CEREBRAL BLOOD FLOW RESPONSE TO ISOCAPNIC HYPOXIA DURING SLOW WAVE SLEEP AND WAKEFULNESS

Guy E. Meadows¹,³, Denise M. O’Driscoll¹,³, Anita K. Simonds¹,³, Mary J. Morrell¹,³, Douglas R. Corfield¹,²

¹ Clinical and Academic Unit of Sleep and Breathing, National Heart and Lung Institute, Imperial College, London, UK. ²MacKay Institute of Communication and Neuroscience, School of Life Sciences, Keele University, Keele, UK. ³Sleep and Ventilation Unit, Royal Brompton Hospital, Sydney Street, London, UK.

Running Head: Brain blood flow during sleep

Address for correspondence:
Douglas R. Corfield
Mackay Institute of Communication and Neuroscience
School of Life Sciences
Keele University, Keele
Staffordshire, ST5 5BG
United Kingdom
Tel: +44 (0) 1782 583 485
Fax: +44 (0) 870 133 8621
E-mail: d.corfield@keele.ac.uk
ABSTRACT

Nocturnal hypoxia is a major pathological factor associated with cardio-respiratory disease. During wakefulness, a decrease in arterial oxygen tension results in a decrease in cerebral vascular tone and a consequent increase in cerebral blood flow; however, the cerebral vascular response to hypoxia during sleep is unknown. In the present study, we determined the cerebral vascular reactivity to isocapnic hypoxia during wakefulness and during stage III/IV, NREM sleep. In 13 healthy individuals, left middle cerebral artery velocity (MCAV) was measured using transcranial Doppler ultrasound, as an index of cerebral blood flow. During wakefulness, in response to isocapnic hypoxia (arterial oxygen saturation –10%), the mean (± sem) MCAV increased by 12.9 ± 2.2% (P < 0.001); during NREM sleep, isocapnic hypoxia was associated with a –7.4 ± 1.6% reduction in MCAV (P < 0.001). Mean arterial blood pressure was unaffected by isocapnic hypoxia (P > 0.05); R-R interval decreased similarly in response to isocapnic hypoxia during wakefulness (−21.9 ± 10.4%, P < 0.001) and sleep (−20.5 ± 8.5%, P < 0.001). The failure of the cerebral vasculature to react to hypoxia during sleep suggests a major state-dependent vulnerability associated with the control of the cerebral circulation and may contribute to the pathophysiologies of stroke and sleep apnea.

Key words: transcranial Doppler ultrasound, middle cerebral artery velocity, cortical blood flow.
INTRODUCTION

Nocturnal hypoxia is a major pathological factor associated with cardio-respiratory
diseases including obstructive sleep apnea (OSA) (14) and congestive heart failure (16).
Reductions in arterial blood oxygen levels will impose stress on all organ systems;
however, the brain is particularly vulnerable to the effects of hypoxia (3). Recently OSA,
a condition in which cognitive function can be substantially impaired, has been
associated with pathological loss of cortical grey matter (19, 22), suggesting that the
nocturnal hypoxia associated with OSA may be sufficient to damage brain tissue directly.

During any hypoxic insult, protection of the brain will depend on an adequate cerebral
vascular response. Normally, perfusion of the brain is dependent on a tight coupling
between its oxygen supply and the metabolic demand (31). During wakefulness, a
decrease in oxygen supply results in a decrease in cerebral vascular tone and a
consequent increase in cerebral blood flow that will mitigate the effects of the systemic
hypoxia. Although the cerebral vascular response to hypoxia is not linearly related to the
fall in arterial PO$_2$, like the ventilatory response to hypoxia, it is linearly related to the fall
in arterial oxygen saturation (15).

The transition from wakefulness to stage III/IV, non-rapid eye movement (NREM) sleep
is accompanied by marked alterations in the control of the cerebral vascular system.
During stage III/IV NREM sleep and despite a relative state of hypercapnia (a potent
cerebral vasodilator), cerebral blood flow decreases along with cerebral metabolism (36).
We recently reported that cerebral vascular reactivity to CO$_2$ is markedly reduced during
NREM sleep (21), a reduction that would permissively allow cerebral blood flow to fall in this state. In this context, we hypothesised that the cerebral vascular response to hypoxia is similarly reduced during NREM sleep; such a reduction, in hypoxic cerebral vascular reactivity, would severely impair the brain’s ability to defend itself against a nocturnal hypoxic insult. The present study tested the hypothesis that, in normal human subjects, isocapnic hypoxic cerebral vascular reactivity is decreased during stage III/IV NREM sleep compared to wakefulness.

METHODS
The study was carried out with local ethical approval (Royal Brompton and Harefield Hospital Ethics Committee) and with written, informed consent from subjects, with no reported history of cardiopulmonary disease; all had normal lung function determined by forced spirometry. Snorers or light sleepers were excluded. Of 17 volunteers (all male), sufficient data were collected from 13 subjects (mean ± sd: age 22 ± 4, body mass index 22 ± 2 kg/m²). There were no anthropometrical differences between included and excluded subjects.

**Determination of middle cerebral artery velocity**

The velocity of blood in the left middle cerebral artery (MCAV) was determined using pulsed Doppler ultrasonography (Scimed Ltd) applied using existing protocols from our laboratory (21). The mean value of the velocity associated with the maximum frequency of Doppler shift was calculated for each cardiac cycle (21).
Isocapnic Hypoxia

Subjects breathed through an apparatus designed to regulate the fraction of inspired oxygen and to maintain the end-tidal partial pressure of carbon dioxide (PETCO₂) within ± 2mmHg of a predetermined level, independent of changes in ventilation (2, 20).

In each subject, the cortical blood flow responses to four separate conditions were tested during wakefulness (lying supine, eyes open, watching a video) and during the first 90-minute cycle of stage III/IV NREM sleep: 1. Eucapnic euoxia (spontaneous air breathing); 2. isocapnic euoxia (air breathing with clamped end tidal CO₂); 3. isocapnic hypoxia (-5% SaO₂; hypoxic breathing mixture with clamped end tidal CO₂); 4. isocapnic hypoxia (-10% SaO₂; hypoxic breathing mixture with clamped end tidal CO₂). To reduce the risk of arousal from sleep and to prevent any overshoot in the stimulus, the level of hypoxia was titrated gradually over a few minutes. Once the target SaO₂ was reached each level of hypoxia was maintained constant for a 5-minute period. To ensure a steady state the data analysis was performed on the last two minutes of this 5 min period.

General measurements

Airflow was measured using a pneumotachograph (model 3700A, Hans Rudolf). PETCO₂ and PETO₂ were determined using rapidly responding gas analyzers (Applied Electrochemistry CD-3A & S-3A). Blood pressure was monitored continuously, using a Finapres BP monitor (2300 Ohmeda); from this, mean arterial blood pressure (MABP) was derived. Cardiac intervals (R-R) were monitored using a Lifetrak ECG monitor (HME Ltd). SaO₂ was monitored using a pulse oximeter (N-200E Nellcor).

Electroencephalograms (EEG: C3A2, C4A1), electro-oculograms (EOG: F7A1, F8A1)
and a submental electromyogram (EMG) were recorded (Grass Telefactor) using the International 10-20 system of electrode placement. Sleep was staged using the approach of Rechtschaffen and Kales (26) and staging was performed with the investigators blinded to the other physiological data. Subsequently, any intervention associated with an arousal (ASDA criteria (1) was excluded from the analysis (18).

**Statistical Analysis**

Results are presented as the group means (± sem). Statistical comparisons between wakefulness and sleep baseline eucapnic euoxic variables were performed using Student’s paired sample t-tests (2 tail). A repeated measures analysis of variance (ANOVA) was used to determine the effect of varying levels of isocapnic hypoxia during wakefulness and sleep on MCAV, VE, MABP and RR interval (Systat Version 8). Where the ANOVA identified significance, t-tests were used to identify any pair wise differences. The significance threshold was set at P < 0.05.

**RESULTS**

**Eucapnic euoxic baseline variables during wakefulness and sleep (Table 1).**

For the group, the baseline eucapnic euoxic MCAV decreased by 4.9 ± 3.4 cm/sec (P < 0.001) and the baseline PCO₂ increased by 2.2 ± 1.0 mmHg from wakefulness to sleep (P < 0.01). Ventilation (VE) decreased slightly but not significantly from wake to sleep (-1.7 ± 1.4 L/min; P > 0.05). Baseline MABP decreased by an average of 20.5 ± 1.9 mmHg (P < 0.001) from wake to sleep; RR interval did not change (p > 0.05). The mean baseline SaO₂ decreased by an average of 2.0 ± 0.2% (P > 0.05).
Effects of isocapnic normoxia during wakefulness and sleep (Table 1).

As expected, the clamping circuit slightly increased the PCO$_2$ above the baseline eucapnic value during both wakefulness and sleep (wake increase: 1.2 mmHg (P < 0.001); sleep increase: 0.9 mmHg (P<0.05). Baseline MCAV, VE, BP, RR and SaO$_2$ values were not significantly different (P > 0.05) during isocapnic euoxia awake or asleep.

Effects of isocapnic hypoxia during wakefulness and sleep

The original traces from one individual show the different effects of isocapnic hypoxia on the MCAV during sleep compared to wakefulness (Fig 1). Isocapnic hypoxia progressively increased the mean MCAV during wakefulness, for –5% SaO$_2$, by 8.83 ± 1.7% and, for -10% SaO$_2$, by 12.9 ± 2.16% (Fig 2a; P < 0.001). In contrast, during sleep, isocapnic hypoxia progressively decreased the mean MCAV, for -5% SaO$_2$, by –6.97 ± 1.6% and, for –10% SaO$_2$, by –7.42 ± 1.6% (Fig 2a; P < 0.001). The percentage changes in MCAV from baseline isocapnic euoxia to isocapnic hypoxia (-10% SaO$_2$) are presented for each individual in Fig 2b. Isocapnia was maintained during hypoxia in each state (Fig 3b). The level time course of the MCAV response over the five minutes of each intervention indicated that a steady state had been achieved (Fig 4); further, there were no statistically significant differences in MCAV between mins 4 and 5 of each intervention.

During wakefulness, isocapnic hypoxia (-5% and -10% SaO$_2$) increased ventilation by 20.7 ± 4% and 26.3 ± 10% (P < 0.001; Fig 3a) respectively, however, during sleep the
increase in ventilation was no longer significant (-5% and -10% SaO₂: 10 ± 11% and 10 ±
9%, P > 0.05; Fig 3a). MABP was unaffected by isocapnic hypoxia (-5 and -10% SaO₂)
during wakefulness or sleep (Fig 3c; P > 0.05). However, during wakefulness and sleep,
isocapnic hypoxia (-10% SaO₂) decreased the RR interval from its mean baseline
isocapnic euoxic value by an average of 21.9 ± 10.4% during wakefulness (Fig 3d; P <
0.001) and 20.5 ± 8.54% during sleep (Fig 3d; P < 0.001).

DISCUSSION

The most important finding of the present study was that the cortical blood flow response
to hypoxia was dramatically altered during sleep compared to wakefulness. During
wakefulness, in response to isocapnic hypoxia, cortical blood flow increased, in contrast,
during sleep, in response to the same degree of isocapnic hypoxia, cortical blood flow
decreased. As the brain is particularly sensitive to the effects of hypoxia, the inability of
the cerebral vasculature to respond to hypoxic stress during sleep suggests a significant
vulnerability of the brain in this state.

Methodological considerations

Validity of transcranial Doppler (TCD) technique

The basic assumption with this methodology is that relative changes in MCAV
directly represent relative changes in blood flow within this artery. The validity of
this assumption depends on whether the middle cerebral artery diameter remains
constant in response to altered PCO₂ and/or blood pressure. This assumption has
been challenged e.g.(4), however, the majority of research suggests that MCAV is a
reliable index of cortical blood flow (24, 29). Poulin et al. (24) using the Doppler signal power as an index of cross sectional area of the MCA, concluded that the caliber of the MCA did not change significantly under conditions of moderate hypercapnia. Further support comes from a recent MRI study by Serrador et al. (29), who demonstrated that MCA dimensions (measured to within 0.1mm with magnetic resonance imaging) are stable under a wide range of PETCO2 and induced orthostatic stress. In addition, research using a rodent model suggests that the arterioles and not the larger arteries are responsible for alterations in brain blood flow during slow wave sleep (12). Although a differential change in the diameter of the MCA, in response to hypoxia during sleep compared to wakefulness, may be a theoretical confound, we consider it unlikely that such an effect would nullify the differences in the MCAV responses observed here.

Isocapnic hypoxia

Carbon dioxide is a potent cerebral vasodilator and in humans, during wakefulness, cortical blood flow is very sensitive to changes in arterial PCO2. The clamping circuit used in the present study ensured only a minimal variation in the PCO2 occurred during all testing conditions. The marked differences in hypoxic cerebral vascular reactivity between wakefulness and sleep can, therefore, not be attributed to variations in PCO2.

Arterial blood pressure

In common with others (18, 32), we report a sleep related reduction in baseline MABP. Cortical blood flow is usually independent of variations in arterial blood pressure, over a
wide range, due to autoregulatory mechanisms. Although the presence of autoregulation has not been tested in humans during sleep, it is preserved in lambs (13). Assuming that cerebral autoregulation is present during sleep, a reduction in blood pressure will be met by a reduction in cerebral vascular tone and the maintenance of cerebral blood flow (15). This action will lead to a reduction in cerebral vascular reserve. However, whilst reduced, the MCAV does increase in response to hypercapnia indicating that some cerebral vascular reserve does remain during sleep (21). A loss of cerebral vascular reserve could therefore not explain the absence of the hypoxic cerebral vascular response during sleep. In addition, isocapnic hypoxia had no specific effect on MABP either awake or asleep suggesting that the magnitude of this potential confound would be minor.

Sleep state

To minimize any potential circadian or sleep state differences that might have influenced our finding, all data were collected during the first cycle of III/IV NREM sleep achieved following a ‘normal’ bed time. In addition, this sleep state was chosen for study as it provides a stable condition in which testing can be performed. Physiologically III/IV NREM sleep is of interest, as it is the state in which brain blood flow is at its lowest. Further, we have previously shown that hypercapnic cerebral vascular reactivity is reduced during III/IV NREM sleep (18). Both cardiovascular and respiratory regulation is altered during REM sleep (30); the control of brain blood flow also appears to differ during REM, compared to NREM, sleep (36). It is therefore possible that differences in cerebral vascular reactivity exist between
these states. However, for the present study, in order to limit the level of discomfort experienced by the subjects it was decided to only test during NREM sleep.

**Potential mechanisms of sleep-related changes in hypoxic reactivity**

The mechanisms underlying hypoxic cerebral vasodilatation during wakefulness remain unclear. Low PO$_2$ may directly act on smooth muscle to induce relaxation and, indirectly, may induce the release of vasodilator metabolites. Adenosine and ATP sensitive potassium channels have been suggested, although the relative importance of each remains unclear (10, 27). There is increasing evidence that the mechanisms of action of hypoxia may involve the production of nitric oxide (NO). During wakefulness, NO is reported to mediate hypoxic cerebral vasodilatation in humans (33). During sleep, Zoccoli et al. (35) has also shown that vasodilatory action of NO plays a major regulatory role within the cerebral circulation of lambs. In humans, circulating blood NO levels are reported to be lowest during the night and in the early hours of the morning (8). This leads us to the speculation that a sleep-related reduction in both endothelial and/or neuronal NO production would reduce the vasodilatory capabilities of the cerebral vasculature and consequently reduce the cortical blood flow response to hypoxia.

A sleep-related reduction in vasodilatory capacity alone could not explain the reduction in cortical blood flow below baseline levels that occurred with hypoxia during sleep in the present study. This hypoxia-related reduction in blood flow would suggest the presence of additional hemodynamic factors or hypoxia-related vasoconstrictors that reduce cortical blood flow when the vasodilatory response to hypoxia is minimized.
during sleep. Hypoxic hyperventilation reduces mean pleural pressure and thus reduces both central venous and cerebral spinal fluid pressure, thereby increasing the hemodynamic gradient. Since the hypoxic ventilatory response is greater during wakefulness than sleep (5) this would tend to produce a greater blood velocity response to hypoxia during this state. It is therefore possible that alterations in hypoxic ventilatory response could in part explain the reported findings, however the changes in ventilation were slight making it unlikely to be the sole contributor. A study in goats, in which the hypoxic ventilatory response was controlled, reported no difference in the hypoxic cerebral blood flow response between wakefulness and NREM sleep (28). However, significant differences in the experimental approach (i.e. the use of carbon monoxide, to both induce the state of hypoxia and depress the hypoxic ventilatory response) make a direct comparison between this study and ours difficult.

Hypoxia constricts the pulmonary circulation, increasing the pulmonary artery pressure and hence increasing central venous pressure (CVP) (6). The increase in CVP will reduce the perfusion pressure gradient and therefore tend to reduce cortical blood flow (7). During wakefulness, this effect will be present but is clearly insufficient to mask the predominant vasodilatory action of hypoxia on the cerebral circulation. During sleep, with the loss of the hypoxia related vasodilatation, the effect of the decreased perfusion gradient to reduce cerebral blood flow may become evident.

Alternatively, or in addition, the hypoxia-related reduction in cerebral perfusion may reflect a more predominant action of vasoconstrictor substances during sleep. One potential candidate, for a hypoxia-related vasoconstrictor substance, is endothelin-1. It has a potent constrictor action on the cerebral circulation (9) and, in animals, intermittent
hypoxia induced by sleep apnea increases systemic endothelin-1 levels (17). However, it is not known if hypoxia induces endothelin-1 synthesis or release in the cerebral circulation. A recent study investigating the circadian variation of endothelin-1 reports that its levels are highest during the night and in the early hours of the morning (8). Whether these circadian variations in endothelin-1 contribute to the altered hypoxic cerebral vascular reactivity during sleep is speculative.

**Clinical implication**

Nocturnal hypoxia and hypercapnia are characteristics of cardio-respiratory diseases such as OSA and congestive heart failure. Our studies have shown that the cerebral vascular responses to both hypercapnia (21) and isocapnic hypoxia are drastically reduced or even abolished in healthy subjects during sleep. Failure of the cerebral circulation to respond to hypoxia and hypercapnia would result in hypo-perfusion of the brain leading to impaired neural function and increased risk of cerebral ischemia and stroke (25), a condition with an increased frequency during the early hours of the morning. Such sleep related reductions in cerebral vascular reactivity may be partly responsible for the pathological loss of grey matter and cognitive dysfunction reported within OSA (19, 22) and heart failure (34). It is noteworthy that daytime hypercapnic cerebral vascular reactivity is reported to be impaired in both OSA (23) and congestive heart failure (11); these pathophysiological changes in the control of the cerebral vascular circulation might be further risk factors for these groups.
In conclusion, the present study reports that, in healthy males, the compensatory increase in cortical blood flow in response to hypoxia is reversed during stage III/IV NREM sleep compared to wakefulness. These finding suggest a major state-dependent vulnerability associated with the control of the cerebral circulation. The sleep-related mechanisms underlying these responses remain to be elucidated; they may include direct effects of sleep on the mechanisms regulating the response of cerebral vessels to hypoxia. Alternatively, or in addition, indirect effects of sleep on the cardio respiratory system may limit the cerebral blood flow response to hypoxia.

This work was supported by The Wellcome Trust
References


Legends to figures

Fig 1. The original trace for one subject displays the MCAV, PCO₂, PO₂, and SaO₂ during four conditions: Wake isocapnic euoxia; Wake isocapnic hypoxia -10% SaO₂; NREM sleep isocapnic euoxia and NREM sleep isocapnic hypoxia -10% SaO₂. During wake, MCAV is seen to increase with isocapnic hypoxia; with sleep this response is absent.

Fig 2. a. MCAV during baseline isocapnic euoxia, -5% and -10% SaO₂ (isocapnic hypoxia) during wakefulness (black bars) and sleep (grey bars; mean ± sem, n = 13).

b. Percentage changes (from baseline isocapnic euoxia to isocapnic hypoxia; -10% SaO₂) in MCAV (cm/sec) during wakefulness and sleep for each individual and for the group mean (symbol with sem bars).

Fig 3. VE (a), PCO₂ (b) MABP (c) RR interval (d) during baseline isocapnic euoxia, -5% and -10% SaO₂ (isocapnic hypoxia) during wakefulness (black bars) and sleep (grey bars; mean ± sem, n = 13).

Fig 4. MCAV (n = 13 ± sem) for baseline isocapnic euoxia and during each 5 minute intervention (mins 1-5) : wake and NREM sleep, –5 and –10% SaO₂.
Table 1. Effect of eucapnic and isocapnic euoxia during wake and sleep on cardio-respiratory variables. Results are the mean ± SE, n = 13.

<table>
<thead>
<tr>
<th></th>
<th>MCAV (cm/sec)</th>
<th>VE (l/min)</th>
<th>PCO₂ (mmHg)</th>
<th>MABP (mmHg)</th>
<th>RR (sec)</th>
<th>SaO₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake eucapnia</td>
<td>60.5 ± 3.7</td>
<td>8.6 ± 1.2</td>
<td>41.5 ± 0.9</td>
<td>95.8 ± 1.9</td>
<td>1.2 ± 0.04</td>
<td>99.6 ± 0.1</td>
</tr>
<tr>
<td>Wake isocapnia</td>
<td>60.7 ± 4.0</td>
<td>10.5 ± 1</td>
<td>42.7 ± 0.9</td>
<td>98.1 ± 1.8</td>
<td>1.2 ± 0.09</td>
<td>99.5 ± 0.2</td>
</tr>
<tr>
<td>Sleep eucapnia</td>
<td>55.6 ± 3.1</td>
<td>6.9 ± 0.8</td>
<td>43.7 ± 1.1</td>
<td>75.3 ± 1.9</td>
<td>1.2 ± 0.06</td>
<td>97.6 ± 0.2</td>
</tr>
<tr>
<td>Sleep isocapnia</td>
<td>55.9 ± 3.1</td>
<td>8.0 ± 0.9</td>
<td>44.6 ± 1.0</td>
<td>68.4 ± 2.7</td>
<td>1.1 ± 0.04</td>
<td>97.5 ± 0.2</td>
</tr>
</tbody>
</table>
Fig 1.

Wake: isocapnic euoxia

Wake: isocapnic hypoxia

Stage III/V NREM sleep: isocapnic euoxia

Stage III/V NREM sleep: isocapnic hypoxia
Fig 2.

(a) MCAV (cm/sec) vs. Δ SaO₂

(b) % change in MCAV with different states (Wake, Sleep)

Wake - Black
Sleep - Gray
Fig 3.

(a) 
Ve (L/min)

(b) 
PCO$_2$ (mmHg)

(c) 
MABP (mmHg)

(d) 
RR (sec)

Δ SaO$_2$
Fig 4.

The graph shows the change in MCAV (cm/sec) over time (mins) from wake and sleep baseline conditions. The plot includes data points for wake baseline, wake -5%, wake -10%, sleep baseline, sleep -5%, and sleep -10%. Error bars indicate the variability in the measurements.

- Wake baseline: Black square, solid line
- Sleep baseline: Black square, dashed line
- Wake -5%: Black triangle, solid line
- Wake -10%: Black circle, solid line
- Sleep -5%: White circle, dashed line
- Sleep -10%: White triangle, dashed line