Overnight Changes in the Cerebral Vascular Response to Isocapnic Hypoxia and Hypercapnia in Healthy Humans
Protection Against Stroke

Guy E. Meadows, PhD; Futoshi Kotajima, PhD; Ali Vazir, MBBS; Konstantinos Kostikas, PhD; Anita K. Simonds, MD; Mary J. Morrell, PhD; Douglas R. Corfield, PhD

Background and Purpose—The reduction in hypercapnic cerebral vascular reactivity that occurs in the morning after sleep is associated with an increased risk of cerebral ischemia and stroke. It is not known if the cerebral vascular response to hypoxia is similarly reduced in the morning, but such a reduction could be considered a further risk factor for cerebral vascular disease.

Methods—To test if the cerebral vascular response to hypoxia is reduced in the morning, the overnight changes in the left middle cerebral artery velocity (MCAV) in response to isocapnic hypoxia (IH) and hypercapnia before and after a normal night sleep were determined in 18 individuals.

Results—From evening to morning, hypercapnic cerebral vascular reactivity decreased significantly (evening 2.0 ± 0.4, morning 1.3 ± 0.2 cm/sec/mm Hg; P < 0.05); in contrast, the increase in MCAV in response to IH (−10% SaO2) was unchanged (evening 9.0 ± 1.4, morning 8.7 ± 2.2%; P > 0.05).

Conclusions—Our findings indicate that substantial differences exist in the regulation of the cerebral circulation in response to hypoxia and hypercapnia on waking from sleep. An intact cerebral vascular response to IH, during this time period, could be interpreted as a protective mechanism against cerebral ischemia and stroke; this is of particular relevance to patients with obstructive sleep apnea who arouse from sleep during hypoxia. (Stroke. 2005;36:2367-2372.)

Key Words: cerebral blood flow | hypercapnia | isocapnic hypoxia | middle cerebral artery velocity | nitric oxide | physiology | transcranial Doppler

Hypercapnic cerebral vascular reactivity is reduced in the morning compared with the afternoon and evening.1 This reduction is suggested to be one cause for the increased risk of cerebral ischemia and stroke,2–4 especially during the morning hours.1 The mechanism responsible for this reduction is unknown. However, during nonrapid eye movement (NREM) sleep, cerebral metabolism, blood flow, and the cerebral vascular response to CO2 are all reported to be reduced.5,6 It is therefore possible that the reduction in cerebral vascular reactivity is determined by changes in cerebral vascular regulation during the proceeding sleep period and that this phenomenon may reflect a “carryover” from the sleeping state.

Our laboratory has also reported that the cerebral blood flow (CBF) response to isocapnic hypoxia (IH) is also abolished during NREM sleep.7 However, it is not known if this response is similarly reduced in the morning. Any such reduction would further limit the ability of the cerebral system to adapt to the metabolic demands of the brain and could therefore be interpreted as an additional risk factor. This is of particular relevance to patients with obstructive sleep apnea (OSA) who wake up from sleep during hypoxia and are known to have increased sudden death during sleep.8

The mechanisms behind the reported changes in cerebral vascular regulation are unknown. Research in lambs suggests that nitric oxide (NO) is essential in determining the level of CBF during sleep,9 and in humans, a trend exists for NO levels to be reduced from evening to morning.10 From this, it is plausible to suggest that NO could play an important role in determining the changes in cerebral vascular reactivity during sleep and on awakening in humans.

The present study tested 2 main hypotheses in healthy humans; first, that the cerebral vascular response to IH, in common with the response to hypercapnia, would be reduced during the morning compared with the evening; and second, that NO levels would be reduced in the morning compared with the evening and that these changes would be linked to changes in vascular reactivity.
Methods
Eighteen healthy, nonsmoking, male individuals (mean±standard deviation: age 24±3 years, body mass index 23±3 kg/m²) were studied. No history of cardiopulmonary disease or abnormal lung function was reported, and each subject provided written and informed consent. The study was performed with local ethical approval (Royal Brompton and Harefield Hospital Ethics Committee).

Protocol
The cerebral vascular responses to IH and hypercapnia were assessed during the evening (7 to 8 PM) and the morning (6 to 7 AM) 1 hour after awakening.

A 2-Mhz pulsed Doppler ultrasound system (TC22; Scimed Ltd) was used to determine the velocity of blood in the left middle cerebral artery (MCA) as an index of cerebral blood flow. The MCA was identified by an insonation pathway through the left temporal window, just above the zygomatic arch, using search techniques previously described. In brief, optimization of the Doppler signals from the MCA was performed by varying the sample volume depth in incremental steps and at each depth, varying the angle of insonance to obtain the best-quality signals from the Doppler frequency. A headband (modification of the Welder TCD Fixation; Nicolet EME GmbH) was used to hold the ultrasonic probe ensuring optimal insonation position and angle for the duration of the experiment. The headband and probe were removed for the overnight sleep period; measurements and markings of the probe and headband position were made to allow accurate repositioning for the morning MCAV determinations. The signals were sampled every 10 ms by using a computerized data acquisition system (Micro 1401, Spike 2, CED). For each cardiac cycle, the mean value for the velocity associated with the maximum frequency of the Doppler shift was calculated.

Hypercapnic Intervention
The respiratory circuit consisted of a face mask (B&D Electromedical) connected to a pneumotachograph (model 3700A; Hans Rudolf Inc); perpendicular to this was fitted a 2-way valve (T-shaped nonreturn valve, model 2600; Hans Rudolf Inc) and a 2-m length of 33-mm diameter corrugated plastic tubing attached to the inspiratory limb, which acted as a reservoir into which CO2 could be bled.

The arterial PCO2 was elevated above the baseline by regulating the inspired CO2 load using a constant flow rate technique. Each subject was supplied with a range of flow rates (0, 100, 200, 400, 600, 800 mL/min 79% CO2/21% O2) each being maintained constant for 5 minutes.

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 ±2 mm Hg of a predetermined level, independent of changes in ventilation.

In each subject, the cerebral vascular responses to 4 separate conditions were tested during wakefulness (lying supine, eyes open, watching a video): (1) eucapnic euoxia (spontaneous air breathing); (2) isocapnic euoxia (air breathing with clamped PETCO2); (3) IH (~5% SaO2; FO2 approximately 15% with clamped PETCO2); and (4) IH (~10% SaO2; FO2 approximately 10% with clamped PETCO2). The level of hypoxia was titrated gradually over a few minutes, and once the target SaO2 was reached, each level of hypoxia was maintained constant for a 5-minute period.

General Measurements
Airflow was measured using a pneumotachograph (model 3700A; Hans Rudolf Inc). PETCO2 and PETO2 were determined using rapidly responding gas analyzers (model CD-3A & S-3A; AEL Technologies). Blood pressure was monitored continuously using a plethysmograph BP monitor (Finapres; 2300 Ohmeda). Cardiac intervals (RR) were monitored using an electrocardiography monitor (Lifetrak; HME Ltd). SaO2 was monitored using a pulse oximeter (N-200E; Nellcor). During the night, electroencephalograms (EEG: C3A2, C4A1, O1A2), electrooculograms (EOG: F7A1, F8A1), and a submental electromyogram (EMG) were recorded (model 12; Grass Instruments Co) using the International 10 to 20 system of electrode placement. Sleep was staged using the approach of Rechtschaffen and Kales, and staging was performed with the investigators blinded to the other physiological data. Nocturnal PETCO2, abdominal and rib cage effort band movements (Respirac; Studley Data Systems) and SaO2 were recorded to exclude abnormal sleeping and respiratory effort patterns. The mean nocturnal PETCO2 in each individual was derived from 10 minutes of wakefulness before sleep and during the first and last 10-minute period of light sleep (ie, stage I).

Data Analysis
To ensure a steady state, the data analysis was performed on the last 2 minutes (fourth and fifth minute) of each 5-min period, a technique previously proven to be efficient at producing steady-state hypercapnic and isocapnic hypoxic responses. The hypercapnic cerebral vascular reactivity was characterized by the slope of the linear regression fitted to the mean measurements of MCAV and PETCO2. The cerebral vascular response to IH in each individual was determined as the percentage change in MCAV from baseline isocapnic normoxia to IH (~5 and ~10% SaO2).

Measurement of Nitric Oxide
The total nitrite+nitrate concentration (µmol/L) of plasma was determined in 11 of the subjects. Resting venous blood samples were obtained from an antecubital fossa vein 30 minutes before the assessment of cerebral vascular responses. Blood samples were immediately centrifuged at 1500 G for 10 minutes, and the plasma was removed and frozen at ~70°C for later analysis. The total nitrite+nitrate concentration (µmol/L) was determined using a nitrite+nitrate assay kit (R&D Systems).

Statistical Analysis
Results are presented as group means (±standard error of mean [SEM]). Statistical comparisons between evening and morning MCAV, PETCO2, Ve, MAP, RR interval, and SaO2 responses to IH and hypercapnia were performed using Student paired-sample t tests with the significance threshold being set at P<0.05 (2-tailed). The relationship between the change in total nitrite+nitrate and hypercapnic cerebral vascular reactivity was assessed by determining the correlation coefficient.

Results
Sleep-Related Variables
All subjects were observed to have normal sleep architecture (Table I, available online only at http://www.strokeaha.org), did not snore, and had no sleep-disordered breathing. As expected, the PETCO2 values measured during sleep were significantly higher than those recorded before sleep onset (wake presleep: 41.4±0.8, first 10 minutes of sleep 45.9±0.9, last 10 minutes of sleep 46.1±1.2 mm Hg±SEM, P=0.03).

Evening to Morning Differences
Baseline MCAV, Ve, PETCO2, MAPB, RR interval, and SaO2 remained unchanged between the evening and the morning (P>0.05; n=18; Table).

Original traces from one individual, highlighting cerebral vascular response to hypercapnia and hypoxia, are displayed in Figure 1A and B, respectively. Hypercapnic cerebral vascular reactivity was significantly reduced by an average of 35% from evening to morning (evening: 2.0±0.4 cm/sec/mm Hg, 3.7%/mm Hg; morning: 1.3±0.2 cm/sec/mm Hg, 3.0%/mm Hg; P=0.03; Figure 2). In contrast, over the same
time period, the increase in MCAV in response to IH remained unchanged (SaO₂ -5%; evening: 4.2±1.4, morning: 3.5±1.4; SaO₂ -10%; evening: 9.0±1.4, morning: 8.7±2.2; % change from baseline, P=0.6). The absolute changes in MCAV from baseline isocapnic euoxia to IH (−5 and −10% SaO₂) are presented in Figure 3A; and the percentage changes in MCAV from baseline isocapnic euoxia to IH (−10% SaO₂) are presented for each individual in Figure 3B. VE, MABP, and RR interval responses to hypercapnia and IH were unchanged between evening and morning (P>0.05; Table).

### Total Nitrate + Nitrite Levels

Total nitrate + nitrite levels were lower in the morning compared with the evening; however, this trend was not significant (evening: 34.6±5.5, morning: 29.8±3.9 μmol/L; P=0.07). No relationship was found between the change in total nitrite + nitrate and the change in hypercapnic cerebral vascular reactivity from evening to morning (r²=0.06, P>0.05).

### Discussion

The major finding of this study is that the cerebral vascular response to IH is no different in the evening compared with the morning, 1 hour after awakening. In contrast, over the same time period, morning hypercapnic cerebral vascular reactivity is markedly reduced, a finding that is in agreement with others.1,16

The similarity in the magnitude of the hypoxic cerebral vascular response between the evening and morning is noteworthy, because we have previously reported that this response is absent during NREM sleep; indeed, hypoxia is associated with a reduction in CBF during NREM sleep.7 Although the mechanism responsible for the abolition of this response is unknown, the present data would suggest that, in the majority of individuals, the return of the hypoxic response on waking occurs rapidly. However, in 3 individuals, CBF increased in response to hypoxia during the evening but decreased in response to the same stimulus in the morning (Figure 3B); these reductions are similar to those reported during sleep7 and may indicate individual variation in the rate of recovery of the response on waking. In common with hypoxia, the cerebral vascular response to hypercapnia is also reduced during NREM sleep; because this response to hypercapnia remains low in the morning, it appears that its recovery after sleep is slower than that of hypoxia. These findings, therefore, suggest that substantial differences exist in the regulation of CBF between waking and sleep and between hypoxia and hypercapnia.

The mechanisms responsible for the differences in hypoxic and hypercapnic cerebral vascular regulation are unknown. However, changes in nocturnal PCO₂ could, in part, explain the compromised morning hypercapnic cerebral vascular reactivity. Chronic hypercapnia is associated with a blunted cerebral vascular reactivity to CO₂ in rabbits; patients with OSA who characteristically retain CO₂ also report low cerebral vascular response to CO₂.16,18–20 The relative state of hypercapnia associated with sleep could, therefore, lead to a downregulation of CO₂ or pH receptors and an impaired morning cerebral vascular response to CO₂.16

This is the first study to simultaneously measure changes in NO and cerebral vascular reactivity. Total nitrite + nitrate levels trended toward a decrease from evening to morning; a similar trend was reported by Elherick et al.10 However, the lack of correlation between total nitrite + nitrate levels and hypercapnic cerebral vascular reactivity suggests that changes in total nitrite + nitrate levels from evening to morning do not explain the fall in hypercapnic cerebral vascular reactivity. Circadian variations in vasoconstrictor activity such as increased levels of morning endothelin 110 and/or increases in alpha adrenergic vasoconstrictor activity21 might also contribute to the observed difference.

### Methodologic Considerations

#### Transcranial Doppler Ultrasound

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 MCA diameter remains constant in response to altered Pco₂/Po₂ and/or blood pressure. This assumption has been challenged; however, the majority of research suggests that MCAV is a reliable index of CBF.24–27

#### Statistical Power

Post hoc power calculations suggest that given the very small difference between the means and the standard deviation of the difference, the hypoxic study would need close to 10 000 patients to demonstrate a statistical significance of this magnitude of difference. In contrast, the assessment of hypercapnic cerebral vascular reactivity was adequately powered, with the
total of 18 subjects producing a probability of 91% that the study will detect a treatment difference at a 2-sided 5% significance level. We are therefore confident that the study has been appropriately powered to support our conclusions.

**Reproducibility**

To determine the reproducibility of both these evening measurements, a subset of 12 subjects performed a second test, at the same time of day, within 1 week of the primary observation. The
variability exists between the evening and morning measurements, findings that are consistent with our observations that cerebral vascular regulation is altered over this time period.

**Approximation of Arterial Pco₂**
The P₄₋₅CO₂ is widely accepted as an accurate approximation of the arterial Pco₂ in healthy individuals. Small systematic differences may exist between arterial and end-tidal measurements that are dependent on factors such as body position. However, the magnitudes of such differences are maintained during hypercapnic stimulation, and the use of P₄₋₅CO₂ in the present study would not be a significant confound.

**Clinical Implications**
Cerebral vascular reactivity is considered an index of the capacity of the cerebral vessels to adapt to the metabolic demands of the brain. Any reduction in this property could be interpreted as an increased risk of cerebral ischemia and stroke. Research suggests that the incidence of stroke is highest during the morning hours, especially on awakening, a finding that has been attributed to a reduction in cerebral vascular reactivity. An intact CBF response to IH, during this time period, could be interpreted as a protective mechanism, acting to maintain CBF during any hypoxic episode such as that reported in patients with OSA. It remains to be established if the present observations, in healthy young males, can be extrapolated to “at-risk” groups such as the elderly or those with OSA.

**Summary**
The current study suggests that substantial differences exist in the regulation of the cerebral circulation in response to hypoxia and hypercapnia on waking from sleep. We speculate that cerebral vascular control on awakening will be determined by the proceeding sleep period, a time when cerebral vascular regulation is at its lowest.

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