**Experimental Physiology**

# Intermittent hypoxia and vascular function: implications for obstructive sleep apnoea

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**Obstructive sleep apnoea (OSA) has been implicated as a risk factor for the development of hypertension, stroke and myocardial infarction. The main cause of cardiovascular and cerebrovascular disease in OSA is thought to be exposure to intermittent hypoxia, which can lead to oxidative stress, inflammation, atherosclerosis, endothelial dysfunction and hypertension. These proposed mechanisms have been drawn from basic research in animal and human models of intermittent hypoxia in addition to clinical investigation of patients with OSA. This review outlines the association between OSA and vascular disease, describes basic mechanisms that may be responsible for this association and compares the results from studies of OSA subjects with those in experimental models of intermittent hypoxia.**

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Obstructive sleep apnoea (OSA) is characteristically associated with repetitive oscillations in oxyhaemoglobin saturation ($S_{aO_2}$) during sleep, thus resulting in chronic exposure to intermittent hypoxia. This condition is thought to be responsible for many of the cardiovascular consequences of OSA, including systemic hypertension, myocardial infarction and stroke (Prabhakar, 2001; Lavie, 2003, 2005). Although the mechanism by which intermittent hypoxia leads to vascular disease in OSA is unknown, it has been proposed that sympathetic nervous system overactivity (Fletcher, 2001), oxidative stress (Lavie, 2003) and endothelial dysfunction (Lavie, 2003) contribute to it. All of these mechanisms are discussed in detail in the review.

Intermittent hypoxia has been shown to affect the control of breathing (Gozal & Gozal, 2001; Mitchell *et al.* 2001; Prabhakar, 2001; Mitchell & Johnson, 2003; Morris *et al.* 2003), the cardiovascular system (Tahawi *et al.* 2001; Phillips *et al.* 2004, 2005) and the autonomic nervous system (Morgan *et al.* 1995; Yasuma & Hayano, 2000; Cutler *et al.* 2004*b*). Recent initiatives in OSA research have included the development of experimental animal and human models of acute and chronic intermittent hypoxia to evaluate potential mechanisms for the association between OSA and vascular disease. The objectives of this review are to outline some of these basic mechanisms and to compare the results from studies involving OSA patients with those from experimental models of intermittent hypoxia.

## Obstructive sleep apnoea

Obstructive sleep apnoea is a chronic medical condition characterized by repeated episodes of apnoea during sleep (Young *et al.* 2002). Each apnoea characteristically lasts about 20 s and is terminated by an abrupt restoration of ventilation. Obstructive sleep apnoea occurs in 2% of middle-aged women and 4% of middle-aged men in the general population (Young *et al.* 1993) and its prevalence is much higher in specific high-risk patient groups, such as those with congestive heart failure (40%; Sin *et al.* 1999), end-stage kidney disease (50%; Hanly & Pierratos, 2001) and stroke (60%; Yaggi *et al.* 2005).

Apnoeas occur because of recurrent closure of the upper airway during sleep (Guilleminault *et al.* 1976). In the majority of patients, the pathogenesis of OSA involves both a structural and a functional abnormality of the pharynx. This has been extensively reviewed elsewhere (Fogel *et al.* 2004; Ryan & Bradley, 2005; White, 2005) and is not discussed here. However, it is important to stress that each cycle of apnoea and resumption of ventilation is accompanied by arterial oxyhaemoglobin desaturation and resaturation. Since most individuals with OSA typically resaturate their haemoglobin into the

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normal range, this exposes them to intermittent hypoxia throughout the night. The development of intermittent hypoxia and the physiological responses this evokes are thought to be responsible for the subsequent clinical manifestations of vascular disease (Lavie, 2005).

Obstructive sleep apnoea presents clinically with complaints of excessive daytime sleepiness, fatigue, irritability and deficits in attention and memory, and with nocturnal symptoms which may include a history of snoring, apnoeas, choking and frequent awakenings (Stierer & Punjabi, 2005). The diagnosis and severity of OSA are assessed by overnight, attended polysomnography, which involves the continuous recording of sleep and breathing. Sleep stages are determined from the electroencephalogram, the electro-oculogram and a submental electromyogram. Breathing is assessed by measuring respiratory effort (typically by monitoring movement of the chest and abdomen by inductance plethysmography), nasal airflow and $S_{aO_2}$. Additional monitoring includes snoring, body position and electrocardiography. Figure 1*A* shows a 2 min excerpt from a polysomnogram of a patient with OSA. There are standardized criteria to score an apnoea and hypopnoea, from which the apnoea/hypopnoea index (AHI) is derived (American Academy of Sleep Medicine, 1999). An apnoea is defined as the absence of airflow for 10 s or more. An

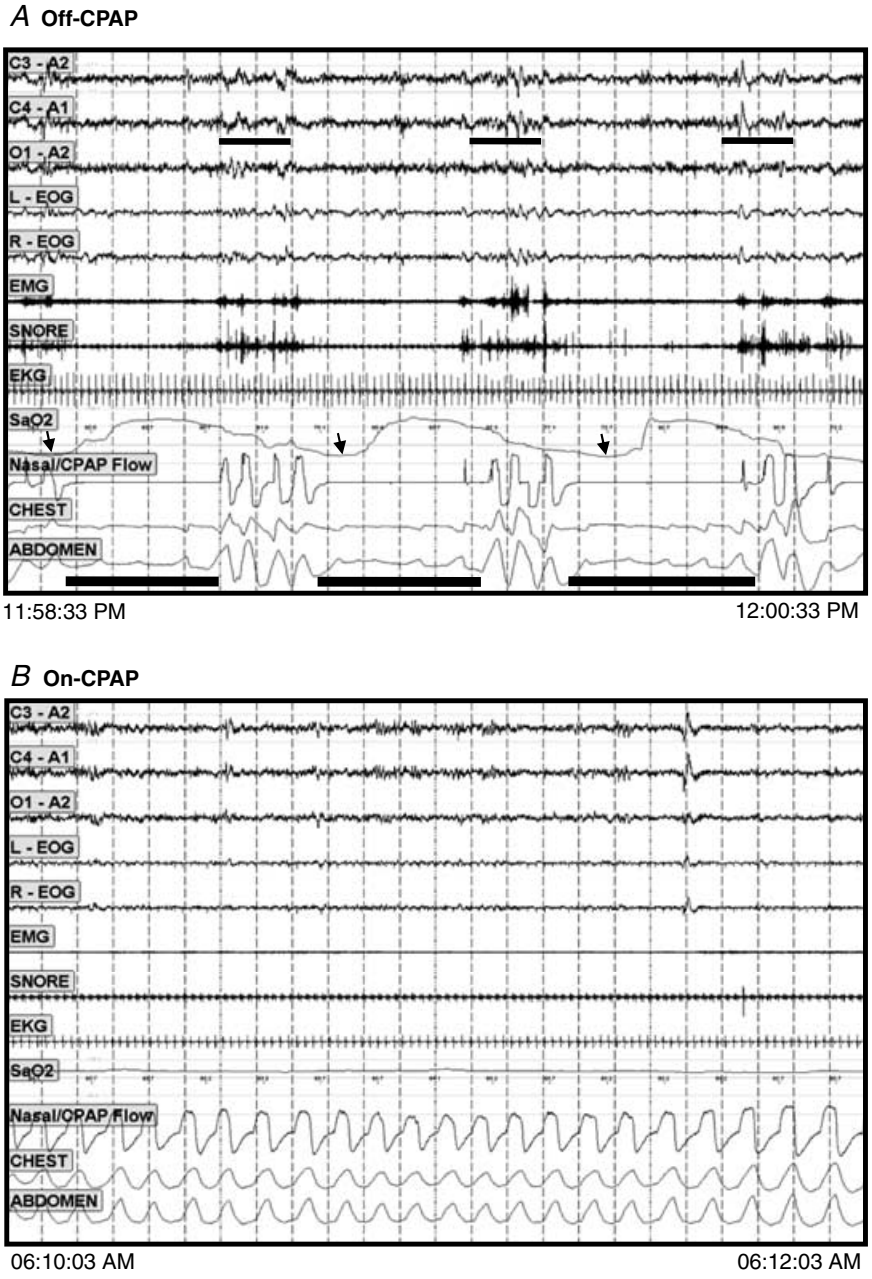


**Figure 1. Polysomnograph of a patient with OSA, off CPAP (*A*) and on CPAP (*B*)**
*A* shows a 2 min polysomnograph obtained from an untreated patient with OSA. Note periods of apnoea (indicated by long filled horizontal bars) where nasal flow ceases and chest and abdominal motion is paradoxical (i.e. opposite to each other), arterial oxyhaemoglobin desaturation (denoted by bold arrows), and EEG activation or arousal (indicated by short filled horizontal bars). Additional features on the figure include a relative bradycardia during apnoea followed by tachycardia at termination of apnoea [observed in electrocardiogram (EKG) channel] and the relative absence of sound during apnoea and loud snort at the end of apnoea when breathing resumes (observed in the SNORE channel). *B* displays a 2 min polysomnograph from the same patient treated with continuous positive airway pressure (CPAP) at a level of 8 $cmH_2O$. Note the correction of apnoeas, hypoxaemia, snoring and arousals from sleep. For both panels, the recordings from top to bottom are a 3-channel electroencephalogram (C3-A2; C4-A1; O1-A2), a left and right electro-oculogram (L-EOG; R-EOG), a submental electromyogram (EMG), snoring (SNORE), an electrocardiogram (EKG), pulse oximetry ($S_{aO_2}$), nasal pressure (Nasal/CPAP Flow), and chest and abdominal movements (Chest and Abdomen).

hypopnoea is defined as a 50% reduction in airflow for 10 s or more or a marked reduction in airflow associated with an arousal and/or a 4% decrease in $S_{aO_2}$. Apnoeas are classified as obstructive if there is evidence of continued effort to breathe, such as paradoxical movement of the chest wall and abdomen, and central if there is a transient loss or reduction of respiratory effort. The AHI indicates OSA severity and is defined as the number of apnoeas and hypopnoeas per hour of sleep. Obstructive sleep apnoea is typically defined as an AHI of 5 or more per hour (Stierer & Punjabi, 2005) and is further classified as mild (AHI 5–15), moderate (AHI 16–30) and severe (AHI > 30; American Academy of Sleep Medicine, 1999).

Once a diagnosis of OSA has been established, the choice of treatment is determined by a combination of factors, including the polysomnographic findings, the severity of associated symptoms and cardiovascular sequelae, the presence of comorbid disease such as respiratory failure or heart failure which may be exacerbated by OSA, and the individual patient's motivation to comply with therapy. The therapeutic options include conservative measures, such as weight reduction, avoidance of alcohol and sedatives at bedtime, sleeping in non-supine positions, and use of a dental appliance to pronate the mandible during sleep or use of continuous positive airway pressure (CPAP; Olson *et al.* 2005). Upper airway surgery in adults is usually reserved for the small number of patients who have an obvious and resectable structural abnormality, such as significantly enlarged tonsils (Li, 2003). Currently, CPAP is the most definitive treatment for OSA and involves the application of a positive pressure to the upper airway during sleep (Sullivan *et al.* 1981; Hirshkowitz & Sharafkhaneh, 2005). Most commonly administered through a nasal mask, the pressure is titrated to maintain a patent airway through which the individual breathes and maintains normal gas exchange. The use of CPAP provides an immediate and effective means to correct OSA and associated hypoxaemia (Fig. 1*B*) and therefore can be utilized in the investigation of its impact on the vascular system.

## Obstructive sleep apnoea and cardiovascular disease

The demonstration of strong epidemiological associations between OSA and vascular disease has led to the consensus that OSA is a risk factor for the development of hypertension (Morrell *et al.* 2000; Nieto *et al.* 2000; Peppard *et al.* 2000), myocardial infarction (Hung *et al.* 1990; Shahar *et al.* 2001) and stroke (Arzt *et al.* 2005; Yaggi *et al.* 2005). Data from the Wisconsin Sleep Cohort Study, which investigated a community-based population, was analysed prospectively for the association between OSA and hypertension (Peppard *et al.* 2000). The investigators found a dose–response relationship between OSA at baseline and the prevalence of hypertension four years later, which was independent of confounding factors such as weight, age, gender, and the consumption of alcohol and nicotine. Obstructive sleep apnoea is also associated with an increased risk for myocardial infarction. In a cross-sectional analysis of the Sleep Heart Health Study, the association between OSA and self-reported myocardial infarction was assessed in 6424 individuals from the general population (Shahar *et al.* 2001). In this study, the relative odds for heart failure, adjusted for confounding factors, were significantly elevated in patients with OSA (2.38), indicating an elevated risk for myocardial infarction. The odds ratios for coronary heart disease (1.58) and stroke (1.27) were also significantly increased. Finally, in a cross-sectional analysis of 1475 subjects from the general population, those subjects with an AHI greater than 20 had increased odds for stroke compared with those without OSA after adjustment for known confounding factors (Arzt *et al.* 2005). Yaggi *et al.* (2005) found similar results in a group of 1022 patients referred to a sleep clinic for assessment of sleep apnoea (697 had OSA). Obstructive sleep apnoea significantly increased the risk of stroke independently of other risk factors, including hypertension.

The strong relationship between OSA and cardiovascular disease has important clinical and public health relevance. The increased prevalence of hypertension, myocardial infarction and stroke increases both cardiovascular morbidity and mortality and the demand on healthcare resources. Observational cohort studies indicate that untreated patients with OSA have an increased risk of fatal and non-fatal cardiovascular events (Marin *et al.* 2005), a heightened risk of sudden cardiac death during the sleeping hours (Gami *et al.* 2005), and an increased risk of stroke or death from any cause (Yaggi *et al.* 2005). Effective treatment of OSA with CPAP may reduce this risk of cardiovascular disease (Marin *et al.* 2005).

## Experimental models of intermittent hypoxia

Models of intermittent hypoxia have been developed to mimic the pattern of hypoxaemia experienced by patients with OSA. A model of intermittent hypoxia that seeks to mimic OSA must emulate its characteristics. These were briefly described above and are described here in further detail. Patients with OSA have repeated apnoeas throughout the night, with each apnoea lasting a minimum of 10 s. However, the average duration of apnoea in a large sleep clinic population is about 20 s (O'Connor *et al.* 2000). Patients with moderate-to-severe OSA have an AHI greater than 30 events per hour of sleep (American Academy of Sleep Medicine, 1999). The

**Table 1. Models of intermittent hypoxia**

| | Subjects | Methodology | Hypoxic intensity | Hypoxia duration |
|---|---|---|---|---|
| A. Acute intermittent hypoxia | | | | |
| Animal models | | | | |
| Altay *et al.* (2004) | Swiss-Webster ND4 Mice | 12 × 30 s periods of apnoea every 5 min; $P_{CO_2}$ uncontrolled | $P_{O_2} = 40$ mmHg | 1 h |
| Human models | | | | |
| Xie *et al.* (2000) | Healthy men | 1 × 20 s period of hypoxia–hypercapnia per minute; $N_2$ and $CO_2$ added to produce an $S_{aO_2} = 80\%$ and $P_{CO_2} = +3$ to $+5$ mmHg | $S_{aO_2}$ nadir 80% | 20 min |
| Cutler *et al.* (2004*a*,*b*) | Healthy men and women | 1 × 30 s voluntary apnoea per minute; $P_{CO_2}$ uncontrolled | $S_{aO_2}$ nadir 80–85% | 20 min |
| Leuenberger *et al.* (2005) | Healthy men and women | 1 × 20 s voluntary end-expiratory apnoea per minute; $P_{CO_2}$ uncontrolled | $S_{aO_2}$ nadir 83.1 ± 1.2% | 30 min |
| Tamisier *et al.* (2005) | Healthy men and women | 6–10 breaths of 100% $N_2$ separated by 3–4 breaths of room air, producing 30–40 drops in $S_{aO_2}$ per hour; $P_{CO_2}$ uncontrolled | $S_{aO_2}$ nadir ∼85% | 2 h |
| B. Chronic intermittent hypoxia | | | | |
| Animal models | | | | |
| Allahdadi *et al.* (2005) | Sprague–Dawley rats | 20 cycles of 5% $O_2$–5% $CO_2$ $h^{-1}$ separated by 21% $O_2$–0% $CO_2$ | $S_{aO_2}$ nadir = ∼70% | 7 h $day^{-1}$; 14 days |
| Chen *et al.* (2005) | Sprague–Dawley rats | 1 × 60 s period of hypoxia every 2 min; $P_{CO_2}$ uncontrolled | Nadir 4–6% $O_2$ | 8 h $day^{-1}$; 5 days $week^{-1}$ for 5 weeks |
| Dunleavy *et al.* (2005) | Wistar rats | 2 × 15 s periods of hypoxia–hypercapnia per minute; $N_2$ and $CO_2$ added to produce nadir 6–8% $O_2$ and peak 10–14% $CO_2$ | Nadir $P_{O_2} = 55$–$65$ mmHg and peak $P_{CO_2} = 64$ mmHg | 8 h $day^{-1}$; 21 days |
| Fletcher *et al.* (1992*a*,*b*,*c*, 1995, 1999, 2002) Bao *et al.* (1997) Fletcher (2000) | Sprague–Dawley rats | 2 × 12 s periods of hypoxia per minute; $N_2$ added to produce nadir 3–5%$O_2$; $P_{CO_2}$ uncontrolled | $S_{aO_2}$ nadir = ∼70% (range 60–80%) | 8 h $day^{-1}$; 35 days |
| Greenberg *et al.* (1999) | Sprague–Dawley rats | 1 × 60 s period of hypoxia every 2 min; $N_2$ added to produce nadir 6.5–7% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 8 h $day^{-1}$; 30 days |
| Julien *et al.* (2003) | C57BL/6J mice | 2 × 6–7 s period of hypoxia per minute; $N_2$ added to produce nadir 3–5% $O_2$; $P_{CO_2}$ uncontrolled | $S_{aO_2}$ nadir 62–79% | 8 h $day^{-1}$; 14 days |
| Kanagy *et al.* (2001) | Sprague–Dawley rats | 90 s period of hypoxia–hypercapnia every 90 s; $N_2$ and $CO_2$ added to produce nadir 5% $O_2$ and peak 5% $CO_2$ | Hypoxic intensity not indicated | 8 h $day^{-1}$; 11 days |
| Lai *et al.* (2006) | Sprague–Dawley rats | 1 × 30 s period of hypoxia every 45 s; $N_2$ added to produce nadir 2–6% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 6 h $day^{-1}$; 30 days |
| Lefebvre *et al.* (2006) | Wistar rats | 1 × 40 s period of hypoxia per minute; air–$N_2$ mix used to achieve 5% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 8 h $day^{-1}$; 35 days |
| Peng & Prabhakar (2004) | Sprague–Dawley rats | 1 × 15 s period of hypoxia every 5 min; $N_2$ added to produce nadir 5% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 8 h $day^{-1}$; 10 days |
| Phillips *et al.* (2004, 2005) | Sprague–Dawley rats | 1 × 60 s period of hypoxia every 4 min; $N_2$ added to produce nadir 10% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 12 h $day^{-1}$; 14 days |
| Tahawi *et al.* (2001) | Sprague–Dawley rats | 2 × 12 s period of hypoxia per minute; $N_2$ added to produce nadir 2–3% $O_2$; $P_{CO_2}$ uncontrolled | Hypoxic intensity not indicated | 6–8 h $day^{-1}$; 35 days |
| Human models | | | | |
| Foster *et al.* (2005) | Healthy men | 1 × 5 min period of hypoxia every 10 min; air–$N_2$ mix used to achieve 12% $O_2$; $P_{CO_2}$ maintained at resting levels | $S_{aO_2}$ nadir ∼90% | 1 h $day^{-1}$; 12 days |

degree of associated hypoxia varies considerably between patients, but each apnoea is associated with arterial oxyhaemoglobin desaturation of at least 4% followed by resaturation to normal levels (American Academy of Sleep Medicine, 1999). The different models of intermittent hypoxia that have been used in animals and humans are discussed below. The considerable variation between them is summarized in Table 1.

**Animal models.** Generally, animal models of intermittent hypoxia are induced by alternating the inspired gas from short periods of hypoxia (20–60 s) to periods of normoxia (45–90 s; Fletcher *et al.* 1992*a*; Peng & Prabhakar, 2004; Phillips *et al.* 2004, 2005). During periods of hypoxia, the fraction of inspired oxygen ($F_{IO_2}$) ranges from 3 to 10%. Short-term intermittent hypoxia lasts for a few hours, whereas chronic intermittent hypoxia continues for 5–8 h per day over 14–35 days.

**Human models.** Models of intermittent hypoxia in healthy human subjects can be separated into short-term and chronic. Short-term intermittent hypoxia models generally use 30 s periods of hypoxia or voluntary apnoeas interspersed with normoxia for 20–60 min (Xie *et al.* 2000; Cutler *et al.* 2004*a*; Tamisier *et al.* 2005). Models of chronic intermittent hypoxia have exposed individuals to an hour of intermittent hypoxia (5 min hypoxia alternating with 5 min normoxia) daily for 2 weeks (Foster *et al.* 2005). In addition, some studies control the level of carbon dioxide (Foster *et al.* 2005), whereas others do not (Tamisier *et al.* 2005).

**Comparison of animal and human models of intermittent hypoxia.** Animal models of intermittent hypoxia generally mimic OSA better than human models for several reasons. Since the animals studied are smaller than humans, changes in $S_{aO_2}$ are more rapid and of greater magnitude. Healthy humans require longer periods of hypoxia to induce arterial oxyhaemoglobin desaturation and thus require longer cycling time. Animals can be safely exposed to chronic intermittent hypoxia without much supervision, while exposing humans to chronic intermittent hypoxia is much more labour intensive. Although data from experimental models of intermittent hypoxia appear to demonstrate similar physiological responses to those seen in OSA patients, these models are limited by the fact that many do not incorporate monitoring of sleep, are not accompanied by apnoea or sleep fragmentation, and do not include exposure to hypercapnia. Exposure to hypercapnia during intermittent hypoxia may not be critical, because the effect of intermittent hypoxia on diurnal blood pressure in rats is similar regardless of whether the level of carbon dioxide is increased or not (Fletcher *et al.* 1995). However, hypercapnic hypoxia does lead to greater sympathetic activation than hypocapnic hypoxia (Tamisier *et al.* 2004). Despite these limitations, intermittent hypoxia models are a useful tool for studying the progression of cardiovascular disease in OSA.

## Intermittent hypoxia and vasomotor function

Intermittent hypoxia alters vasomotor function. Specifically, chronic exposure to intermittent hypoxia attenuates vasodilator function and, in most cases (but not all), vasoconstrictor function is enhanced. The response to short-term intermittent hypoxia has not been studied in detail. The balance between vasodilatation and vasoconstriction is physiologically important because it determines blood flow to metabolically active tissues, which has implications for the pathogenesis of hypertension and ischaemia of the heart and brain.

## Vasoconstrictor function and the sympathetic nervous system

Intermittent hypoxia is hypothesized to stimulate chemoreceptor activity, increasing sympathetic nervous system activity, which leads to vasoconstriction and, potentially, systemic hypertension. Figure 2 shows a theoretical schematic diagram that outlines the pathways by which intermittent hypoxia may lead to hypertension and should be viewed as a supplement to this section. Since sympathetic nervous system activity remains elevated after removal of hypoxia (Xie *et al.* 2001), it has been hypothesized that intermittent hypoxia 'ramps up' sympathetic activity and increases sympathetic responsiveness to subsequent bouts of hypoxia (Greenberg *et al.* 1999; Cutler *et al.* 2004*b*). Elevated sympathetic nervous system activity results in activation of vascular smooth muscle, leading to vasoconstriction and elevated systemic blood pressure (Fletcher *et al.* 2002). Furthermore, elevated sympathetic nervous system activity is thought to upregulate the production of angiotensin II by stimulating the production of renin in the kidney (Fletcher *et al.* 2002). Angiotensin II is a potent vasoconstrictor which is important in the regulation of systemic blood pressure. Other possibilities leading to altered vasoconstrictor function and systemic hypertension include baroreflex impairment (Cortelli *et al.* 1994; Lai *et al.* 2006) and increased production of the vasoconstrictor endothelin-1 (Phillips *et al.* 1999).

**Patients with OSA.** Patients with OSA have elevated muscle sympathetic nerve activity (MSNA; Narkiewicz *et al.* 1998, 1999), increased plasma levels of catecholamines (Mills *et al.* 2006) and reduced $\alpha$- and $\beta_2$-adrenergic vascular responses (Grote *et al.* 2000), thus suggesting a functional downregulation of vascular sympathoadrenergic receptors.

The renin–angiotensin system is highly involved in controlling systemic blood pressure. Renin is produced in the kidneys and converts angiotensinogen to angiotensin I, which is then converted to the vasoactive mediator angiotensin II by angiotensin-converting enzyme (Perazella & Setaro, 2003). Circulating angiotensin II has two effects: (a) it causes vasoconstriction; and (b) it

stimulates the adrenal cortex to secrete aldosterone (Perazella & Setaro, 2003). Aldosterone decreases sodium excretion, causing water retention, and is therefore important in the control of blood volume and blood pressure (Perazella & Setaro, 2003). The vasoconstrictor response to angiotensin II is increased in patients with OSA (Fig. 2) and is likely to contribute to the secondary hypertension observed in approximately 50% of OSA patients (Millman *et al.* 1991; Kraiczi *et al.* 2000).

Moller *et al.* (2003) measured 24 h blood pressure and the plasma concentrations of angiotensin II and aldosterone in a group of patients with OSA and in healthy controls. Patients with OSA had elevated diurnal and nocturnal blood pressure and also elevated plasma levels of angiotensin II and aldosterone. Therapy with CPAP reduced blood pressure and was associated with a decrease in the activity of the renin–angiotensin system. However, the results from this study should be interpreted with caution because control subjects were not carefully matched for body mass index and obesity. Since obesity is a risk factor for hypertension, this could have confounded interpretation of the results. In addition, increased angiotensin II concentration in OSA patients could have resulted from comorbid hypertension rather than OSA. As indicated above, interestingly, vasoconstrictor sensitivity to angiotensin II is enhanced in patients with OSA (Kraiczi *et al.* 2000). During infusion of angiotensin II, mean forearm conductance was 40% lower in OSA patients than in the control subjects (Kraiczi *et al.* 2000).

Another possible mechanism leading to altered vasoconstrictor function, which is shown in Fig. 2, involves endothelin-1, a vasoconstrictive neuropeptide which is produced by endothelin-converting enzyme from the precursor peptide, big endothelin-1 (Greenberg *et al.* 1992). Endothelin-1 is produced in vascular endothelial cells in response to hypoxia and sheer stress, and the cleavage of endothelin-1 from big endothelin is inhibited by NO (Greenberg *et al.* 1992; Marasciulo *et al.* 2006). Since intermittent hypoxia increases sheer stress and reduces NO bioavailability, this may be responsible for increased endothelin-1 production. However, it is not fully understood how production of endothelin-1 is upregulated (Phillips *et al.* 1999). Subsequent studies suggest that plasma endothelin-1 is not elevated in OSA patients but that big endothelin-1 is elevated, and that it is reduced following CPAP therapy (Grimpen *et al.* 2000; Jordan *et al.* 2005). Further study is required to clarify these inconsistencies.

**Human models.** Healthy human models of chronic intermittent hypoxia have not yet been used to investigate the effects of intermittent hypoxia on MSNA, plasma catecholamines, or $\alpha$- and $\beta_2$-adrenergic vascular responses, nor have they been used to investigate the effects

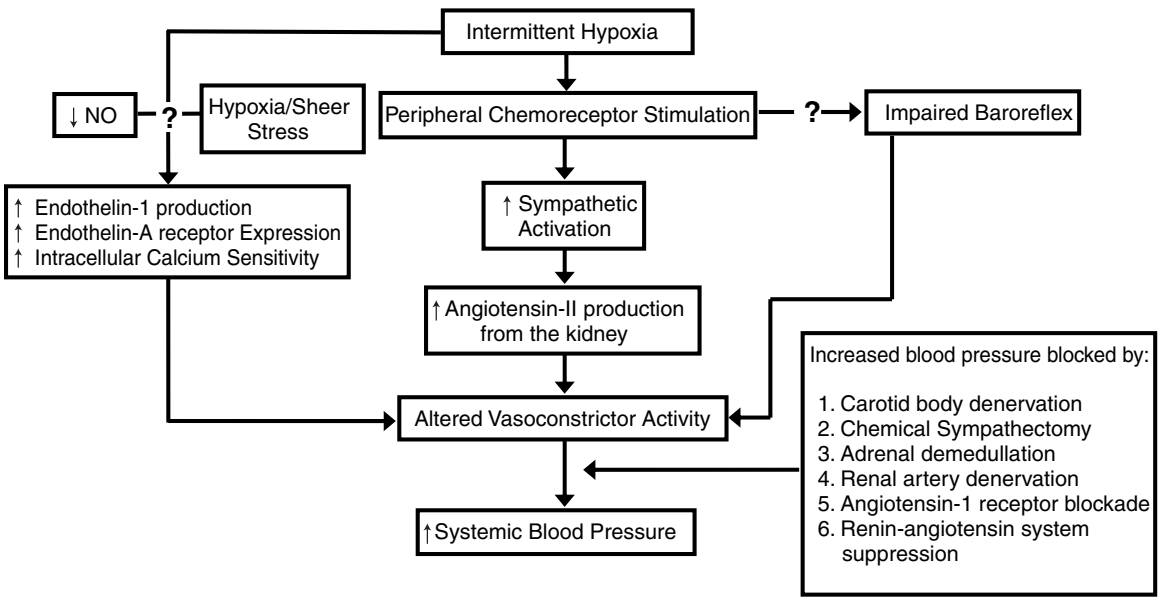


**Figure 2. Schematic diagram outlining the hypothesized pathways by which intermittent hypoxia leads to hypertension**
Chronic intermittent hypoxia activates the peripheral chemoreceptor and reflexively increases sympathetic nervous system activation and angiotensin II production, and enhances vasoconstrictor activity. An impaired baroreflex and increased endothelin production may also alter vasoconstrictor activity and promote an increase in systemic blood pressure. Definition of abbreviations: ? indicates that the complete mechanism of action is not completely understood.

of intermittent hypoxia on the renin–angiotensin system or on endothelin-1-mediated vasoconstriction. However, changes in MSNA following short-term intermittent hypoxia and acute hypoxia have been extensively studied.

Twenty minutes of *sustained* hypoxia and hypercapnia [$S_{aO_2}$, 80% and end-tidal partial pressure of $CO_2$ ($P_{ET,CO_2}$), +5 mmHg] in healthy, awake humans caused a 220% increase in MSNA, which persisted following removal of the stimulus (Morgan *et al.* 1995). This important finding has implications for the pathogenesis of chronically elevated sympathetic nervous system activity that accompanies OSA and has provided the basis for subsequent studies. Xie *et al.* (2000) determined that *intermittent* hypoxia and hypercapnia had a similar effect. Twenty minutes of intermittent hypoxia–hypercapnia [20 s hypoxia–hypercapnia ($S_{aO_2}$, 80% and $P_{ET,CO_2}$, +5 mmHg) alternating with 40 s of normoxia) led to a 175% increase in MSNA, which remained 150% above baseline 20 min following the end of the intermittent hypoxia–hypercapnia protocol. In order to determine the relative contributions of hypoxia and hypercapnia to the development of persistent sympathoexcitation, an additional study was done in which healthy humans were exposed to 20 min of sustained hypoxia and hypercapnia separately (Xie *et al.* 2001). Hypoxia elicited prolonged sympathetic activation even after removal of the stimulus, but hypercapnia did not.

Cutler *et al.* (2004*a*,*b*) used a model of intermittent hypoxia, which was induced by voluntary apnoea (1 × 30 s apnoea per minute, for 20 min), to determine whether the cessation of breathing is important in prolonged sympathetic activation. Their results indicated that MSNA was significantly elevated for up to 180 min following the end of intermittent hypoxia. This was found for exposure to 20 min of hypercapnic hypoxia and isocapnic hypoxia (no apnoeas), and suggests that hypoxia is the primary mediator of this response. Leuenberger *et al.* (2005) also found sustained sympathetic activation and a transient elevation of blood pressure following 30 min of voluntary end-expiratory apnoeas that were primed with a hypoxic gas mixture and lasted for 20 s out of each minute.

To determine whether the pattern and intensity of the hypoxic exposure was important in determining sympathoexcitation, Tamisier *et al.* (2005) compared a 2 h continuous hypoxic protocol with a 2 h intermittent hypoxia protocol. Their results show persistent sympathoexcitation following continuous hypoxia but not following cyclic hypoxia. This contrasts with the other studies discussed previously, possibly because the hypoxic protocols were hypocapnic, and hypocapnic hypoxia does not lead to a significant post-stimulus sympathoexcitation (Tamisier *et al.* 2004).

**Animal models.** Animal models have been used to demonstrate that chronic intermittent hypoxia alters adrenergic vascular responses. Phillips *et al.* (2005) used video microscopy to measure the diameter of the isolated gracilis muscle resistance arteries before and during exposure to noradrenaline at various intraluminal pressures in rats exposed to 14 days of chronic intermittent hypoxia (60 s period of $F_{IO_2} = 10\%$ every 4 min for 12 h day$^{-1}$). They found that resting tone, myogenic activation and vasoconstrictor responses to noradrenaline were reduced in these animals. Interestingly, treatment of the rats' drinking water with a superoxide scavenger restored myogenic responses and noradrenaline-induced constriction, suggesting a role for superoxide production in the attenuated vasoconstrictor responsiveness to noradrenaline in intermittent hypoxia.

Fletcher and colleagues have intensively studied the effects of intermittent hypoxia on the development of hypertension and the role of the sympathetic nervous system, peripheral chemoreceptors and the renin–angiotensin system in this response (Fletcher *et al.* 1992*a*, 1999, 2000, 2002). They first determined that intermittent hypoxia (2 × 12 s periods of $F_{IO_2} = 2$–3% per minute, 8 h day$^{-1}$ for 35 days) led to a 13 mmHg increase in mean arterial blood pressure (MAP; Fletcher *et al.* 1992*c*), and subsequently prevented the increase in MAP by carotid body denervation (Fletcher *et al.* 1992*a*), chemical sympathectomy (Fletcher *et al.* 1992*b*), adrenal demedullation (Bao *et al.* 1997) and renal artery denervation (Bao *et al.* 1997). In addition, they demonstrated that intermittent eucapnic and hypercarbic hypoxia had no additional effects on diurnal blood pressure compared with hypocapnic intermittent hypoxia (Fletcher *et al.* 1995). Using this model of intermittent hypoxia, they have shown that blockade of the angiotensin-1 receptor with losartan prevented the rise in blood pressure following chronic intermittent hypoxia (Fletcher *et al.* 1999) and that suppression of the renin–angiotensin system (by salt loading) prevented the increase in MAP (Fletcher *et al.* 2002). This work has generated the hypothesis that secondary hypertension in OSA is a result of adrenergic and renin–angiotensin system overactivity, as outlined in Fig. 2.

Interestingly, rats exposed to intermittent hypoxia 8 h day$^{-1}$ for 11 days (90 s period of 5% $O_2$–5% $CO_2$ every 90 s) increased plasma endothelin-1, as measured by radioimmunoassay (Kanagy *et al.* 2001). These rats also demonstrated a significant increase in MAP (Kanagy *et al.* 2001). Using a 35 day chronic intermittent hypoxia protocol (1 × 40 s period of $F_{IO_2} = 5\%$ per minute for 8 h day$^{-1}$), Lefebvre *et al.* (2006) reported a 17% increase in carotid artery contraction in response to endothelin-1, and cyclo-oxygenase inhibition by indomethacin reduced contraction of the carotid artery

by 24%. Allahdadi *et al.* (2005) subjected rats to chronic intermittent hypoxia–hypercapnia (20 cycles of 5% $O_2$–5% $CO_2$ per hour; 7 h $day^{-1}$ for 14 days) and measured the diameter and vessel wall calcium concentration simultaneously in mesenteric arteries with endothelial function disabled. The intermittent hypoxia–hypercapnia protocol led to increased constrictor sensitivity to endothelin-1 and increased calcium sensitivity compared with control animals. They also reported an increase in endothelin-A receptor expression. This suggests that intermittent hypoxia alters signalling components unique to endothelin-1 at the receptor and post-receptor level that increase calcium sensitivity during endothelin-A receptor activation (Allahdadi *et al.* 2005).

## Vasodilator function

Intermittent hypoxia provokes a cascade of events that ultimately lead to endothelial dysfunction, inflammation and atherosclerosis. Figure 3 is a schematic diagram outlining this hypothetical pathway and should be used throughout this section as a supplement to the text. It is hypothesized that intermittent hypoxia leads to the formation of free radicals or reactive oxygen species (ROS; Jordan *et al.* 2006) which react with nitric oxide (NO), an important vasodilator, to produce peroxynitrite (Lavie, 2003). This reaction can diminish the bioavailability of NO and thereby attenuate NO-dependent vasodilatation (Cohen, 1995). Oxidative stress is also capable of increasing transcription factor production and adhesion molecule expression and is discussed in the following section.

**Patients with OSA.** Several reports suggest that endothelium-dependent vasodilatation is altered in OSA patients. The increase in forearm blood flow after infusion of acetylcholine is reduced by 39% in OSA patients (Carlson *et al.* 1996). Acetylcholine acts on the endothelium and causes vasodilatation through a NO-dependent pathway (Faraci & Brian, 1994). Similar results were found in a separate study on a group of non-hypertensive OSA patients (Kato *et al.* 2000). Furthermore, altered vascular reactivity was found only in the resistance vessels of the forearm, since the brachial artery (a conductance vessel) was unaffected (Kato *et al.* 2000). After removal of the OSA-related intermittent hypoxia by treatment with CPAP, a significant improvement in vascular function was observed (Imadojemu *et al.* 2002; Lattimore *et al.* 2006). Vascular reactivity in the forearm remains intact to endothelium-independent stimuli such as NO donors, L-arginine (substrate for NO) and calcium channel blockers (Kato *et al.* 2000; Lattimore *et al.* 2006), suggesting that vascular smooth muscle function is not affected in OSA.

Plasma levels of NO derivatives are decreased in OSA patients and increase following CPAP therapy, thus supporting the hypothesis that NO bioavailability is reduced in OSA (Ip *et al.* 2000; Schulz *et al.*

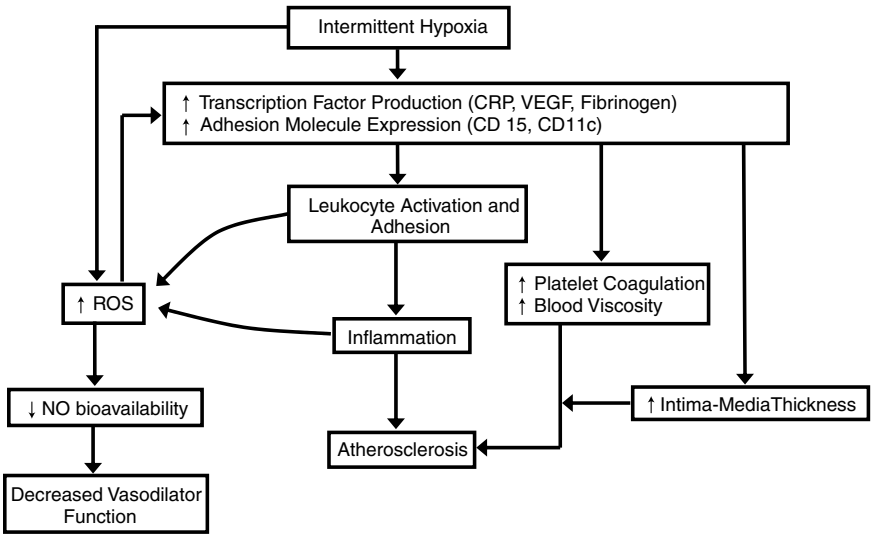


**Figure 3. Schematic diagram outlining the hypothesized pathways by which intermittent hypoxia leads to endothelial dysfunction, inflammation and atherosclerosis**
Chronic intermittent hypoxia increases reactive oxygen species (ROS) and increases production of transcription factors and adhesion molecules. Elevated ROS decreases nitric oxide (NO) bioavailability leading to endothelial dysfunction. Leukocyte activation and adhesion to the endothelium leads to atherosclerosis. Definition of abbreviations: ROS, reactive oxygen species; NO, nitric oxide; CRP, C-reactive protein; and VEGF, vascular endothelial growth factor.

2000*b*). In addition, basal NO production is increased in OSA patients following CPAP therapy, reflected by a significantly greater reduction in forearm blood flow by NO synthase blockade with $N^G$-monomethyl-L-arginine (L-NMMA; Lattimore *et al.* 2006). However, other studies do not support the notion that NO-mediated oxidative/nitrosative stress occurs in patients with OSA (Svatikova *et al.* 2004). Since superoxide-mediated scavenging of NO generates peroxynitrite, which can nitrosylate membrane proteins and oxidize lipids, Svatikova *et al.* (2004) measured circulating nitrotyrosine as an indicator of peroxynitrite formation. Their results showed no difference in nitrotyrosine levels between control and OSA groups and suggest that peroxynitrite formation does not occur in OSA. Instead, it has been proposed that an endogenous competitive inhibitor of NO synthase reduces NO production (Suzuki *et al.* 2006). Further studies are required to clarify these conflicting results.

Cerebrovascular reactivity to carbon dioxide is reduced in patients with OSA (Diomedi *et al.* 1998; Placidi *et al.* 1998; Xie *et al.* 2005). Two reports have measured cerebrovascular reactivity to hypercapnia in OSA patients by means of transcranial Doppler ultrasonography of the middle cerebral artery during breath-holds (Diomedi *et al.* 1998; Placidi *et al.* 1998). Both studies measured the breath-hold index (BHI), which is calculated by dividing the increase in middle cerebral artery blood flow velocity during a breath-hold by the duration of the breath-hold. These studies report a 60% reduction in breath-hold index in OSA patients compared with control subjects during wakefulness (Diomedi *et al.* 1998; Placidi *et al.* 1998). Following CPAP therapy, the BHI increased in OSA patients to values comparable to controls (Diomedi *et al.* 1998).

**Human models.** A single study (Foster *et al.* 2005) has assessed 'the cerebral vascular' response to chronic intermittent hypoxia in humans. Foster *et al.* (2005) assessed cerebral tissue oxygenation using two protocols of intermittent hypoxia. Subjects were exposed to either 30 min of hypoxia [12% $O_2$; long duration intermittent hypoxia (LDIH)] per day or to a 5 min hypoxia (12% $O_2$) to 5 min normoxia cycle [short duration intermittent hypoxia (SDIH)] for an hour per day for 12 days. Throughout both protocols, cerebral tissue oxygenation was assessed using near-infrared spectroscopy during a progressive hypoxic challenge. The change in cerebral tissue oxygenation indexed to the change in $S_{aO_2}$ during the hypoxic challenge was used as a measure of the sensitivity of cerebral oxygen saturation to hypoxia. Regardless of the protocol used, the authors observed an initial increase in cerebral oxygen saturation sensitivity on day 3, followed by a subsequent decline, which was evident by the 12$^{th}$ day (Fig. 4*A*). Although both protocols demonstrated similar findings, SDIH had a greater effect than LDIH (Fig. 4*B* and *C*). These findings suggest that the vascular processes required to maintain homeostasis of cerebral blood flow and tissue oxygenation are altered by intermittent hypoxia and that this effect is greater following exposure to short cycles of desaturation–resaturation.

In contrast, a study from our laboratory found a different cerebral vascular response to hypoxia following *discontinuous hypoxia*. Kolb *et al.* (2004) exposed healthy human subjects to 8 h of *sustained hypoxia* (∼13.8% $O_2$) for five consecutive nights and found that the cerebral blood flow sensitivity to hypoxia, which was assessed during a stepwise reduction in end-tidal oxygen pressure ($P_{ET,O_2}$), was increased by 116% compared with baseline values. This model of sustained hypoxia stimulates an adaptive process that increases cerebral vasodilatation in response to hypoxia.

**Animal models.** Data from animal models of intermittent hypoxia have provided further insight into how vascular function may be altered in patients with OSA. Tahawi *et al.* (2001) exposed rats to 35 days of intermittent hypoxia ($2 \times 12$ s periods of $F_{IO_2}$∼2–3% per minute, 8 h day$^{-1}$) and measured *in vivo* arteriolar reactivity in the cremaster muscle by video microscopy. They found a 16 mmHg increase in MAP and an attenuated response to acetylcholine-induced, arteriolar vasodilatation. In addition, the vasoconstrictor response to NO blockade by $N^G$-nitro-L-arginine methyl ester (L-NAME) was reduced by 83% in arterioles of rats exposed to intermittent hypoxia, implying lower basal release of NO. More recently, Phillips *et al.* (2004) exposed rats to a different pattern of intermittent hypoxia ($1 \times 60$ s period of $F_{IO_2} = 10\%$ every 4 min, 12 h day$^{-1}$ for 14 days). They studied vascular reactivity *ex vivo* in the resistance vessel of the gracilis muscle and the middle cerebral artery by video microscopy. Acetylcholine-induced dilatation of the gracilis artery and middle cerebral artery was severely attenuated, while the responses to sodium nitroprusside (a NO donor) and to calcium-free physiological saline solution were similar between rats exposed to intermittent hypoxia and control animals. Furthermore, dilatation of both the gracilis artery and the middle cerebral artery in response to hypoxia was virtually abolished in intermittently hypoxic animals. Taken together, these data imply that exposure to intermittent hypoxia reduces the bioavailability of NO in resistance vessels of the cerebral and skeletal muscle circulation and severely blunts the vasodilator response to acute hypoxia. While the evidence seems strong, these conclusions are not shared by all studies. For example, acetylcholine-induced relaxation of vascular smooth muscle in the aorta, carotid artery and mesenteric vascular bed was not altered following 35 days of chronic intermittent hypoxia ($1 \times 40$ s period of $F_{IO_2} = 5\%$ per minute, 8 h day$^{-1}$; Lefebvre *et al.* 2006). These findings

may indicate that conductance vessels and resistance vessels react differently to chronic intermittent hypoxia.

### Vessel inflammation and atherosclerosis

Intermittent hypoxia is thought to cause vessel inflammation and lead to the progression of atherosclerosis (Lavie, 2005). It is hypothesized to upregulate transcription factor production and adhesion molecule expression, which can aid in the production of ROS and exacerbate endothelial dysfunction, as illustrated in Fig. 3.

**Patients with OSA.** Leukocyte accumulation and adhesion to the endothelium with cell surface receptors and the initiation of leucocyte–endothelial cell interactions can critically impair endothelial cell function and promote the atherogenic process (Fig. 3; Dyugovskaya *et al.* 2002). Dyugovskaya *et al.* (2002) determined that OSA was associated with increased expression of adhesion molecules CD15 and CD11c on monocytes, increased adherence of monocytes in culture to human endothelial cells, and increased intracellular ROS production and upregulation of CD15 expression due to hypoxia *in vitro* in monocytes of control subjects. Further studies implicate cytokine production of CD4 and CD8 T cells of OSA patients in atherogenesis and plaque development (Dyugovskaya *et al.* 2005). Apparently, CD4 and CD8 T cells undergo phenotypic and functional changes, resulting in a shift towards type-2 cytokine dominance with increased interleukin (IL)-4 and decreased IL-10 expression (Dyugovskaya *et al.* 2005). In addition, CD8 T cells of OSA patients exhibit a fourfold increase in tumour necrosis factor-$\alpha$ and CD40 ligand (Dyugovskaya *et al.* 2005). Serum levels of IL-6 and IL-18 are also elevated in OSA patients and are significantly correlated with duration of OSA-related hypoxia and severity of OSA (Minoguchi *et al.* 2005).

Biomarkers of oxidative stress (plasma malondialdehyde and urinary *o,o′*-ditryosine) are elevated in OSA and correlate well with the severity of hypoxaemia (Jordan *et al.* 2006). Increased oxidative stress and immune cell activation in OSA lead to increased

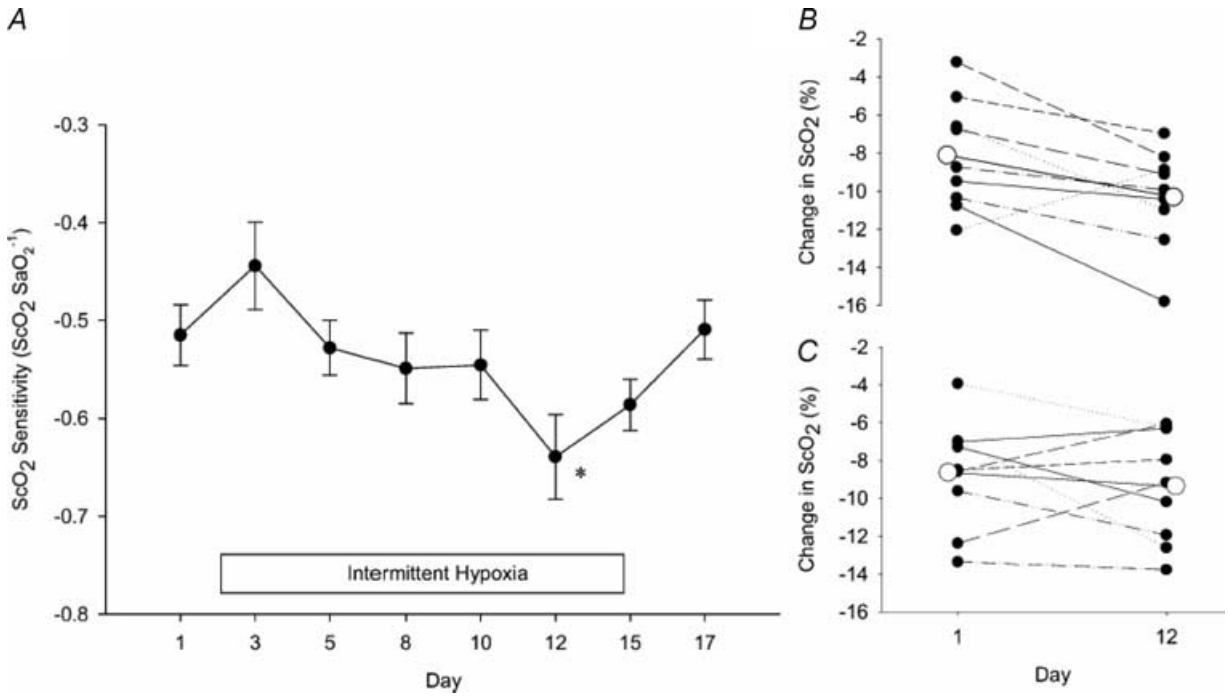


**Figure 4. Effects of intermittent hypoxia on human cerebral tissue oxygenation**
*A*, the effects of intermittent hypoxia on the ratio of the fall in cerebral tissue oxygen saturation to the fall in arterial oxyhaemoglobin saturation ($\Delta S_{cO_2}/\Delta S_{aO_2}$) during progressive hypoxia in SDIH and LDIH subjects combined. Data points are means ± S.E.M. *$P < 0.001$ significantly different from day 1. *B*, the absolute change in $S_{cO_2}$ on days 1 and 12 at iso-$S_{aO_2}$ (83 ± 3%) for each subject in SDIH. All but one subject showed a greater reduction in $S_{cO_2}$ at day 12. *C*, the absolute change in $S_{cO_2}$ on days 1 and 12 at iso-$S_{aO_2}$ (83 ± 3%) for each subject in LDIH. Five of nine subjects show a greater reduction in $S_{cO_2}$ at day 12. In *B* and *C*, the large open circle data points represent the group means. Definition of abbreviations: $S_{cO_2}$, cerebral oxygen saturation; $S_{aO_2}$, arterial oxyhaemoglobin saturation; SDIH, short duration intermittent hypoxia; LDIH, long duration intermittent hypoxia. Reproduced with permission from Foster *et al.* (2005).

ROS production in monocytes and polymorphonuclear neutrophils, overexpression of adhesion molecules and cytotoxicity of monocytes (Schulz *et al.* 2000*a*; Dyugovskaya *et al.* 2002, 2005). This further reduces NO bioavailability and increases monocyte and platelet adhesion, thus aiding in the progression of atherosclerosis and vascular dysfunction (Fig. 3). Middle-aged patients with OSA who are free of clinically overt cardiovascular diseases have early signs of atherosclerosis (Drager *et al.* 2005). For example, patients with OSA have a significantly increased pulse wave velocity, increased intima–media thickness and decreased carotid diameter compared with matched controls (Altin *et al.* 2005; Drager *et al.* 2005; Saletu *et al.* 2006). In addition, serum inflammatory markers are elevated in OSA patients; these have been proposed as significant predictors of atherosclerosis and its complications (Hackam & Anand, 2003).

Intermittent hypoxia may also stimulate the production of vascular endothelial growth factor (VEGF), a potent angiogenic cytokine which, because of its ability to stimulate smooth muscle proliferation, is implicated in the progression of atherosclerosis. Serum levels of VEGF are elevated in patients with OSA and correlate strongly with the degree of nocturnal hypoxaemia (Lavie *et al.* 2002; Schulz *et al.* 2002). Levels of VEGF decrease in patients successfully treated with CPAP (Schulz *et al.* 2002). In addition to increased levels of VEGF, OSA patients have increased fibrinogen levels, platelet coagulation, blood viscosity and C-reactive protein (Fig. 3; Wessendorf *et al.* 2000; Minoguchi *et al.* 2005; Saletu *et al.* 2006). The increases in C-reactive protein and fibrinogen levels are strongly correlated with indices of OSA severity (Wessendorf *et al.* 2000; Minoguchi *et al.* 2005; Saletu *et al.* 2006).

**Human models.** Healthy human models of intermittent hypoxia have not been used to assess the relationship between hypoxia and vessel inflammation and atherosclerosis. Future research in this area of study will undoubtedly be useful.

**Animal models.** Intermittent hypoxia in animal models also leads to oxidative stress and inflammation. Rats exposed to 5 weeks of chronic intermittent hypoxia ($1 \times 60$ s period of $F_{IO_2} = 4$–$5\%$ every 2 min, 8 h day$^{-1}$ for 5 days week$^{-1}$) have greater myocardial lipid peroxides and lower levels of myocardial superoxide dismutase (Chen *et al.* 2005). This suggests that intermittent hypoxia, in addition to increasing ROS production, decreases a major antioxidant system. In a model of chronic intermittent hypoxia–hypercapnia ($2 \times 15$ s periods of $F_{IO_2} = 6$–$8\%$ and $CO_2 = 10$–$14\%$ per minute; 8 h day$^{-1}$ for 3 weeks), platelet reactivity was significantly elevated, as were platelet adhesion and aggregation (Dunleavy *et al.* 2005).

Altay *et al.* (2004) undertook a novel study using knockout mice to determine the role of nitric oxide in the inflammatory response to intermittent hypoxia. Animals were mechanically ventilated and subjected to 12 cycles of intermittent hypoxia by turning the ventilator off for 30 s every 5 min, over 1 h. The mice were wild type, neuronal NO synthase (nNOS) knockouts, or endothelial NO synthase (eNOS) knockouts. Following intermittent hypoxia, leucocyte–endothelial cell adherence in the cortical venular microcirculation was measured by using epifluorescence videomicroscopy, and hippocampal CA1 pyramidal cell injury was evaluated by light microscopy by counting viable pyramidal cells present in the CA1 sector. They found that this brief exposure to intermittent hypoxia was sufficient to trigger a rapid and prolonged inflammatory response in the cerebral microcirculation. Leucocytes became adherent to cortical venular endothelium within 4 h. Neuronally derived NOS modulated leucocyte–endothelial cell interactions because leucocyte–endothelial cell adherence was absent in nNOS knockout mice. Cerebrovascular inflammation was accentuated in eNOS knockout mice, which suggests that NO produced by eNOS is important in limiting cerebrovascular inflammation and prevents injury/death to hippocampal pyramidal cells.

Together, these results suggest that OSA is indeed a disorder of oxidative stress, leads to inflammation and may accelerate the progression of atherosclerosis.

## Conclusion

This review has highlighted the association between OSA and vascular disease, outlined some potential basic mechanisms for this association, and compared the results from studies on OSA patients with those from experimental human and animal models of intermittent hypoxia. It is clear that intermittent hypoxia leads to hypertension and that sympathetic overactivity and the renin–angiotensin system are important in this response. In addition, altered vascular function and baroreceptor function could be involved. Intermittent hypoxia leads to a systemic inflammatory response which may contribute to the progression of atherosclerosis and decreased vascular function, which are highly important responses relating to an increased risk of stroke and myocardial infarction in patients with OSA. To date, the small number of studies on experimental human models of intermittent hypoxia have demonstrated sustained sympathetic activation and altered cerebral oxygenation. Animal studies have provided the bulk of our knowledge regarding basic mechanisms responsible for the development of hypertension following chronic intermittent hypoxia, and are beginning to provide valuable data regarding the role of intermittent hypoxia

and vascular dysfunction. Despite these advances, further research is required to determine the basic mechanisms that link OSA and vascular disease. We propose that advances will be led by collaborative clinical and basic science research on patients with OSA and experimental models of intermittent hypoxia. It is anticipated that such studies will provide an improved understanding of this important clinical problem and form the basis for effective therapeutic strategies.

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