Influence of controlled breathing patterns on cerebrovascular autoregulation and cardiac baroreceptor sensitivity

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ABSTRACT

Transfer function analysis has become one of the main techniques to study the dynamic relationship between cerebral blood flow and arterial blood pressure, but the influence of different respiratory rates on cerebral blood flow has not been fully investigated. In 14 healthy volunteers, middle cerebral artery blood flow velocity, recorded using transcranial Doppler ultrasound, non-invasive beat-to-beat Finapres blood pressure, ECG and end-tidal CO₂ (ṖETCO₂) levels were recorded with subjects resting supine and breathing spontaneously or at controlled rates of 6, 10 and 15 breaths/min. Transfer function analysis and impulse and step responses were computed at each respiratory rate. ṖETCO₂ levels tended to fall slightly during paced respiration, especially at 15 breaths/min. Controlled breathing rates did not alter transfer function analysis in the frequency range below 0.08 Hz but, above this frequency, the coherence function contained significant peaks corresponding to the respiratory frequencies. The impulse response was similar at all breathing rates, but the step response was characteristic of more efficient autoregulation with reduced ṖETCO₂ levels associated with increasing respiratory rate. The effects of breathing rate and rhythmicity and ṖETCO₂ levels must be considered in studies of cerebral autoregulation.

INTRODUCTION

Through continual adjustment of cerebrovascular resistance, cerebral autoregulation (CA) maintains cerebral blood flow (CBF) at a relatively constant level, despite changes in mean arterial pressure (MAP) in the range 60–150 mmHg [1], with a latency of a few seconds [2–4]. Early research techniques did not allow the time course of the autoregulatory response to be studied but, with the advent of transcranial Doppler ultrasound (TCD), beat-to-beat CBF velocity (CBFV), an acceptable surrogate measure for CBF [5–8], can now be easily recorded in man with temporal resolution that allows the dynamics of CA to be characterized. It has been suggested that the latency of dynamic CA may be the more vulnerable aspect of the mechanism and may be affected first in pathological situations [4,9].

One of the main techniques to study dynamic CA has been the use of transfer function analysis of spontaneous fluctuations in arterial blood pressure (ABP) and CBFV [3,10–13]. In frequency terms, it has been suggested by several authors [11,12,14] that dynamic CA acts as a high-pass filter, dampening CBFV changes relative to ABP changes in the low-frequency range below approx. 0.1 Hz, and allowing a more passive response above this level. Frequency domain analysis has also confirmed previous observations of Diehl et al. [14] and Birch et al. [15] that, under steady-state conditions, CBFV fluctuates with a positive phase in relation to periodic or quasi-periodic changes in ABP.

Key words: cerebral autoregulation, controlled respiration, paced breathing, transcranial Doppler ultrasound, transfer function analysis.

Abbreviations: ABP, arterial blood pressure; BP, blood pressure; BRS, baroreceptor sensitivity; CA, cerebral autoregulation; CBF, cerebral blood flow; CBFV, CBF velocity; LED, light-emitting diode; MAP, mean arterial pressure; MCA, middle cerebral artery; ṖETCO₂, end-tidal partial pressure of CO₂; Ṗaco₂, arterial CO₂ partial pressure; TCD, transcranial Doppler ultrasound.

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Although the well known influence of arterial CO\textsubscript{2} partial pressure (Paco\textsubscript{2}) on CA has also been characterized in the frequency domain \cite{12,16}, there is little information about the effect of respiratory rate on transfer function analysis of dynamic CA. Previous work \cite{17} has shown respiratory rate to have powerful physiological effects, for example, on cardiac baroreceptor sensitivity (BRS) independent of direct effects on ABP. Physiological studies have employed various controlled respiratory rates in the spectral analysis estimates of, for example, CA using 6 breaths/min \cite{13,14}, and cardiac BRS using 15 breaths/min \cite{17}. However, work using the Valsalva and Mueller manoeuvres \cite{18–20} has shown that changes in intrathoracic pressure can have a strong influence on the CBFV response to transient changes in ABP. As the changes in intrathoracic pressure are likely to differ at different breathing rates, we postulate that this may influence estimates of dynamic CA. Clarification of this point is relevant to the design of future studies in CA, especially if, for example, concurrent measurement of cardiac BRS is planned as often happens. To date, it is not clear whether synchronized breathing leads to the same results as transfer function analysis of spontaneous fluctuations in ABP and CBFV, obtained during normal non-controlled breathing, or what the effects of synchronized breathing at higher rates might be on CA. Breathing at different frequencies will induce oscillations in ABP that will stimulate CA in different spectral bands. At frequencies above 0.1 Hz (i.e. 6 breaths/min), it is expected that CBFV will show a passive response, but the reliability and reproducibility of transfer function analysis estimates in lower frequency bands, where dynamic CA is more active \cite{12}, might be affected. These considerations led to the main hypothesis of our present study that synchronized breathing at different rates will have a significant influence on transfer function analysis estimates of dynamic CA and their reproducibility compared with results obtained during spontaneous breathing.

\section*{METHODS}

\subsection*{Subjects}

Fifteen healthy volunteers aged between 23 and 51 years were recruited from the Leicester University Departments of Medicine for the Elderly and Medical Physics. None of the volunteers had a history of cardiovascular or neurological disease or was taking any medication known to affect cardiovascular or cerebrovascular responses.

\subsection*{Ethical approval and consent}

The research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and the local Leicestershire Ethical Committee approved the study. All participants gave written informed consent.

\section*{Protocol}

Subjects avoided caffeine, nicotine and alcohol for 12 h prior to the CA recordings and were studied at least 2 h post-prandially. Studies were conducted in a dedicated research room kept at a constant temperature (20–24 °C) with external stimuli minimized.

The middle cerebral arteries (MCAs) were insonated bilaterally as described by Aaslid et al. \cite{21}, and CBFV was measured using TCD (QVL 842X; SciMed, Bristol, U.K.). A three-lead surface ECG was fitted and beat-to-beat ABP was measured non-invasively by a servo-controlled plethysmograph (Finapres 2300; Ohmeda, Englewood, CO, U.S.A.) on the middle finger of the left hand, supported at mid-axillary level throughout the studies. Expired CO\textsubscript{2} was measured via a closely fitting facemask and an infrared capnograph (Capnogard; Novametrix Medical Systems, Wallingford, CI, U.S.A).

Three 5-min recordings were made at respiratory rates of 6, 10 or 15 breaths/min, guided by a light-emitting diode (LED) that was on for inspiration and off for expiration, and a 10-min recording was made with the patient breathing spontaneously. During paced respiration, subjects were asked to try to avoid deep breathing. The four recordings were performed in random order with a 5-min interval between them to allow mean Paco\textsubscript{2} levels to normalize if they had been affected by the fixed respiratory rates.

TCD, Finapres BP, ECG and expired CO\textsubscript{2} output signals were continuously recorded on digital tape (Sony PC-108M).

\section*{Data analysis}

The digital audiotape recording was downloaded on to a dedicated microcomputer and a fast Fourier transform was used to extract the maximum frequency velocity envelope with the use of a time window of 5 ms. BP, ECG and expired CO\textsubscript{2} recordings were also sampled at a rate of 200 samples/s. The BP signal was calibrated at the start of each recording, all signals being visually inspected for artefacts or noise. Narrow spikes on the CBFV signals were removed by linear interpolation and the four signals were low-pass filtered with a zero-phase eighth-order Butterworth digital filter with a cut-off frequency of 20 Hz. The beginning and end of each cardiac cycle was detected from the ECG and beat-to-beat estimates of mean CBFV and mean BP were obtained by integration. The end-tidal partial pressure of CO\textsubscript{2} (Petco\textsubscript{2}) was detected by a special algorithm under visual inspection. Beat-to-beat values of mean CBFV and MAP and breath-by-breath values of Petco\textsubscript{2} were interpolated with a third-order polynomial and resampled at 0.2 Hz to produce signals with a uniform time-base.
Baseline demographic data for 14 subjects

Data are presented as means ± S.D. (range). Spontaneous respiration is equivalent to 13 ± 2 breaths/min. BPs and heart rate were measured using Finapres. PETCO$_2$ and CBFV data represent the means for the whole recording. Mean CBFV was significantly lower at controlled respiratory rates 6, 10 and 15 breaths/min compared with spontaneous respiration. Mean CBFV was also significantly lower at 15 breaths/min compared with CBFV at 6 breaths/min.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6 breaths/min</th>
<th>10 breaths/min</th>
<th>15 breaths/min</th>
<th>Spontaneous respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124 ± 15 (100–153)</td>
<td>126 ± 14 (104–150)</td>
<td>128 ± 17 (104–168)</td>
<td>123 ± 15 (98–152)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 ± 7 (55–81)</td>
<td>66 ± 10 (50–90)</td>
<td>68 ± 8 (58–88)</td>
<td>65 ± 8 (51–81)</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>83 ± 9 (70–103)</td>
<td>85 ± 11 (69–109)</td>
<td>87 ± 10 (73–112)</td>
<td>84 ± 9 (71–102)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64 ± 8 (53–80)</td>
<td>67 ± 9 (53–85)</td>
<td>66 ± 12 (54–92)</td>
<td>64 ± 9 (49–94)</td>
</tr>
<tr>
<td>PETCO$_2$ (mmHg)</td>
<td>41 ± 6 (29–49)</td>
<td>38 ± 8 (23–47)</td>
<td>36 ± 7 (22–44)</td>
<td>41 ± 5 (29–48)</td>
</tr>
<tr>
<td>CBFV (cm/s)</td>
<td>58 ± 14 (31–78)</td>
<td>58 ± 15 (31–77)</td>
<td>54 ± 13 (29–76)</td>
<td>62 ± 13 (39–79)</td>
</tr>
</tbody>
</table>

CA

Cross-spectral analysis of CBFV and ABP recordings was performed as described previously by Panerai et al. [3]. The impulse response was obtained by the inverse Fourier transform using the same fast Fourier transform algorithm and the impulse response was numerically integrated for values of time > 0 to obtain the step response.

Using these analyses, comparison between right and left CBFVs, different respiratory rates and first and second halves of each recording were made.

BRS calculation

Cardiac BRS (combined α) was estimated using spectral analysis techniques explained previously [22] involving fast Fourier transform for pulse interval and systolic BP.

Statistical methods

Demographic data are presented as means ± S.D. (range).

The difference in PETCO$_2$ between the beginning and end of the data collection period was compared using Student’s paired t test, and the difference in PETCO$_2$ at different respiratory rates and between patients was compared using a two-way ANOVA.

For each group of records for comparison, i.e. right compared with left CBFV and first compared with second half at each respiratory frequency, and all records at each respiratory rate, the mean and standard error of squared coherence and gain frequency response were computed for each of the first 30 harmonics up to 0.28 Hz. Differences between the right and left CBFVs and the first and second halves of the recording at each respiratory frequency were tested with Student’s t test for comparisons of individual harmonics.

A linear mixed effects model including patients as random effects was used to explain the variation in coherence and gain of the transfer function for each harmonic. Linear mixed effects models were also used to explain the variation in step (values taken at 4 s) and of the maximum and minimum deflections of the impulse response function between the four respiratory rates. In comparison of the step and impulse responses, the models included patients as random effects and breathing rates and PETCO$_2$ as fixed explanatory variables.

The statistical packages SPSS 10.0.5 for Windows and ‘R’ (version 1.7.1) were used, and statistical significance taken at the 5% level.

RESULTS

Fifteen volunteers were studied but, in one subject, there were technical problems with the recording and data from this subject were excluded from the analysis. In 12 subjects, bilateral CBFV signals were obtained for comparison of right and left CBFVs, and in a further two subjects only right CBFV signals were obtained, giving data from 14 subjects (7 male; aged 32 ± 9 years (range, 23–51 years)). Finapres BP, heart rate, PETCO$_2$ and CBFV at the different respiratory rates are shown in Table 1.

CBFV during spontaneous respiration was 62 ± 14 and 65 ± 12 cm/s for the right and left MCAs respectively, with no significant difference between the two sides for the 12 subjects with bilateral signals (P = 0.977). CBFV was lower at all controlled respiratory rates than during spontaneous respiration and at 15 compared with 6 breaths/min.

During controlled respiration, both the ABP and CBFV traces showed a sinusoidal modulation at the respiratory frequency of the recording with an apparent phase lead in CBFV (Figure 1). The respiratory modulation was confirmed by well-defined peaks in the ABP and CBFV power spectra at 0.1, 0.17 and 0.25 Hz and their multiple harmonics in the recordings made at 6, 10 and 15 breaths/min respectively (Figures 2a–2c).

PETCO$_2$ and respiratory rate

Two-way ANOVA showed that PETCO$_2$ (Table 1) differed significantly between patients (F = 16.5, P < 0.001) and between the four respiratory rates (F = 7.5, P < 0.001). On average, PETCO$_2$ fell steadily during the 5-min
recordings at all controlled respiratory rates, although not in all patients, but did not fall during the 10-min recording with spontaneous respiration. The mean difference in $P_{\text{ETCO}}_2$ between a 10 s mean value at the beginning and end of the recordings was $1.5 \pm 2.7$ mmHg ($P = 0.053$), $1.2 \pm 2.0$ mmHg ($P = 0.044$) and $2.8 \pm 3.0$ mmHg ($P = 0.003$) at respiratory rates of 6, 10 and 15 breaths/min respectively, and $-0.3 \pm 1.3$ mmHg ($P = 0.363$) with spontaneous respiration. However, $P_{\text{ETCO}}_2$ fell by 5 mmHg or more on four occasions, in three patients at 15 breaths/min ($-5$ mmHg twice and $-11$ mmHg) and again in one of these patients at 6 breaths/min ($-9$ mmHg). The average respiratory rate calculated for each 10-min spontaneous respiration recording was $13 \pm 2$ (range, 10–18) breaths/min ($0.22 \pm 0.04$ Hz). The CBFV power spectrum for spontaneous respiration contained a muted peak just above 0.2 Hz that was not apparent in the MAP spectrum, although it would coincide with the median spontaneous respiratory rates (Figure 2d).

**Transfer function analysis**

In the 12 subjects with bilateral TCD data, the coherence and gain frequency spectra and impulse and step responses were not significantly different from one another whether the right or left MCA CBFV was used, and the averages of right and left sides were used in further analysis, giving data for 14 volunteers for further comparisons.

At respiratory rates of 6, 10 and 15 breaths/min, the results of cross-spectral analysis of CBFV and ABP showed marked peaks in the coherence function at the fundamental respiratory frequencies used in the recordings, 0.10, 0.17 and 0.25 Hz, and their multiple harmonic frequencies in comparison with one another and with the coherence function for spontaneous respiration (Figure 3a). The coherence functions were not significantly different from one another below 0.08 Hz but, above this, differed at the peaks and troughs described (Figure 3a). The coherence was greater than 0.90 at the fundamental respiratory frequencies for 6, 10 and 15 breaths/min and at the first multiple harmonic of the respiratory frequency at 6 breaths/min. The ratio of the size of the fundamental to the first harmonic was $0.98 \pm 0.04$, $1.07 \pm 0.05$ and $1.17 \pm 0.04$ at 6, 10 and 15 breaths/min respectively, i.e. at 6 breaths/min, the first multiple harmonic of the respiratory frequency was slightly larger than the peak at the fundamental breathing frequency.

The gains of the transfer functions were similar at the different respiratory rates and during spontaneous respiration (Figures 3b and 3c). The phase leads of CBFV
at the different respiratory rates were $42 \pm 24^\circ$, $37 \pm 14^\circ$ and $18 \pm 12^\circ$ at 6, 10 and 15 breaths/min respectively, taken from the phase frequency response of the transfer function analysis at each respiratory rate.

The phase frequency response is represented in Figure 3, but was not statistically compared between the breathing rates, because of the difficulties in comparing angular measurements.

**Impulse and step responses**
The impulse response was not significantly different between data recorded under any of the controlled respiratory rates or during spontaneous respiration (Figure 4). Using the linear mixed effects model to explain the variation in step response where no correction for $P_{ETCO_2}$ was used, the effect of respiratory rate alone on step response was not significant ($P = 0.0508$). However, there was a visible trend for the step response to return to lower values as the breathing rate increased (Figure 5). $P_{ETCO_2}$ was found to have a significant effect on step response even when correcting for breathing rate ($P = 0.03$), whereas the effect of breathing rate when correcting for $P_{ETCO_2}$ on step was not significant ($P = 0.40$; Figures 5 and 6).

**Short-term reproducibility**
There were minor differences in the transfer function analysis for the first and second halves of the recording, mostly over very short frequency ranges (see below). During controlled respiration, coherence functions between first and second halves of the recordings were slightly lower in the second half of the recording within a short frequency range of 0.1–0.14 Hz. At 6 breaths/min,
Cardiac BRS
Cardiac BRS (combined α) was $31.2 \pm 20.7$, $20.3 \pm 11.4$, $18.4 \pm 12.5$ and $24.0 \pm 15.9$ ms/mmHg during spontaneous respiration and at 6, 10 and 15 breaths/min respectively, and was significantly greater at a respiratory rate of 6 breaths/min than when measured under any of the other conditions of the study. Cardiac BRS measured at 15 breaths/min was lower than during spontaneous respiration ($P = 0.047$).

DISCUSSION

Different controlled respiratory rates have been used in physiological studies where CA [13,14] or cardiac BRS [20] have been measured. Cardiac BRS and CA are often measured concurrently and, although respiratory rate is well known to have profound effects on estimates of cardiac BRS, to our knowledge, this is the first study comparing the effect of several different respiratory rates (6, 10 and 15 breaths/min and spontaneous respiration) on the transfer function between ABP and CBFV. In the present study, we were not endeavouring to stimulate dynamic CA with these breathing patterns, but gauge the influence of controlled respiration compared with spontaneous breathing on dynamic CA measurements.

Below 0.08 Hz where, according to the high-pass filter model [11,12,14], dynamic CA should act, the coherence and gain of the transfer functions were remarkably similar at all breathing rates (Figure 3). The step response indicated a trend towards a more efficient return of CBFV.
to baseline as $\text{PETCO}_2$ decreased, which was associated with increasing respiratory rate (Figure 5).

A large part of the variability in CBFV is related to ABP variability, as supported by a coherence function above 0.5 over much of the frequency range, and some of the CBFV variability independent of ABP can be explained by breath-by-breath fluctuations in $\text{PETCO}_2$ [23]. Disproportionately large changes in CBFV relative to the change in ABP have been recorded during the release phase of the Valsalva manoeuvre [19] and, on a smaller scale, changes in intrathoracic pressure during normal breathing may also add to CBFV variability on a breath-by-breath basis independent of their effects on ABP and $\text{PETCO}_2$. Other sources of CBFV variability could be associated with systemic changes in mean $\text{PaCO}_2$ or oxygen tension, central modulating mechanisms, intrinsic cerebral vasomotion or mental activation [24–27]. Controlled respiratory rates might potentially control some of these variables by reducing the randomness of the respiratory effects and by ‘clamping’ mental activation through concentration on the LED.

In the case of patients with significant carotid artery stenosis, although Reinhard et al. [13] were able to grade dynamic CA using spontaneous respiration and deep breathing at 6 breaths/min, the inter-method agreement was poor-to-moderate.

The coherence functions for the different controlled respiratory frequencies were significantly different from one another at the fundamental respiratory frequencies and their multiple harmonics, and the coherence function for all of the controlled respiration data was periodically higher and lower compared with the data recorded with spontaneous respiration. The observation that at 6 breaths/min, in contrast with the other respiratory frequencies, the peak in coherence at the fundamental frequency was lower than its first harmonic could be interpreted as a sign of autoregulation in that frequency range that is less effective with increasing frequency, particularly above 0.17 Hz, as this is the next peak we can look at.

Although we decided not to compare phase frequency responses statistically, because of the difficulties in comparing angular measurements, the effect of phase is incorporated in the time domain measures. The step responses suggested that, in response to a step in ABP, CBFV would subsequently fall towards baseline in slightly less than 5 s in all recordings, consistent with active CA, as reported from previous observations [3] (Figure 5). The step responses were characteristic of improved CA [3], with decreasing $\text{PETCO}_2$ associated with the increasing respiratory rate (Figure 6), although the effect of respiratory rate alone was just not significant.

In other studies, changes thought to reflect impairment of CA with mean $\text{PaCO}_2$ levels have been seen using transfer function and time domain analysis when $\text{PETCO}_2$ increased by 5 mmHg or more [12,16]. Coherence function and gain frequency response increased for frequencies below 0.05 Hz, and phase frequency response decreased in the frequency ranges 0.02–0.1 Hz [16] and 0.07–0.20 Hz [12].

The mean $\text{PETCO}_2$ for the recordings fell as the respiratory rate increased with a difference of 5 mmHg between respiratory rates of 6 and 15 breaths/min, a difference that would be expected to cause changes in the transfer function analysis and may explain why the step response at 15 breaths/min was suggestive of improved autoregulation. The absence of a significant difference in the other components of the transfer function may indicate that the step response is the most sensitive and discriminating parameter produced by this analysis. The change in $\text{PETCO}_2$ for the different breathing rates may also explain the falling mean CBFV with increased breathing rate.

There is no evidence from previous studies to suggest that, under basal conditions in normal subjects, dynamic CA differs between right and left hemispheres. The components of the transfer functions and impulse and step responses obtained in the present study were very similar between the two sides recorded, confirming the good quality of the signals. As a measure of reproducibility of the technique, the first and second halves of the recordings were compared and, despite small reductions in $\text{PETCO}_2$ seen at controlled respiratory rates, there were only minor differences in the components of the transfer functions, and impulse and step responses were not significantly different between the two halves.

**Methodological limitations**

The Finapres non-invasive finger BP monitor was used, rather than intra-arterial measurements, in the present study for ethical reasons and has been shown by various authors to be successful in accurately mirroring dynamic beat-to-beat BP changes [28] and MAP variability.

The use of CBFV as a surrogate for CBF is only valid if the diameter of the insonated vessel remains constant. Direct imaging has shown [6,8] only minor or no alteration in MCA diameter during changes in ABP or $\text{PETCO}_2$ levels larger than those found in the present study. It is therefore unlikely that there would be any significant changes in MCA diameter during the smaller BP oscillations induced by controlled respiration.

The coherence function is a linear measure of the power in the CBFV spectrum, which is caused by power in the BP spectrum and is influenced by non-linearity of the relationship between the two, the signal-to-noise ratio and factors that independently influence either BP or CBFV. Although dynamic CA is unlikely to be a linear system, the BP oscillations we have induced are small enough to assume approximate linearity, and the high values of coherence function seen verified this approach.
During a protocol where respiration is altered, it is very difficult to avoid fluctuations in PetCO2. Although all patients received the same instruction on breathing in time with the LEDs and were asked to avoid breathing too deeply, the effect of the different respiratory rates on PetCO2 did differ between patients with implications for CA in the individual.

Conclusion
Synchronized breathing rates above 6 breaths/min do not influence frequency domain estimates of dynamic CA, as reflected by the coherence function and gain frequency responses below 0.08 Hz, despite the shift in ABP variability to higher frequencies. In the time domain, differences in CBFV step responses to the ABP change can be observed at higher breathing rates (15 breaths/min), but these resulted from accompanying reductions in PetCO2, rather than the mechanical effects of respiration. These results also have implications for the design of clinical or research protocols where breathing rate might need to be controlled, to allow simultaneous studies of BRS, or in conjunction with cognitive tasks to exert control on the metabolic component of the CBFV response. The need to monitor CO2 closely when studying dynamic CA is reinforced.

ACKNOWLEDGMENTS
We thank Dr Lingke Fan for his work in developing the CBF Doppler analyser.

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Received 3 June 2003/7 August 2003; accepted 1 October 2003
Published as Immediate Publication 1 October 2003, DOI 10.1042/CS20030194

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