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SERIES "THE GENETIC AND CARDIOVASCULAR ASPECTS OF OBSTRUCTIVE SLEEP APNOEA/HYPOPNOEA SYNDROME"
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Cardiovascular disease in obstructive sleep apnoea syndrome: the role of intermittent hypoxia and inflammation

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ABSTRACT: **There is increasing evidence that intermittent hypoxia plays a role in the development of cardiovascular risk in obstructive sleep apnoea syndrome (OSAS) through the activation of inflammatory pathways. The development of translational models of intermittent hypoxia has allowed investigation of its role in the activation of inflammatory mechanisms and promotion of cardiovascular disease in OSAS. There are noticeable differences in the response to intermittent hypoxia between body tissues but the hypoxia-sensitive transcription factors hypoxia-inducible factor-1 and nuclear factor-κB appear to play a key role in mediating the inflammatory and cardiovascular consequences of OSAS. Expanding our understanding of these pathways, the cross-talk between them and the activation of inflammatory mechanisms by intermittent hypoxia in OSAS will provide new avenues of therapeutic opportunity for the disease.**

KEYWORDS: **Cardiovascular disease, inflammation, hypoxia, nuclear factor-κB, obstructive sleep apnoea syndrome, review**

Obstructive sleep apnoea syndrome (OSAS) is a highly prevalent disorder characterised by repetitive upper airway obstruction during sleep that leads to intermittent hypoxia, sleep fragmentation and excessive daytime sleepiness. Over the past 30 yrs our understanding of the features and consequences of this sleep-disordered breathing has progressed significantly and it is now recognised as a major healthcare problem affecting $\geqslant$4% of males and $\geqslant$2% of females in the developed world [1]. Given the modern pandemic of obesity in Western society, the prevalence of this disorder will probably continue to rise.

Recent studies have shown that OSAS is associated with an increase in all-cause and cardiovascular mortality, complementing existing evidence that OSAS has a causal relationship in the development of cardiovascular disease [2, 3]. The data are strongest for systemic arterial hypertension, with a number of large population-based studies showing an association between OSAS and development of systemic hypertension, independent of confounding factors such as sex, age and obesity [4, 5]. There are also studies supporting an independent association with ishaemic heart disease, atrial fibrillation, stroke and heart failure, and long-term follow-up studies of OSAS patients effectively treated with continuous positive airway presure (CPAP) have shown a significant benefit in reducing cardiovascular mortality and nonfatal cardiovascular events [6–11].

The pathogenesis of cardiovascular disease in OSAS is not completely understood, but is thought to be multifactorial in origin [12]. Proposed mechanisms by which OSAS predisposes to cardiovascular disease include sympathetic excitation, vascular endothelial dysfunction and metabolic dysregulation, as well as oxidative stress and inflammation induced by cyclical

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intermittent hypoxia [13]. There is increasing evidence that intermittent hypoxia plays a role in the pathobiology of cardiovascular complications in OSAS through activation of pro-inflammatory pathways (fig. 1) [14]. The development of cell culture and animal models of intermittent hypoxia in recent years have allowed investigation of the role of intermittent hypoxia in the activation of inflammatory mechanisms and development of atherosclerosis in OSAS.

The present manuscript highlights the role of intermittent hypoxia in the pathophysiology of cardiovascular complications in OSAS through activation of pro-inflammatory pathways. The hypoxia-sensitive transcription factors that probably contribute to the inflammatory and cardiovascular consequences of intermittent hypoxia are discussed. There is a review of the data garnered from translational studies involving cell culture and animal models of intermittent hypoxia, which complement data from studies of OSAS patients. The effects of intermittent hypoxia in specific tissue types are explored, with the aim of identifying the target organs of intermittent hypoxia in OSAS. The therapeutic potential of targetting inflammatory mechanisms in the treatment of OSAS is also reviewed.

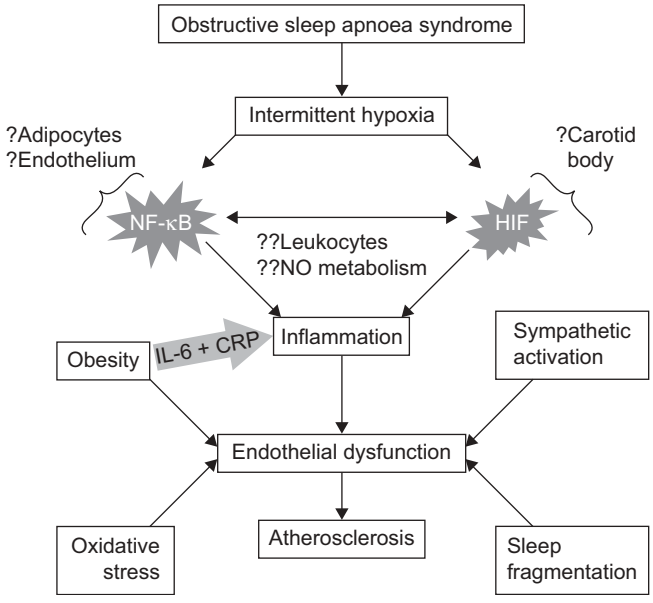

FIGURE 1. Activation and interaction of inflammatory pathways in response to intermittent hypoxia in obstructive sleep apnoea syndrome (OSAS). Proposed mechanisms by which OSAS predisposes to the development of endothelial dysfunction and cardiovascular disease include sympathetic excitation, vascular endothelial dysfunction, oxidative stress and inflammation. Intermittent hypoxia, the characteristic feature of OSAS, activates inflammatory mechanisms directly through the hypoxia-sensitive transcription factors nuclear factor (NF)-κB and hypoxia-inducible factor (HIF)-1. The co-existence of obesity in many OSAS patients augments the pro-inflammatory state through increased production of interleukin (IL)-6 and C-reactive protein (CRP) by adipose tissue. There are differences in the response to intermittent hypoxia between body tissues, with NF-κB having its largest apparent influence in endothelial cells, and adipocytes and HIF-1 playing a key role in the carotid body response. Activation of leukocytes and nitric oxide (NO) involvement in the inflammatory response to intermittent hypoxia in OSAS may be regulated by cross-talk between the NF-κB and HIF-1 pathways.

PATTERNS OF INTERMITTENT HYPOXIA

OSAS is characterised by a unique form of intermittent hypoxia, with short, repetitive cycles of hypoxia and reoxygenation. However, patterns of cyclical intermittent hypoxia are not uniform across all patients with OSAS or in translational models of the disease. For example, rapid eye movement (REM) sleep is associated with longer apnoeas and greater apnoea-related desaturation than non-REM sleep [15], which may predispose some patients to having periods of sustained hypoxia in REM sleep, during which oxygen saturation fails to return to baseline in between apnoeas. This is an important point for consideration when examining the pathobiological effects of OSAS, particularly in translational studies, as prolonged periods of sustained hypoxia allow for development of an adaptive response aimed at increasing tissue perfusion and oxygenation, whereas shorter intermittent hypoxic exposures may preferentially activate inflammatory pathways [16].

A number of studies have indicated such an adaptive response in patients with more severe OSAS-related nocturnal hypoxaemia. CAHAN and co-workers [17, 18] noted a marked increase in the variability of erythropoietin (EPO) levels in OSAS patients and suggested that mean EPO levels are in part related to the presence of OSAS, the degree of hypoxaemia, and body weight. WINNICKI *et al.* [19] showed similar EPO levels in patients with OSAS and healthy controls, but EPO levels increased in patients with severe OSAS during sleep and were decreased with CPAP therapy. EPO levels remained stable in patients with mild disease throughout the night. IMAGAWA *et al.* [20] found an increase in haemoglobin concentration, an increase in serum EPO levels and a marked increase in serum vascular endothelial growth factor (VEGF) levels in patients with severe OSAS. SCHULZ *et al.* [21] showed that OSAS patients with severe nocturnal hypoxia had markedly increased VEGF serum levels compared with OSAS patients presenting with moderate hypoxia and the control subjects. LAVIE *et al.* [22] showed a similar elevation in VEGF concentration in sleep apnoea patients with a mean apnoea/hypopnoea index of 53.4 events·h^{-1}.

The induction of protective responses in a subset of OSAS patients may also partly explain the decline in mortality risk associated with OSAS in those aged >50 yrs [23]. In the largest study to date, excess mortality was associated with OSAS only in males aged <50 yrs [24]. This trend was apparent even in patients with severe OSAS (respiratory disturbance index >50), the majority of whom were also obese with a mean body mass index (BMI) of 32.8 kg·m^{-2}. Similar findings have been reported in other mortality studies in OSAS [25–27]. Although the cyclical intermittent hypoxia that characterises OSAS may not provide sufficient hypoxic stimulus to induce cardioprotection through ischaemic preconditioning, periods of sustained hypoxia during REM sleep in a subset of OSAS patients, as described above, at least theoretically, have the scope to induce such a phenomenon.

A variety of cell culture, animal and human models of intermittent hypoxia have been developed as tools to investigate its role in the pathophysiology of OSAS [28]. However, despite seeking to mimic the cyclical variation in oxygenation seen in OSAS, models vary in terms of duration of hypoxic exposure, the number of hypoxic episodes and the degree of

hypoxia experienced. Therefore, it is important to note that these are generally models of intermittent hypoxia rather than models of OSAS. Cell culture models are relatively low cost, are not labour intensive and allow investigation of multiple cell types, but the severity of hypoxia is usually far greater than that experienced in OSAS. Animal models better imitate the pattern of intermittent hypoxia in OSAS and the use of transgenic animals allows investigation of the possible effects of specific genes in the pathophysiology of the disorder. However, hypoxic events in animal models are usually not linked to sleep state and are not accompanied by sleep fragmentation and the arousals that are typical in OSAS. Most animal models are based on systems that cyclically alter the fraction of inspired oxygen to mimic the intermittent hypoxia in OSAS, and are therefore not accompanied by upper airway occlusion and the changes in intrathoracic pressure associated with apnoea. Nonetheless, translational models of intermittent hypoxia are now essential tools in the investigation of disease mechanisms specific to OSAS, and complement clinical studies which are often confounded by the presence of comorbidities in the patient population.

INFLAMMATION IN OSAS: CYTOKINES AND INFLAMMATORY CELLS

The evidence for a direct role of OSAS in the regulation of inflammation is strongest for tumour necrosis factor (TNF)-α. Several studies have demonstrated elevated TNF-α levels in OSAS patients and, although both T-cells and monocytes have been identified as potential sources in OSAS patients, it should be remembered that the endothelium and adipose tissue can also secrete TNF-α. A four-fold increase in the percentage of γδ T-cells containing intracellular TNF-α was observed by DYUGOVSKAYA *et al*. [29]. MINOGUCHI *et al*. [30] showed that spontaneous production of TNF-α by monocytes and serum levels of TNF-α are elevated in patients with moderate-to-severe OSAS and that these levels are decreased by CPAP therapy. VGONTZAS *et al*. [31] found that TNF-α levels were highest among patients with OSAS when compared with other subjects with conditions associated with excessive daytime somnolence (EDS), including narcolepsy and idiopathic hypersomnolence. It was proposed that TNF-α may mediate sleepiness in conditions of EDS, and a significant and marked decrease in objective sleepiness was subsequently demonstrated in eight obese OSAS patients following treatment with the TNF-α antagonist etanercept [32]. The propensity of TNF-α to mediate sleepiness was also observed in the largest study to date, by RYAN *et al*. [33], who showed that TNF-α levels were higher in sleepy nonapnoeic snorers compared with normal control subjects, but still lower than in subjects with OSAS. CPAP was also found to significantly lower TNF-α levels. Similarly, levels of interleukin (IL)-8 showed similar differences between groups and also fell after CPAP therapy. Importantly, all subjects in the study were matched in terms of age, BMI, blood pressure and lipid profile, were free of cardiovascular comorbidity and were not taking any medications. The inability of other studies to find similar elevations of TNF-α and IL-8 levels in OSAS patients may be accounted for by the failure to control for confounding factors in this manner [34]. It should also be noted that, in the OSAS group, oximetry recordings during polysomnography demonstrated frequent transient oxygen desaturations during apnoeas with resaturation to normal levels in the periods between apnoea.

There is also evidence supporting the activation of circulating leukocytes in OSAS patients. Several studies have demonstrated upregulation of leukocyte adhesion molecule expression in OSAS [29, 35–39]. This increased expression of adhesion molecules is associated with increased avidity toward endothelial cells and a subsequent enhancement in inflammation-mediated endothelial dysfunction. It has been shown that neutrophil apoptosis is delayed in OSAS patients, further augmenting their inflammatory potential [35].

In brief, intermittent hypoxia may directly promote cytokine production and inflammatory cell activation in OSAS patients. It should be noted, however, that only one study to date has shown a direct association between this inflammatory activation and vascular change [40]. The potential roles of two hypoxia-sensitive transcription factors in the regulation of this inflammatory response will be discussed in the following section.

HYPOXIA-RESPONSIVE TRANSCRIPTION FACTORS IN OSAS

Nuclear factor-κB

The eukaryotic transcription factor nuclear factor (NF)-κB is a key mediator of the inflammatory response. NF-κB is composed of members of the Rel family of proteins and was first described as a nuclear factor necessary for immunoglobulin κ light chain transcription in B-cells, hence its name [41]. NF-κB exists in most cells in an inactive form bound to the inhibitor, IκB, which retains it in the cytoplasm. IκB is targeted for ubiquitin-mediated degradation upon sensation of an appropriate endogenous or exogenous inflammatory stimulus [42]. NF-κB is released from IκB and translocates to the nucleus, where it can upregulate transcription of specific pro-inflammatory genes responsible for encoding of inflammatory cytokines, chemokines and surface adhesion molecules. NF-κB plays a central role in the inflammatory response and orchestrates expression of a range of factors, including cytokines (TNF-α, IL-6 and IL-8), adhesion molecules (intercellular adhesion molecule-1) and enzymes (cyclo-oxygenase-2) [43–45].

There is evidence in the literature supporting an association between intermittent hypoxia in cell culture and animal models, and OSAS in humans, with increases in NF-κB activity and its downstream products, particularly TNF-α. In an *in vitro* model using HeLa cells and bovine aortic endothelial cells, intermittent hypoxia activated NF-κB in a dose-dependent manner [16, 46]. In another study, serum TNF-α levels were measured in OSAS patients and compared with levels in age-, sex- and BMI-matched controls [33]. Serum TNF-α levels were higher in OSAS patients compared with controls and reverted to control levels after 6 weeks of CPAP therapy. Similarly, in a mouse model, exposure to intermittent hypoxia activated NF-κB in cardiovascular tissue with a concomitant increase in expression of inducible nitric oxide synthase (iNOS) protein, a recognised NF-κB-dependent gene product. OSAS patients were also found to have markedly elevated monocyte NF-κB activity that decreased significantly with CPAP therapy [43]. The same group have also demonstrated increased NF-κB activity in circulating neutrophils and raised plasma levels of

the NF-κB-controlled gene products, soluble E-selectin and soluble vascular cell adhesion molecule-1 in OSAS patients, with a reduction in NF-κB activity to control levels following CPAP therapy [47].

Hypoxia-inducible factor-1

Hypoxia-inducible factor (HIF)-1 is a heterodimeric transcription factor consisting of a constitutively expressed β-subunit and an α-subunit that contains an oxygen-dependent degradation (ODD) domain [48]. Under normoxic cellular conditions, the ODD domain is hydroxylated in an oxygen-dependent manner, rendering the α-subunit vulnerable to proteasomal degradation [49]. Therefore, HIF-1 is suppressed in normoxia. However, in hypoxia, HIF-1 is stable and active, capable of binding to the regulatory regions of its target genes and inducing their expression.

HIF-1 is the major regulator of oxygen homeostasis within the cell, affecting and regulating dozens of genes as cellular oxygen concentrations change. In general, such factors allow an adaptation to hypoxia that is directed towards increasing tissue perfusion and oxygenation and, hence, overcoming the initial hypoxic insult. In normoxia, most cells produce ATP *via* oxidative phosphorylation and HIF-1 regulates the shift to increased glycolysis and anaerobic metabolism at low oxygen tensions [50]. By binding to the hypoxia response element in the EPO gene, HIF-1 activates its transcription, increasing red blood cell production and enhancing blood oxygen-carrying capacity [51]. Through VEGF transcription, HIF-1 regulates migration of mature endothelial cells towards hypoxic areas of tissue, thereby promoting angiogenesis [52]. Elevated serum levels of HIF-1 gene products, such as EPO and VEGF, have been demonstrated in OSAS patients, particularly patients with severe nocturnal hypoxaemia [17–20]. Cell culture experiments have also shown upregulation of HIF-1 in intermittent hypoxia [53, 54].

However, not all HIF-1-mediated effects are protective. HIF-1 promotes enhanced survival of myeloid inflammatory cells, such as granulocytes, monocytes and macrophages, resulting in their functional longevity and potentiation of inflammation [55]. Therefore, HIF-1 may also be viewed as a pro-inflammatory contributor to the hypoxic response by promoting inflammatory cell survival. Delayed neutrophil apoptosis has recently been demonstrated in OSAS patients; however, the mechanisms that underlie this response remain unexplained [35].

Interaction between the NF-κB and HIF-1 pathways

It has become clear that, as well as having independent roles in the regulation of gene expression in response to hypoxia, there is also significant crosstalk between NF-κB and HIF-1, resulting in a mutual dependence between the pathways (fig. 2.) There is an active NF-κB binding site contained in the proximal promoter site of the HIF-1 gene and NF-κB regulates basal levels of HIF-1 gene expression [56, 57]. Hypoxia up-regulates HIF-1 transcription through a NF-κB-dependent mechanism [58]. Conversely, HIF-1 can also influence the NF-κB pathway. WALMSLEY *et al.* [59] showed that the hypoxic induction of NF-κB transcription is dependent on the presence of HIF-1 and that HIF-1 is directly involved in regulating neutrophil survival in hypoxia through the modulation of NF-κB signalling. Overexpression of HIF-1 results in increased NF-κB activity and an enhanced inflammatory response [60]. HIF-1 and NF-κB also share some common gene products. For example, nitric oxide is a potent vasodilator, and both HIF-1 and NF-κB can enhance its bioavailability through increased expression of iNOS [61, 62]. In summary, it is likely that cross-talk between NF-κB and HIF-1 plays a central but complex role in modulating the inflammatory response to intermittent hypoxia in OSAS [63].

DIFFERENTIAL RESPONSES TO INTERMITTENT HYPOXIA IN SPECIFIC TISSUE TYPES

Intermittent hypoxia and carotid bodies

A number of physiological responses to hypoxia have been shown to depend on the oxygen-sensing ability of the peripheral arterial chemoreceptors, particularly the carotid bodies. For example, the role of peripheral arterial chemoreceptors in modulating ventilatory acclimatisation to altitude is well recognised [64]. Little is known about the effect of intermittent hypoxia on the carotid body in the context of OSAS. Physiological responses to intermittent hypoxia as it relates to altitude training differ significantly from responses to the characteristic cycles of intermittent hypoxia in OSAS [65]. The propensity for development of periodic breathing during sleep with increasing altitude is well recognised, but it should be acknowledged that normoxia generally follows apnoea at sea level in OSAS, whereas hypoxia persists after apnoea at high altitude [66]. In OSAS, the duration of hypoxic exposure is short but hypoxic episodes occur very frequently throughout the night. OSAS effects a chronic low-grade inflammatory state associated with endothelial dysfunction, whereas acclimatisation to altitude is associated with a protective effect on the vasculature, lowering blood pressure and reducing cardiovascular mortality [67, 68].

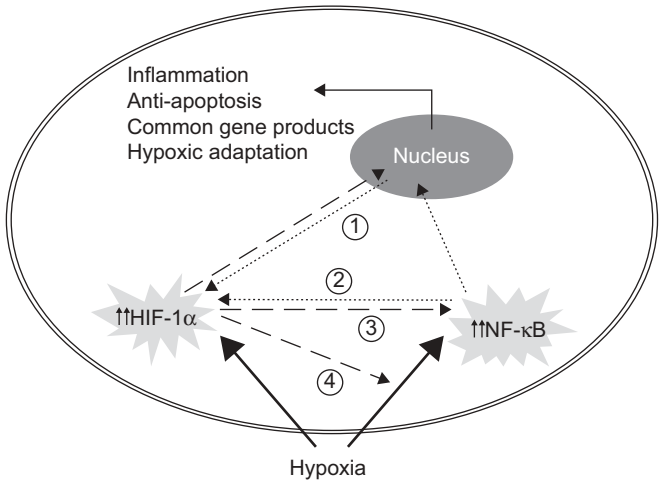

FIGURE 2. Interaction between hypoxia-inducible factor (HIF)-1α and nuclear factor (NF)-κB in hypoxia. Tissue hypoxia leads to the activation of the transcription factors HIF-1α and NF-κB. Activation of HIF-1α facilitates an adaptive response to hypoxia, whereas upregulation of NF-κB leads to inflammatory and anti-apoptotic gene expression. HIF-1α and NF-κB also share some gene products, *e.g.* inducible nitric oxide synthase. NF-κB regulates basal levels of HIF-1 gene expression and upregulation of HIF-1 transcription occurs through a NF-κB-dependent mechanism (1 and 2). Conversely, hypoxic induction of NF-κB transcription is dependent on the presence of HIF-1α (3) and HIF-1α is directly involved in regulating apoptosis through the modulation of NF-κB signalling (4).

Thus, although a substantial body of data exists on the effects of sustained or prolonged periods of hypoxia on the carotid body, its relevance to OSAS largely remains to be determined [69]. It has been shown, however, that patients with OSAS have an exaggerated peripheral chemoreflex response to hypoxia, independent of the effect of obesity [70–72]. This may contribute to enhanced sympathetic activity, increased blood pressure and decreased baroreflex sensitivity in OSAS patients [73].

In vitro studies

There is a growing body of evidence supporting an essential role for the transcription factor HIF-1α in oxygen sensing by the carotid body [74]. YUAN and co-workers [53, 54] showed that stabilisation of HIF-1α occurs as a result of the increased generation of reactive oxygen species by reduced nicotinamide adenine dinucleotide phosphate oxidase in an *in vitro* model of intermittent hypoxia using PC12 rat phaeochromocytoma cells (30 s of hypoxia alternating with 4 min of reoxygenation). These cells share similar properties with carotid body glomus cells, including expression of tyrosine hydroxylase, the rate-limiting enzyme for catecholamine production and oxygen-related neurotransmitter release. The increase in HIF-1α was dependent on the number of cycles of intermittent hypoxia and, although HIF-1α is prone to rapid degradation upon reoxygenation, its degradation after 4 min of oxygenation was not detected. BERRA *et al.* [75] showed similar results with persistence of HIF-1α after 5 min of reoxygenation in CCL39 cells, an established line of lung fibroblasts.

In vivo studies

The most robust data supporting a role for HIF-1α in oxygen sensing by the carotid body come from mice partially deficient in HIF-1α expression. Complete HIF-1α deficiency is lethal at the embryonic stage of development, but *hif1a*$^{+/-}$ heterozygous (HET) mice develop normally [76]. Carotid body-mediated cardiorespiratory changes in response to intermittent hypoxia are absent or markedly attenuated in HET mice [77]. The impaired physiological responses to intermittent hypoxia in HET mice are associated with the absence of HIF-1α upregulation and increased reactive oxygen species generation. HET mice do not develop hypertension in response to intermittent hypoxia in contrast to their wild-type litter-mates who develop hypertension and show increased HIF-1 expression within their carotid bodies [78].

LAM *et al.* [79] recently demonstrated increased rat carotid body expression of the HIF-2α and HIF-3α subtypes in response to intermittent hypoxia, in contrast to the increase in the HIF-1α subtype seen in sustained hypoxia. The authors concluded that differential regulation of the HIF-α subtypes could account for the differences in carotid body responses to intermittent and sustained hypoxia.

A number of studies involving various animal models of intermittent hypoxia have examined its effect on carotid body function. FLETCHER and co-workers [80, 81] developed a rat model of intermittent hypoxia similar to the pattern seen in OSAS (20 s of 5% inspiratory oxygen fraction; 9 episodes·h^{-1}; 8 h·day^{-1}; 30 days) in which the animals developed hypertension and increased sympathetic nerve activity following exposure to intermittent hypoxia, and obliteration of this response occurred following denervation of the carotid bodies. REY *et al.* [82] have shown a reduction in baroreflex sensitivity in normotensive cats similar to that seen in OSAS patients following stimulation of the carotid bodies by intermittent hypoxia. PAWAR *et al.* [83] have demonstrated differences in carotid body responses to intermittent hypoxia between neonatal and adult rats. The data obtained by PAWAR *et al.* [83] suggested that neonatal carotid bodies are more sensitive to intermittent hypoxia than adult carotid bodies and that intermittent hypoxia stimulates glomus cell hyperplasia in neonatal carotid bodies. In contrast to adult carotid bodies, hypoxic sensitisation was not reversed in neonatal carotid bodies after re-exposure to normoxia.

In brief, the carotid body plays an important role in the perception of oxygen tension in intermittent hypoxia. The subsequent responses to intermittent hypoxia mediated by the carotid body differ to those provoked by sustained hypoxia and appear to be largely dependent on HIF-1α. Further work is required in this area, however, to define the downstream molecular mechanisms involved in the pathogenesis of cardiovascular comorbidities associated with intermittent hypoxia.

Intermittent hypoxia and endothelial cells

In vitro studies

A limited number of studies have examined the effects of intermittent hypoxia in endothelial cell culture models. RYAN *et al.* [46] showed selective activation of NF-κB in bovine aortic endothelial cells in response to intermittent hypoxia and proposed that this activation was dependent on p38 map kinase activation using data from a similar HeLa cell *in vitro* model. In contrast, intermittent hypoxia did not lead to significant HIF-1 activation, while sustained hypoxia led to a robust HIF-1 response.

Although HIF-1α is unstable in normoxia (and consequently during the reoxygenation phase of intermittent hypoxia), there is evidence to support modulation of HIF-1α expression in endothelial cells by intermittent hypoxia *in vitro*. However, it should be noted that these studies investigated the effects of intermittent hypoxia in the context of vascularised tumours rather than OSAS and, therefore, their relevance to OSAS is unknown. TOFFOLI *et al.* [84] showed a progressive increase in HIF-1α during the hypoxic phases of intermittent hypoxia (1 h of 1% O_2 alternating with 30 min 20% O_2) in two endothelial cell lines, EAhy926 and human microvascular endothelial cell-1. As HIF-1α was degraded during the normoxic phase of these cycles, it appears that enhanced HIF-1α stabilisation occurred with intermittent hypoxia in each hypoxic period, although the mechanism of this stabilisation is not apparent. This observation has also been made in human umbilical vein endothelial cells [85]. Progressive increases in the phosphorylated form of HIF-1α also occurred in endothelial cells in a protein kinase (PK)A-dependent manner in response to intermittent hypoxia, and HIF-1α transcriptional activity was reduced by PKA inhibition [84]. The physiological relevance of this progressive HIF-1α phosphorylation remains unclear.

Clinical studies

The endothelium forms an ~1-kg functional organ in the adult human and its position at the interface of tissues and circulating blood makes it readily susceptible to the effects of intermittent hypoxia [86]. The endothelium shows little evidence of disturbance to its integrity in the absence of disease. If specifically looked for, apoptotic endothelial cells

are rarely found in the intima of normal blood vessels [87]. It is proposed that apoptotic cells play an early role in the development of endothelial dysfunction and atherosclerosis and known cardiac risk factors increase endothelial apoptosis *in vitro* and *in vivo* [86]. Apoptotic endothelial cells have also been shown in experimental and human atherosclerotic plaques [87–89]. These cells also release IL-1 which can activate NF-κB in neighbouring endothelial cells, thereby increasing pro-inflammatory cytokine production (TNF-α and IL-8) and promoting leukocyte adhesion molecule expression and release [91].

EL SOLH *et al*. [91] demonstrated that OSAS patients have increased number of circulating apoptotic endothelial cells compared with non-OSAS controls, and that levels correlated with abnormal brachial artery flow-mediated dilation, a marker of endothelial dysfunction. The number of apoptotic endothelial cells in the circulation was lowered by therapy with nasal CPAP. However, contrasting results were reported in another recent study [92].

There is evidence that circulating endothelial progenitor cells (EPCs) derived from bone marrow are involved in the repair of damaged blood vessels and decreased levels of EPCs have been found to be predictive of future cardiovascular events in coronary artery disease. While lower numbers of EPCs have been noted in OSAS patients [93, 94], one study has shown no statistical difference in either circulating endothelial cells or circulating EPCs in a group of OSAS patients, free of pre-existing vascular disease, compared with age- and weight-matched controls [92].

Intermittent hypoxia and white adipose tissue

Qualifying the mechanisms which underlie the relationship between OSAS and the pathogenesis of cardiovascular disease is difficult in OSAS patient populations, owing to the extensive range of co-existing recognised cardiovascular risk factors, in particular obesity. There is a two-fold increase in the risk of developing OSAS with every 10 kg increase in body weight and a four-fold increase in risk with every 6 $kg \cdot m^{-2}$ increase in BMI [95]. In recent years, white adipose tissue has emerged as a major secretory organ, to such an extent that, in the obese, adipose tissue is often the largest endocrine organ. Obesity induces a chronic low-grade inflammatory state and many of the inflammatory pathways proposed to be activated by intermittent hypoxia in OSAS are also activated in adipose tissue. Adipose tissue expresses high levels of inflammatory cytokines, such as TNF-α and IL-6 [96–98]. Induction of these inflammatory cytokines in adipose tissue can also have further downstream metabolic effects often associated with OSAS. For example, changes in TNF-α associated with obesity can induce subsequent changes in insulin sensitivity and glucose homeostasis [99].

Rather than being mutually exclusive contributors to systemic inflammation, OSAS-related intermittent hypoxia may actually modify inflammatory cytokine production by white adipose tissue. It has been hypothesised that white adipose tissue is poorly oxygenated in the obese and that relative hypoxia develops within groups of adipocytes that have become distant from the vasculature with growth of the adipose tissue mass, leading to dysregulation of inflammatory adipokine production [100, 101]. However, the limited number of studies to date exploring this hypothesis have focussed mainly on the effects of sustained hypoxia rather than intermittent hypoxia. As obesity is closely associated with OSAS, intermittent hypoxia may be of greater relevance to adipokine metabolism than sustained hypoxia, although further studies are required in this area.

In vitro/in vivo studies

A number of studies have examined adipokine production in OSAS but, as mentioned previously, there is a paucity of translational studies examining the effects of intermittent hypoxia *per se* on adipose tissue. Adiponectin (APN), the most abundant adipokine, is secreted exclusively by adipocytes, possesses anti-atherogenic properties, and low levels are associated with increased cardiovascular risk. Levels of APN are inversely correlated with the proportion of body fat in adults. 3T3-L1 adipocytes exposed to intermittent hypoxia for 48 h had a significant decrease in the secretion of total and high-molecular-weight APN compared with control adipocytes maintained in 21% O_2 under identical conditions [102]. NAKAGAWA *et al*. [103] demonstrated lower levels of circulating APN in patients with severe OSAS and sustained hypoxia reduced APN concentrations in C57BL/6J mice and 3T3-L1 adipocytes.

Clinical studies

Several other studies have demonstrated lower levels of APN in OSAS patients and subsequent improvement following CPAP therapy [104–110]. However, not all studies have shown an association between OSAS and APN [111–115]. WOLK *et al*. [116] showed that patients with OSAS had significantly higher plasma APN levels compared with non-OSAS BMI-matched controls, concluding that low APN levels are unlikely to account for the association between OSAS and cardiovascular disease.

It is also still unclear whether OSAS is independently associated with increased levels of the acute-phase reactant C-reactive protein (CRP). A number of studies have examined this question, demonstrating elevated levels of CRP associated with OSAS, but many have been limited by methodologies used, failing to properly control for BMI, and including patients with established cardiovascular and/or metabolic disease [117–120]. In contrast, when patients are group-matched for BMI, free of co-morbidities and of similar sex, age, smoking status and lipid profile, an independent relationship was identified between CRP levels and BMI, but not with severity of OSAS [121]. Therefore, in OSAS, the data suggest that CRP levels correlate with adipose tissue mass and obesity rather than OSAS, an observation readily supported by the mechanisms of CRP production.

CRP is synthesised mainly in the liver under the regulation of IL-6, although IL-1 and TNF-α can also induce hepatic CRP mRNA expression. However, CRP production may be augmented by adipose tissue in two ways. First, CRP production has also been demonstrated in adipose tissue, although more weakly than in hepatic tissue. However, adipose tissue forms a much larger organ than the liver in obese individuals and the contribution of adipose-derived CRP is, therefore, probably of greater significance in obesity than in normal subjects. Secondly, adipose tissue is a major contributor to IL-6 production in the body, thereby further enhancing hepatic

TABLE 1 Tissue-specific effects of intermittent hypoxia

Tissue	Mechanism	Effects
Carotid body	Oxygen sensing by HIF-1α	Enhanced sympathetic activity Increased blood pressure Decreased baroreflex sensitivity
Endothelium	Activation of NF-κB	Inflammatory cytokine production Surface adhesion molecule expression Increased apoptotic endothelial cells? Decreased circulating endothelial progenitor cells?
Adipose tissue	Activation of NF-κB	Dysregulation of adipokine production

HIF: hypoxia-inducible factor; NF-κB: nuclear factor-κB.

CRP production. Thus, although CRP levels are elevated in OSAS patients, this probably reflects obesity and increased adipose tissue mass, rather than an effect of intermittent hypoxia.

In summary, OSAS is associated with a low-grade inflammatory state and a role may also exist for OSAS-related intermittent hypoxia in the modulation of adipose tissue cytokine metabolism. There are also noticeable differences apparent in the response to intermittent hypoxia between body tissues (table 1). HIF-1 plays a key role in oxygen-sensing by the carotid bodies and subsequent carotid body-mediated cardiovascular responses. In contrast, NF-κB appears to be primarily responsible for inflammatory signalling by endothelial cells in OSAS. It is likely that NF-κB may also regulate inflammatory signalling in the adipose tissue of OSAS patients. A greater understanding of the mechanisms controlling these transcription factors and the cross-talk between them will provide new avenues of therapeutic opportunity for the condition.

THERAPEUTIC POTENTIAL

Currently, CPAP treatment is the mainstay of OSAS therapy. It effectively ameliorates the symptoms of the disease and reduces the associated long-term cardiovascular morbidity and mortality [10]. However, many patients stop using it because of discomfort and compliance rates vary [122, 123]. Current therapeutic alternatives to CPAP therapy are often suboptimal, particularly for patients with severe disease. Therefore, scope exists for the development and utilisation of therapeutic modalities directed toward the attenuation of inflammatory mechanisms in OSAS. VGONTZAS *et al*. [32] have already demonstrated that the TNF-α antagonist etanercept effectively reduced daytime sleepiness in a small cohort of OSAS patients. Research into agents which modulate the NF-κB and HIF-1 pathways may lead to novel treatment strategies and improved cardiovascular outcomes for OSAS patients in the future.

CONCLUSION

Obstructive sleep apnoea syndrome is a common medical disorder that is growing in prevalence worldwide. It is characterised by recurrent cycles of intermittent hypoxia and there is increasing evidence that intermittent hypoxia plays a role in the development of cardiovascular risk in obstructive sleep apnoea syndrome patients through the activation of inflammatory pathways. Scope exists for further studies demonstrating a direct linkage between inflammation and markers of atherosclerosis and cardiovascular disease in obstructive sleep apnoea syndrome, as currently only one study exists in the field [40]. The hypoxia-sensitive transcription factors hypoxia-inducible factor-1 and nuclear factor-κB appear to play a key role in mediating the inflammatory and cardiovascular consequences of the disease. Expanding our understanding of these pathways, the interaction between them and the potentiation of inflammation by intermittent hypoxia will yield novel therapeutic targets with the scope to reduce cardiovascular risk in obstructive sleep apnoea syndrome.

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