# Detection of impaired cerebral autoregulation improves by increasing arterial blood pressure variability

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**Running headline:** Assessment of dynamic cerebral autoregulation

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**Number of words**: 3,514
**Tables**: 2
**Figures**: 3
**References**: 28

## Acknowledgements

This study was supported by the UK EPSRC grants EP/G008787/1and EP/G010420/1

**Abstract**

Although the assessment of dynamic cerebral autoregulation (CA) based on measurements of spontaneous fluctuations in arterial blood pressure (ABP) and cerebral blood flow (CBF) is a convenient and much used method, there remains uncertainty about its reliability. We tested the effects of increasing ABP variability, using a modification of the thigh cuff method, on the ability of the autoregulation index (ARI) to discriminate between normal and impaired CA, using hypercapnia as a surrogate for dynamic CA impairment. In 30 healthy volunteers ABP (Finapres) and CBF velocity (CBFV, transcranial Doppler) were recorded at rest and during 5% $CO_2$ breathing, with and without pseudo-random sequence inflation and deflation of bilateral thigh cuffs. The application of thigh cuffs increased ABP and CBFV variabilities and was not associated with a distortion of the CBFV step response estimates for both normocapnic and hypercapnic conditions ($p = 0.59$ and $p = 0.96$ respectively). Sensitivity and specificity of CA impairment detection were improved with the thigh cuff method, with the area under the receiver-operator curve increasing from 0.72 to 0.85 ($p = 0.023$). We conclude that the new method is a safe, efficient and appealing alternative to currently existing assessment methods for the investigation of the status of cerebral autoregulation.

**Keywords:** arterial blood pressure; cerebral autoregulation; cerebral blood flow; pseudorandom binary sequences

## Introduction

Cerebral autoregulation (CA) is a complex homeostatic mechanism whose action maintains a relatively constant flow of blood in the face of perturbations in arterial blood pressure (ABP), and protects the cerebral parenchyma from hyper- or hypo-perfusion injury (Paulson *et al* 1990; van Beek *et al* 2008).

The advent of instrumentation that allows continuous noninvasive monitoring of cerebral blood flow (CBF) and ABP with excellent temporal resolution, has caused a shift in our understanding of CA dynamics (Aaslid *et al* 1989). Instead of relying on vasoactive drugs to induce large stable changes in ABP, as required by the traditional 'static' approach (Paulson *et al* 1990; Tiecks *et al* 1995), the dynamic CA response can be identified using different maneuvers such as the sudden release of compressed thigh cuffs (Aaslid *et al* 1989), rhythmic hand grip (Kwan *et al* 2004), Valsalva maneuver (Tiecks *et al* 1996), changes in posture (Claassen *et al* 2009; Lipsitz *et al* 2000) and others that can induce transient alterations in ABP. Most of these maneuvers are difficult to implement in a clinical setting due to the need for patient cooperation, parallel increases in sympathetic activity, or safety of protocols in vulnerable patients. For these reasons, one important alternative, which has been favoured by many centers, is the use of spontaneous fluctuations in ABP and CBF coupled with system identification techniques to derive parameters that can reflect the efficacy of dynamic CA (Panerai 1998). In particular, transfer function analysis using ABP as input and CBF velocity (CBFV) as output yields estimates of the gain and phase frequency responses which have been shown to be markers of dynamic CA in multiple clinical conditions such as stroke, carotid artery disease, severe head injury, diabetes and subarachnoid haemorrhage (Panerai 2008; van Beek *et al* 2008). Moreover, the ARI, an index of dynamic CA initially validated using thigh cuff maneuvers (Tiecks *et al* 1995), can also be derived from

spontaneous fluctuations (Panerai *et al* 1998). The ARI ranges from 0 (absence of CA) to 9 (best observed CA) and has also been shown to discriminate between different groups of patients (Panerai 2008).

Despite encouraging results, most studies described in the literature on the use of spontaneous fluctuations in ABP and CBF to assess dynamic CA were based on differences between groups rather than individual subjects. Consequently, very limited information is available about the sensitivity and specificity of this approach and hence its overall diagnostic accuracy. One ongoing concern about the use of spontaneous fluctuations for assessment of dynamic CA is the potential lack of substantive variability in ABP and CBF which could lead to poor signal-to-noise ratio and limitations in reproducibility (Brodie *et al* 2009; Claassen *et al* 2009; Gommer *et al* 2010; Liu *et al* 2005; Zhang *et al* 1998b). Following the recent development of a new approach to increase ABP variability (Katsogridakis *et al* 2012), we have made use of the well known effect of hypercapnia as a surrogate of CA impairment (Aaslid *et al* 1989; Panerai *et al* 1999; Paulson *et al* 1990) to test the hypothesis that detection of impaired CA is improved by increases in ABP variability.

## Materials and Methods

### Subjects and measurements

Ethical approval was obtained from the local ethics committee prior to commencing the study. Volunteers were recruited if they were normotensive and their medical history was free of known cardiovascular and neurological disorders. On the day of the trial, participants were reminded of the protocol and written informed consent was obtained.

Participants were asked to assume a supine position, and following a brief settling down period brachial ABP was measured by means of oscillometric sphygmomanometry. Bilateral thigh cuffs and the face mask were then attached, and a trial inflation/ deflation cycle was performed to familiarize volunteers with the procedure and to ensure that the flow of air to the thigh cuffs was uninterrupted. The thigh cuff inflation system was described in detail in a previous communication (Katsogridakis *et al* 2012). In summary, the system comprises a compressor and high-pressure reservoir that holds the air required to inflate bilateral thigh cuffs. The flow of air to the cuffs is controlled by the coordinated action of a pressure regulator, a boost valve, an adaptive controller and a deflation valve. Cuffs are alternately inflated and deflated (to a maximum pressure of 150 mmHg) following a pseudo-random binary sequence (PRBS) which increases ABP variability over a wider frequency range ( > 0.05Hz) than would be obtained by using a fixed inflation/deflation frequency (Katsogridakis *et al* 2012). Delivery of 5% $CO_2$ in air is achieved through a face mask (Vital Signs Inc., Totowa, NJ), which is connected to the $CO_2$ delivery subunit. The subunit comprises a y-valve that controls whether carbon dioxide or air is being administered and a 200 litre Douglas bag is used to store the $CO_2$/air mixture. Hardware and software included necessary safety features to protect the subjects from unintended exposure to $CO_2$ and excessive thigh cuff inflation pressure (Katsogridakis *et al* 2012).

Arterial blood pressure was monitored non-invasively using the arterial volume clamping method (Finometer, Finapres Medical Systems, Amsterdam). Freehand transcranial Doppler (Companion III, Viasys Healthcare) identification of both middle cerebral arteries was performed and probes were then held in place with a custom-built head frame. The face mask was connected to the $CO_2$ delivery system and to the capnograph (Datex, Normocap 200) to monitor end-tidal $CO_2$ ($EtCO_2$) levels. A 3-lead surface electrocardiogram (ECG) was also recorded.

Following a 10 min period of supine rest, participants underwent a 5 min baseline recording. Three additional five-minute recordings were then performed in random order to cover all possible combinations of $CO_2$ administration (no $CO_2$ administration and 5% constant $CO_2$ administration) and thigh cuff inflation (no thigh cuff inflation and PRBS driven thigh cuff inflation).

**Data analysis**

Signals were sampled at a rate of 500Hz and stored on a dedicated personal computer for offline analysis. All recordings were visually inspected, the ABP signal was calibrated and narrow spikes (<100ms) and artefacts were removed. Subsequently, all signals were filtered in the forward and reverse direction using an eighth-order Butterworth low pass filter with a cut-off frequency of 20Hz.

The beginning and end of each cardiac cycle were detected from the ECG signal and the heart rate (HR) was estimated to obtain mean beat-to-beat values for the recorded signals. Estimates were then interpolated using a third-order polynomial and resampled at 5Hz to create a time series with a uniform time base.

Auto- and cross-power spectral densities were estimated using the Welch averaged periodogram method, employing a 102.4s window (512 samples), with a 50% overlap. The complex transfer function *H(f)* between ABP and CBFV was estimated as:

$$H(f) = \frac{Syx(f)}{Sxx(f)}$$

where *Syx(f)* is the cross-power spectral density between signals *y(n)* and *x(n)* and *Sxx(f)* the auto-power spectral density of signal *x(n).* The inverse Fourier transform was subsequently used to estimate the CBFV impulse response function from *H(f),* whilst the CBFV step response function was obtained by integrating the CBFV impulse response (Zhang *et al*

1998a). These were in turn used to derive the autoregulation index (ARI) using Tiecks model, employing the mean square error criterion (Tiecks *et al* 1995).

**Statistics**

The Shapiro-Wilk test was used to test for normality. Non-normally distributed data were log-transformed. Repeated measures ANOVA was used to test the effect of different maneuvers on measured and derived parameters (ABP, CBFV, $EtCO_2$ and ARI). Right and left MCA estimates of ARI were averaged if not significantly different as assessed with the paired Student's t-test. To assess whether the use of thigh cuffs led to distorted estimates of dCA, compared to those obtained from the use of spontaneous fluctuations, the value of the CBFV step response function at 6s was obtained for every volunteer and paired Student's t–tests were performed between the two estimates for normocapnic and constant hypercapnic conditions.

Sensitivity and specificity were used as measures of the performance of ARI in detecting hypercapnia induced dCA impairment (Panerai *et al* 1999; Paulson *et al* 1990). Sensitivity *(Sn)* was estimated as:

$$Sn = \frac{TP}{FN + TP}$$

where TP is the number of true positive volunteers (impaired dCA) and FN the number of false negatives. Specificity *(Sp)* was estimated as:

$$Sp = \frac{TN}{FP + TN}$$

where TN is the number of true negative volunteers (intact dCA) and FP the number of false positives. The receiver-operator characteristic (ROC) curve was plotted as *Sn* versus (1-*Sp*)

by using all discrete values of ARI between 0 and 9 as thresholds (cutpoints) for impairment of CA.Values of ARI<threshold were assumed to indicate positive cases (CA impairment). For normocapnia these represented FP and for hypercapnia TP. The reverse classification applied to ARI values above threshold. ROC curves were constructed for baseline and also for PRBS driven thigh cuff inflation and deflation. The improvement in ROC detection characteristics due to increased ABP variability was assessed by testing the areas under the curve with the method proposed by DeLong et al. (DeLong *et al* 1988). Values of $p<0.05$ were considered to indicate statistical significance.

## Results

A total of 30 healthy adult volunteers (13 female) aged 22 to 55 years were recruited. The procedure was well-tolerated and good quality, complete sets of data were obtained for all subjects. Participant demographics and population-averaged values for the main parameters are given in Tables 1 and 2, respectively. Figure 1 shows representative recordings obtained for baseline and the three different maneuvers. Significant changes in all main parameters in Table 2 were observed as the combined result of hypercapnia and operation of the thigh cuffs (ANOVA). Post hoc analysis indicated that heart rate changed due to hypercapnia but not due to PRBS inflation and deflation of thigh cuffs.

The use of PRBS inflation and deflation of thigh cuffs resulted in increased ABP variability both during normocapnia ($9.2 \pm 10.5$ to $17.6 \pm 14.5$ $mmHg^2/Hz$, $p=0.011$) and hypercapnia ($11.0 \pm 8.6$ to $22.3 \pm 18.4$ $mmHg^2/Hz$, $p=0.001$), as assessed by average power spectral values over the frequency range 0-0.1 Hz. The increase in variability did not affect estimates

of CBFV step responses (Fig. 2). Impairment of dynamic CA due to hypercapnia was confirmed by the incomplete return of the CBFV step response baseline (Fig. 2B) with correspondingly smaller values of ARI (Table 2). Step response values at 6s were not different for baseline compared to thigh cuff operation for both normocapnia (0.03 ± 0.33 vs. 0.02 ± 0.25, p=0.56) and hypercapnia (0.42 ± 0.43 vs. 0.42 ± 0.37, p=0.96). ARI values from the right and left MCAs were not significantly different and were averaged for the ROC analysis to follow. The change in right and left MCA averaged ARI values induced by PRBS controlled thigh cuffs was not significant either for normocapnia (-0.15 ± 1.19; p=0.49) or hypercapnia (0.43 ± 1.90; p=0.27).

Both ROC curves in Fig. 3 showed good classification characteristics in comparison with the line of indifference (random choice of impaired/unimpaired). Clearly superior results with improvements in sensitivity for any specificity (except near *Sp*=0 – and of little practical importance) were obtained due to increased ABP variability induced by PRBS cuff inflation and deflation. The area under the ROC curve increased from 0.72 for baseline to 0.85 during cuff operation (p=0.023). The optimal operating point, often defined as the point closest to *Sp*=1 *Sn*=1, also displays higher sensitivity and specificity for the thigh-cuff inflations.

## Discussion

### Main findings

The current study provided confirmation that PRBS controlled inflation/deflation of bilateral thigh cuffs induces significant increases in ABP variability compared to spontaneous fluctuations recorded at rest. Of note, this study enrolled a much larger population and none of the subjects were the same as those who participated in the original pilot study (Katosogridakis et al 2012). The main new finding of relevance though is that increased ABP led to improved detection of CA impairment induced by hypercapnia. This improvement did not depend on the selection of a specific value of the autoregulation index (ARI), but was clearly apparent from the shift of the ROC curve (Fig. 3).

One important additional finding, was that dynamic CA, either expressed by the entire CBFV step response curve or the ARI index, did not change significantly due to the random inflation and deflation of thigh cuffs. At first sight this might look contradictory, but closer examination showed that small changes in individual values of ARI did take place, these did not influence the population distribution at either normocapnia or hypercapnia, but significantly improved the consistency of the ARI drop from hypocapnia to hypercapnia which led to the changes observed in the ROC curve.

**Role of ABP variability**

Concerns about the influence of ABP on the reliability of parameters used for assessment of dynamic CA have been raised in previous studies (Gommer *et al* 2010; Liu *et al* 2005; Zhang *et al* 1998b) with the suggestion that different protocols, such as the sit-to-stand maneuver (Claassen et al 2009) could be beneficial to address this potential problem. In particular, Liu et al (2005) demonstrated that selecting recordings with high spontaneous ABP variability led to more robust estimates of dynamic CA parameters.

Apart from our own pilot study based on a smaller and different group of subjects (Katsogridakis *et al* 2012), the use of PRBS to drive the inflation and deflation of bilateral thigh cuffs has not been described previously. Aaslid et al. (Aaslid *et al* 2007) used a square wave sequence to drive the inflation of thigh cuffs in a group of adult and paediatric neurosurgical patients under mild hypocapnic conditions. Changes in ABP variability due to the repeated inflation and deflation of thigh cuffs were not reported and neither the use of different square wave frequencies. The influence of thigh cuff inflation on heart rate or other indicators of sympathetic outflow was not described either.

Compared to the classical single inflation/release of thigh cuffs (Aaslid *et al* 1989) or the fixed frequency square wave inflation/deflation approach (Aaslid *et al* 2007), PRBS controlled inflation/deflation of thigh cuffs present several important advantages. First of all, the method allows the time during which the cuffs remain inflated to be much reduced compared to the conventional thigh-cuff test (Katsogridakis *et al* 2012), thus reducing patient discomfort and minimizing the possibility of increased sympathetic activity, as indicated by the stable HR values in Table 2 (van Lieshout and Secher 2008). Several of our subjects expressed some degree of discomfort from the face mask, but none complained about the thigh cuffs. This was in stark contrast with our previous extensive experience with the use of the classical single inflation/release of thigh cuffs, which can be very painful due to inflation pressures of 20 mmHg or more above systolic ABP during at least 3 min (Mahony *et al* 2000). Second, due to its temporal variability, PRBS have a much broader frequency spectrum than a single frequency square wave and for this reason the increased ABP variability is also likely to show a broader spectrum thus bringing additional improvements to the quality of transfer function analysis estimates (Katsogridakis *et al* 2012).

Despite potential limitations due to insufficient ABP variability, estimates of dynamic CA based on spontaneous fluctuations should be regarded as the best ‘physiological reference’

method due to the lack of intervening covariates. For this reason, whenever alternative methods for assessment of CA are proposed, for example using changes in posture, it is important to demonstrate that these yield at least similar results to the approach based on spontaneous fluctuations. Finally, it should also be highlighted that the PRBS instrumentation system is portable and can thus readily be made available by the patients' bedside.

**Accuracy of dynamic CA indices**

The ROC curve has been used previously in clinical studies of dynamic CA, but not to describe the diagnostic accuracy of ARI for detection of impaired CA resulting from hypercapnia. In severe head injury, Panerai et al (Panerai *et al* 2004) obtained the ROC curve for the ARI as a predictor of mortality. Hu et al (Hu *et al* 2008) showed ROC curves for two different techniques to derive the phase difference between CBFV and ABP, another indicator of dynamic CA, as predictors of type 2 diabetes. More recently Budohoski et al (Budohoski *et al* 2012) also presented ROC curves comparing two different indices of CA based on correlation coefficients, as predictors of intracranial hypertension caused by severe head injury. Unfortunately, none of these previous studies reported on levels of ABP variability, thus making comparisons with our results difficult. However, it is possible to speculate that ABP variability could be elevated in patients with diabetes due to depression of the baroreflex in this patient group, whilst severe head injury patients in intensive care might have their ABP variability elevated due to artificial ventilation.

**Study limitations**

Measurements of CBFV can reflect changes in CBF as long as the diameter of the insonated vessel remains constant. Several studies have demonstrated that the cross-sectional area of

the MCA changes minimally during thigh cuff inflation/deflation or due to large changes in ABP or $PaCO_2$ (Giller *et al* 1993; Newell *et al* 1994; Serrador *et al* 2000).

For the purposes of this study, a maximum thigh cuff pressure setting (MTCP) of 150mmHg was selected, and was used uniformly for all volunteers. Future studies could include the personalized selection of the MTCP pressure settings in their design, to ensure that maximum ABP variability is induced. This is particularly important when investigating CA function in elderly subjects or patients with elevated systolic BP.

One potential limitation of deriving values of ARI from thigh cuff maneuvers is the need of *a priori* knowledge of the critical closing pressure (CrCP) of the cerebral circulation as part of the mathematical formulation originally proposed by Tiecks *et al* (195). However, in our study this was circumvented by direct fitting of the template functions proposed by Tiecks *et al* (1995) using a lest squares approach which automatically matches the amplitudes of the measured and theoretical (template) step responses without the need to estimate the CrCP.

Only healthy volunteers were included in this study, and it is therefore not certain whether similar results would be obtained in patients with cardiovascular conditions. The age range of the volunteers may also constitute a limitation that hinders the extrapolation of our findings to an older population.

The sample size of the study (n=30) was calculated to detect a change in ARI = 1.2, with 80% power at p=0.05 (Brodie *et al* 2009). Changes in ARI above this target were observed due to hypercapnia, both during baseline and PRBS controlled inflation/deflation of thigh cuffs, but not due to the effect of thigh cuffs, considering the changes mentioned in Results of -0.15 in normocapnia and 0.43 in hypercapnia. Therefore, it is possible that with a larger sample size, the effect of random inflation/deflation on dynamic CA would have manifested.

To assess the sensitivity and specificity of ARI in detecting impairments of autoregulation, we decided to induce a state of autoregulatory inefficiency by means of administering $CO_2$ at

a concentration of 5%, which was used as a surrogate of dynamic CA impairment. Though sufficient evidence exists to suggest that this assumption is not unreasonable (Panerai *et al* 1999; Paulson *et al* 1990), caution needs to be exercised before extrapolating our findings to impairment of dCA caused by different pathologies.

## Conclusions

We have demonstrated the feasibility and efficacy of using pseudorandom binary stimuli for the integrated assessment of cerebral haemodynamics with good acceptability by the volunteers. This approach resulted in increased ABP variability without distortion of dynamic CA estimates. Moreover, the use of PRBS to drive the inflation of thigh cuffs resulted in improved sensitivity, specificity and accuracy of the ARI method in detecting dynamic CA impairment. Further work is needed to investigate the reliability of estimates of dynamic CA obtained with this new method of assessment in a larger population and to test its applicability in a clinical setting.

## Disclosure/Conflict of interest

The authors declare no conflicts of interest.

## References

Aaslid R, Lindegaard KF, Sorteberg W, Nornes H (1989) Cerebral autoregulation dynamics in humans. *Stroke* 20:45-52.

Aaslid R, Blaha M, Sviri G, Douville CM, Newell DW (2007) Asymmetric dynamic cerebral autoregulatory response to cyclic stimuli. *Stroke* 8:1465-1469.

Brodie FG, Atkins ER, Robinson TG, Panerai RB (2009) Reliability of dynamic cerebral autoregulation measurement using spontaneous fluctuations in blood pressure. *Clin Sci* 116:513-520.

Budohoski KP, Czosnyka M, de Riva N, Smielewski P, Pickard JD, Menon DK, Kirkpatrick PJ, Lavinio A (2012) The Relationship Between Cerebral Blood Flow Autoregulation and Cerebrovascular Pressure Reactivity After Traumatic Brain Injury. *Neurosurgery* [Epub ahead of print] 2012 May 30.

Claassen JA, Levine BD, Zhang R (2009) Dynamic cerebral autoregulation during repeated squat-stand maneuvers. *J Appl Physiol* 106:153-160.

DeLong E, DeLong D, Clarke-Pearson D (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 44:837-845.

Giller CA, Bowman G, Dyer H, Mootz L, Krippner W (1993) Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* 32:737-741.

Gommer ED, Shijaku E, Mess WH, Reulen JP (2010) Dynamic cerebral autoregulation: different signal processing methods without influence on results and reproducibility. *Med Biol Eng Comput* 48:1243-1250.

Hu K, Peng CK, Huang NE, Wu Z, Lipsitz LA, Cavallerano J, Novak V (2008) Altered Phase Interactions between Spontaneous Blood Pressure and Flow Fluctuations in Type 2 Diabetes Mellitus: Nonlinear Assessment of Cerebral Autoregulation. *Physica A* 387:2279-2292.

Katsogridakis E, Bush G, Fan L, Birch AA, Simpson DM, Allen R, Potter JF, Panerai RB (2012) Random perturbations of arterial blood pressure for the assessment of dynamic cerebral autoregulation. *Physiol Meas* 33:103-116.

Kwan J, Lunt M, Jenkinson D (2004) Assessing dynamic cerebral autoregulation after stroke using a novel technique of combining transcranial Doppler ultrasonography and rhythmic handgrip. *Blood Press Monit* 9:3-8.

Lipsitz LA, Mukai S, Hamner J, Gagnon M, Babikian V (2000) Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke 3*1:1897-1903.

Liu J, Simpson DM, Allen R (2005) High spontaneous fluctuation in arterial blood pressure improves the assessment of cerebral autoregulation. *Physiol Meas* 26:725-741.

Mahony PJ, Panerai RB, Deverson ST, Hayes PD, Evans DH (2000) Assessment of the thigh cuff technique for measurement of dynamic cerebral autoregulation. *Stroke* 31:476-480.

Newell DW, Aaslid R, Lam A, Mayberg TS, Winn HR (1994) Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke* 25:793-797.

Panerai RB (1998) Assessment of cerebral pressure autoregulation in humans--a review of measurement methods. *Physiol Meas* 19:305-338.

Panerai RB, White RP, Markus HS, Evans DH (1998) Grading of cerebral dynamic autoregulation from spontaneous fluctuations in arterial blood pressure. *Stroke* 29:2341-2346.

Panerai RB, Deverson ST, Mahony P, Hayes P, Evans DH (1999) Effects of CO2 on dynamic cerebral autoregulation measurement. *Physiol Meas* 20:265-275.

Panerai RB, Kerins V, Fan L, Yeoman PM, Hope T, Evans DH (2004) Association between dynamic cerebral autoregulation and mortality in severe head injury. *Br J Neurosurg* 18:471-479.

Panerai RB (2008) Cerebral autoregulation: from models to clinical applications. *Cardiovasc Eng* 8:42-59.

Paulson OB, Strandgaard S, Edvinsson L (1990) Cerebral autoregulation. *Cerebrovasc Brain Metab Rev* 2:161-192.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL (2000) MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31:1672-1678.

Tiecks FP, Lam AM, Aaslid R, Newell DW (1995) Comparison of static and dynamic cerebral autoregulation measurements. *Stroke* 26:1014-1019.

Tiecks FP, Douville C, Byrd S, Lam AM, Newell DW (1996) Evaluation of impaired cerebral autoregulation by the Valsalva maneuver. *Stroke* 27:1177-1182.

van Beek AH, Claassen JA, Rikkert MG, Jansen RW (2008) Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab* 28:1071-1085.

van Lieshout JJ, Secher NH (2008) Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Point: Sympathetic activity does influence cerebral blood flow. *J Appl Physiol* 105:1364-1366.

Zhang R, Zuckerman JH, Giller CA, Levine BD (1998) Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol* 274:H233-241.

Zhang R, Zuckerman JH, Levine BD (1998) Deterioration of cerebral autoregulation during orthostatic stress: insights from the frequency domain. *J Appl Physiol.* 85:1113-1122.

## Figure legends

Figure 1. Representative recordings of ABP (thick line, mmHg), CBFV (thin solid lines, cm/s) and $EtCO_2$ (dotted line, mmHg) from a 27 year-old male volunteer. Subplots (A) and (C) represent the use of spontaneous variability under normocapnic and hypercapnic conditions respectively. Subplots (B) and (D) represent recordings obtained from the use of the thigh cuffs under normocapnic and hypercapnic conditions respectively. ABP, CBFV and $EtCO_2$ are given in units of mmHg, $cm.s^{-1}$ and mmHg, respectively.

Figure 2. Group averaged CBFV step response functions obtained from the use of thigh cuffs (dashed line) and spontaneous variability (solid line) under normocapnic (A) and hypercapnic conditions (B). Error bars represent largest ± 1 SEM. During normocapnia (A) the rapid return of the step response to baseline, at approximately 4 s indicates an efficient dynamic CA which is not observed during hypercapnia (B) when CA is significantly impaired (Table 2).

Figure 3. Receiver operating characteristic (ROC) curves for the detection of CA impairment induced by hypercapnia. Using different threshold values for ARI, sensitivity and specificity (see Methods) were obtained for spontaneous fluctuations in ABP (grey line, squares) and for PRBS controlled inflation/deflation of thigh cuffs (black line, circles). Not all cutoff points can be distinguished due to the superposition of values of sensitivity and 1-specificity. The increased area under the curve, in relation to the ‘line of indifference’ (dashed line) indicates the superior discrimination between normal and impaired CA resulting from the use of thigh cuffs to increase ABP variability.

**Table 1. Volunteer demographics (mean ± SD)**

| | |
|---|---|
| Age (years) | 31.4 ± 12 |
| Height (cm) | 174 ± 8 |
| Weight (kg) | 72.2 ± 12.8 |
| BMI ($kgm^{-2}$) | 23.6 ± 3.3 |
| Systolic BP (mmHg) | 123.0 ± 11.6 |
| Diastolic BP (mmHg) | 75.8 ± 8.3 |

**Table 2. Population averages for measured and derived parameters (mean ± SD)**

| Parameter | Normocapnia / NTC | Normocapnia / TC | Hypercapnia / NTC | Hypercapnia/ TC | p-values (ANOVA) |
|---|---|---|---|---|---|
| mABP (mmHg) | 88.4 ± 14.7 | 91.0 ± 13.1 | 93.5 ± 16.9 | 94.7 ± 16.1 | $p = 0.020$ |
| $CBFV_L$ (cm/s) | 53.6 ± 12.2 | 55.2 ± 12.1 | 65.4 ± 15.6 | 70.7 ± 14.2 | $p < 10^{-4}$ |
| $CBFV_R$ (cm/s) | 52.7 ± 14.1 | 54.6 ± 11.9 | 63.9 ± 15.9 | 66.6 ± 14.3 | $p < 10^{-4}$ |
| $EtCO_2$ (mmHg) | 39.8 ± 3.1 | 38.0 ± 3.1 | 45.9 ± 2.9 | 46.8 ± 2.8 | $p < 10^{-4}$ |
| HR (bpm) | 69.1 ± 8.0 | 68.3 ± 7.3 | 70.9 ± 6.6 | 71.4 ± 6.7 | $p = 0.001$ |
| $ARI_L$ | 6.1 ± 1.5 | 6.2 ± 1.0 | 4.4 ± 1.5 | 4.1 ± 1.4 | $p < 10^{-4}$ |
| $ARI_R$ | 6.2 ± 1.3 | 6.1 ± 0.9 | 4.8 ± 1.9 | 4.3 ± 1.6 | $p < 10^{-4}$ |

NTC: no thigh cuffs
TC : thigh cuffs
$ARI_L$ : ARI value obtained from CBFV recordings of the left MCA.
$ARI_R$ : ARI value obtained from CBFV recordings of the right MCA