *Proc Natl Acad Sci USA* 1989; **86**: 4695–4699.

43. Bolli R, Jeroudi MO, Patel BS et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. *Circ Res* 1989; **65**: 607–622.

44. Villari B, Ambrosio G, Golino P et al. The effects of calcium channel antagonist treatment and oxygen radical scavenging on infarct size and the no-reflow phenomenon in reperfused hearts. *Am Heart J* 1993; **125**: 11–23.

45. Jolly SR, Kane WJ, Bailie MB et al. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* 1984; **54**: 277–285.

46. Terada LS, Dormish JJ, Shanley PF, Leff JA, Anderson BO, Repine JE. Circulating xanthine oxidase mediates lung neutrophil sequestration after intestinal ischae-mia-reperfusion. *Am J Physiol* 1992; **263**: L394–L401.

47. Terada LS, Guidot DM, Leff JA et al. Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. *Proc Natl Acad Sci USA* 1992; **89**: 3362–3366.

48. Findley LJ, Boykin M, Fallon T, Belardinelli L. Plasma adenosine and hypoxaemia in patients with sleep apnea. *J Appl Physiol* 1988; **64**: 556–561.

49. Mann J, Holgate S, Renwick A, Cushley M. Airway effects of purine nucleosides and nucleotides and release with bronchial provocation in asthma. *J Appl Physiol* 1986; **61**: 1667–1676.

50. Sollevi A. Cardiovascular effects of adenosine in man; possible clinical implications. *Prog Neurobiol* 1986; **27**: 319–349.

51. Eldridge FL, Millhorn DE, Kiley JP. Antagonism by theophylline of respirator inhibition induced by adenosine. *J Appl Physiol* 1985; **59**: 1428–1433.

*The most important references are denoted by an asterisk.

52. Mueller RA, Widerlov E, Breese GR. Attempted antagonism of adenosine analogue induced depression of respiration. *Pharmacol Biochem Behav* 1984; **21**: 289–296.

53. Hasday JD, Grum CM. Nocturnal increase of urinary uric acid: creatinine ratio. A biochemical correlate of sleep-associated hypoxaemia. *Am Rev Respir Dis* 1987; **135**: 534–538.

54. McKeon JL, Saunders NA, Murree-Allen K *et al*. Urinary uric acid: creatinine ratio, serum erythropoietin, and blood 2,3-diphosphoglycerate in patients with obstructive sleep apnea. *Am Rev Respir Dis* 1990; **142**: 8–13.

55. Braghiroli A, Sacco C, Erbetta M *et al*. Overnight urinary uric acid: Creatinine ratio for detection of sleep hypoxaemia. Validation study in chronic obstructive pulmonary disease and obstructive sleep apnea before and after treatment with nasal continuous positive airway pressure. *Am Rev Respir Dis* 1993; **148**: 173–178.

56. Sahebjani H. Changes in urinary uric acid excretion in obstructive sleep apnea before and after therapy with nasal continuous positive airway pressure. *Chest* 1998; **113**: 1604–1608.

*57. Semenza GL. Oxygen-regulated transcription factors and their role in pulmonary disease. *Respir Res* 2000; **1**: 159–162.

58. Piacentini L, Karliner JS. Altered gene expression during hypoxia and reoxygenation of the heart. *Pharmacol Ther* 1999; **83**: 21–37.

59. Yuan G, Adhikary G, Prabhakar NR. Activation of HIF-2a by intermittent hypoxia requires CaMKII (Abstract). *FASEB J* 2001; **15**: A154.

60. Imagawa S, Yamaguchi Y, Higuchi M *et al*. Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea-hypopnea syndrome. *Blood* 2001; **98**: 1255–1257.

61. Schulz R, Hummel C, Heinemann S *et al*. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. *Am J Respir Crit Care Med* 2002; **165**: 67–70.

62. Gozal D, Lipton AJ, Jones KL. Circulating vascular endothelial growth factor levels in patients with obstructive sleep apnea. *Sleep* 2002; **25**: 59–65.

63. Lavie L, Kraiczi H, Hefetz *et al*. Plasma Vascular Endothelial Growth Factor (VEGF) in sleep apnea syndrome: effects of nCPAP treatment. *Am J Respir Crit Care Med* 2002; **165**: 1624–1628.

*64. Schultz A, Lavie L, Hochberg I *et al*. Interindividual heterogeneity in the hypoxic regulation of VEGF: significance for the development of the coronary artery collateral circulation. *Circulation* 1999; **100**: 547–552.

65. Gunsilius E, Petzer AL, Gastl GA. Blood levels of vascular endothelial growth factor in obstructive sleep apnea-hypopnea syndrome. (Letter to Editor) *Blood* 2002; **99**: 393–394.

66. Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE. Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. *Clin Sci* 1998; **94**: 395–404.

67. Carlson JT, Hedner J, Fagerber B *et al*. Secondary polycythaemia associated with nocturnal apnoea – a relationship not mediated by erythropoietin? *J Intern Med* 1992; **231**: 381–387.

68. Pokala P, Llanera M, Sherwood J *et al*. Erythropoietin response in subjects with obstructive sleep apnea. *Am J Respir Crit Care Med* 1995; **151**: 1862–1865.

69. Cahan C, Decker MJ, Arnold JL *et al*. Diurnal variations in serum erythropoietin levels in healthy subjects and sleep apnea patients. *J Appl Physiol* 1992; **72**: 2112–2117.

70. Cahan C, Decker MJ, Arnold JL *et al*. Erythropoietin levels with treatment of obstructive sleep apnea. *J Appl Physiol* 1995; **79**: 1278–1285.

71. Knaupp W, Khilnani S, Sherwood J *et al*. Erythropoietin response to acute normobaric hypoxia in humans. *J Appl Physiol* 1992; **73**: 837–840.

72. Ehlenz K Herzog P, von Wichert P, Kaffarnik H, Peter JH. Renal excretion of endothelin in obstructive sleep apnea syndrome. In: Gautier C, Escourrou P, Curzi-Dascalova (eds) *Colloque IMSERM*. John Libbey Ltd 1991; **217**: 226.

73. Saarelainen S, Seppala E, Laasonen K, Hasan J. Circulating endothelin-1 in obstructive sleep apnoea. *Endothelium* 1997; **5**: 115–118.

74. Phillips BG, Narkiewicz K, Pesek CA *et al*. Effects of obstructive sleep apnea on endolethelin-1 and blood pressure. *J Hypertens* 1999; **17**: 61–66.

75. Trakada G, Mnangos M, Spiropoulos K. Mechanisms of endothelin-1 elevation in chronic obstructive pulmonary disease patients with nocturnal oxyhaemoglobin desaturation. *Respiration* 2001; **68**: 134–139.

76. Tahawi Z, Orolinova N, Joshua IG *et al*. Altered vascular reactivity in arterioles of chronic intermittent hypoxic rats. *J Appl Physiol* 2001; **90**: 2007–2013.

77. Kanagy NL, Walker BR, Nelin LD. Role of endothelin in intermittent hypoxia-induced hypertension. *Hypertension* 2001; **37**: 511–515.

78. Grimpen F, Kanne P, Schulz E *et al*. Endothelin-1 plasma levels are not elevated in patients with obstructive sleep apnoea. *Eur Respir J* 2000; **15**: 320–325.

79. Saarelainen S, Hasan J. Circulating endothelin-1 and obstructive sleep apnoea. *Eur Respir J* 2000; **16**: 794–795.

80. Chin K, Ohi M, Shimzu K *et al*. Increase in bilirubin levels of patients with obstructive sleep apnea in the morning – a possible explanation of induced heme oxygenase-1. *Sleep* 2001; **24**: 218–223.

81. Vgontzas AN, Papanicolaou DA, Bixler EO *et al*. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbances and obesity. *J Clin Endocrinol Metab* 1997; **82**: 1313–1316.

82. 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.

83. Entzian P, Linnemann K, Schlaak M, Zabel P. Obstructive sleep apnea syndrome and circadian rhythms of hormones and cytokines. *Am J Respir Crit Care Med* 1996; **153**: 1080–1086.

84. Chin K, Nakamura T, Shimzu 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.

85. 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.

86. El-Solh AA, Mador MJ, Sikka P, Dhillon RS, Amsterdam D, Grant BJ. Adhesion molecules in patients with coronary artery disease and moderate-to-severe obstructive sleep apnea. *Chest* 2002; **121**: 1541–1547.

87. Sen CK. Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol* 1998; **55**: 1747–1758.

88. Noguchi T, Chin K, Ohi M *et al.* Heat shock protein 72 level decreases during sleep in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 1997; **155**: 1316–1322.

89. Lavie L, Ghandour H, Pillar G, Lavie P. Production of heat shock protein 70 by peripheral blood monocytes from patients with sleep apnea syndrome. *J Sleep Res* 1998; **7**(suppl 2): 150.

90. Ghandour H, Lavie P, Lavie L. Expression of heat shock proteins in obstructive sleep apnea syndrome. *Sleep* 1999; **22**: S331.

91. Forgione MA, Leopold JA, Loscalzo J. Roles of endothelial dysfunction in coronary artery disease. *Curr Opin Cardiol* 2000; **15**: 409–415.

*92. 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.

*93. Walzog B, Gaehtgens P. Adhesion molecules: the path to a new understanding of acute inflammation. *News Physiol Sci* 2000; **15**: 107–113.

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

*95. Tailor A, Granger DN. Role of adhesion molecules in vascular regulation and damage. *Curr Hypertens Rep* 2000; **2**: 78–83.

96. Rairigh RL, Storme L, Parker TA *et al.* Role of neuronal nitric oxide synthase in regulation of vascular and ductus arteriosus tone in the ovine fetus. *Am Physiol Lung Cell Mol Physiol* 2000; **278**: L105–L110.

97. Rairigh RL, Le Cras TD, Ivy DD *et al.* Role of inducible nitric oxide synthase in regulation of pulmonary vascular tone in the late gestation ovine fetus. *J Clin Invest* 1998; **101**: 15–21.

98. Hoffmann A, Gloe T, Pohl U. Hypoxia-induced upregulation of eNOS gene expression is redox-sensitive: a comparison between hypoxia and inhibitors of cell metabolism. *J Cell Physiol* 2001; **188**: 33–44.

*99. Phelan MW, Faller DV. Hypoxia decreases constitutive nitric oxide synthase transcript and protein in cultured endothelial cells. *J Cell Physiol* 1996; **167**: 469–476.

100. Faller DV. Endothelial cell responses to hypoxic stress. *Clin Exp Pharmacol Physiol* 1999; **26**: 74–84.

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

102. 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.

103. Carlson J, Hedner J, Patterson A. Increased plasma concentration of ADMA a naturally occurring nitric oxide synthesis inhibitor in OSA patients. *Am J Respir Crit Care Med* 1997; **155**: A869.

*104. Ross R. Atherosclerosis – an inflammatory disease. *New Eng J Med* 1999; **340**: 115–126.

105. Steinberg D. Low density lipoprotein oxidation and its pathological significance. *J Biol Chem* 1997; **272**: 20963–20966.

106. Koo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. *Arteriosclerosis* 1988; **8**: 348–358.

107. Navab M, Berliner JA, Watson AD *et al.* The Yin and Yang of oxidation in the development of the fatty streak: a review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol* 1996; **16**: 831–842.

108. Morel DW, Hessler JR, Chisholm GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. *J Lipid Res* 1983; **24**: 1070–1076.

109. Griendling KK, Alexander RW. Oxidative stress and cardiovascular disease. *Circulation* 1997; **96**: 3264–3265.

110. Wali SO, Bahammam AS, Massaeli H *et al.* Susceptibility of LDL to oxidative stress in obstructive sleep apnea. *Sleep* 1998; **21**: 290–296.

111. Saarelainen S, Lehtimaki T, Jaak-kola O *et al.* Autoantibodies against oxidised low-density lipoprotein in patients with obstructive sleep apnoea. *Clin Chem Lab Med* 1999; **37**: 517–520.

112. Salonen JT, Yla-Herttuala S, Yamamoto R *et al.* Autoantibody against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 1992; **339**: 899–900.

113. Barcelo A, Miralles C, Barbe F, Vila M, Pons S, Agusti AG. Abnormal lipid peroxidation in patients with sleep apnoea. *Eur Respir J* 2000; **16**: 644–647.

114. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1969; **56**: 111–128.

115. Graham IM, Daly EL, Refsum HM *et al*. Plasma homocysteine as a risk factor for vascular disease; The European concerted action project. *JAMA* 1997; **277**: 1775–1781.

116. Boushey CJ, Beresford SAA, Omenn GS *et al*. A qualitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *JAMA* 1995; **274**: 1049–1957.

117. Nygard O, Nordrehaug JE, Refsum H *et al*. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997; **337**: 230–236.

118. Lentz SR, Sobey CG, Piegors DJ *et al*. Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 24–29.

119. Tawakol A, Omland T, Garhard W, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. *Circulation* 1997; **95**: 1119–1121.

120. Bellamy MF, McDowell IF, Ramsey MW *et al*. Hyperhomocysteinemia after an oral methionine load acutely impairs endothelial function in healthy adults. *Circulation* 1998; 1848–1852.

121. Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *Circulation* 1999; **99**: 1156–1160.

122. Selhub J, Jacques PF, Bostom AG *et al*. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995; **332**: 286–291.

*123. Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 5–7.

124. Heinecke JW, Rosen H, Sukuki LA, Chait A. The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem* 1987; **262**: 10098–10103.

125. Eberhardt RT, Forgione MA, Cap A *et al*. Endothelial dysfunction in a murine model of mild hyperholocyst(e)inemia. *J Clin Invest* 2000; **106**: 483–491.

126. Lavie L, Perelman A, Lavie P. Plasma homocysteine levels in obstructive sleep apnea: association with cardiovascular morbidity. *Chest* 2001; **120**: 900–908.

127. Joannides R, Haefeli WE, Linder L *et al*. Nitric oxide is responsible for flow dependent dilation of human peripheral conduit arteries *in vivo*. *Circulation* 1995; **91**: 1314–1319.

128. Celermajer DS, Sorensen KE, Gouch VM *et al*. Non invasive detection of endothelial dysfunction in children and in adults at risk of atherosclerosis. *Lancet* 1992; **340**: 1111–1115.

129. 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.

130. Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling: association with the severity of apnea-induced hypoxaemia during sleep. *Chest* 2001; **119**: 1085–1091.

GLOSSARY OF TERMS

AP-1: activator protein-1; CAD: cardiovascular diseases; ECs: endothelial cells; EPO: erythropoietin; ET-1: endothelin-1; H/R: hypoxia/reperfusion or hypoxia/reoxygenation; H_2O_2: hydrogen peroxide; Hcy: homocysteine; HIF-1: hypoxia inducible factor-1; HSP70: heat shock protein 70; ICAM-1: intracellular adhesion molecule-1; NFκB: nuclear factor kappa B; NO: nitric oxide; NOS: nitric oxide synthase; $O_2^{\bullet-}$: superoxide anions; $OH^{\bullet}$: hydroxyl radical; $OONO^{\bullet}$: peroxynitrite; OSA: obstructive sleep apnoea syndrome; Ox-LDL: oxidized LDL; PMA: phorbol myristate acetate; ROS: reactive oxygen species; VCAM-1: vascular cell adhesion molecule -1; VEGF: vascular endothelial growth factor.