

## **Title**

Is Dynamic Cerebral Autoregulation Measurement Using Transcranial Doppler Ultrasound Reproducible in the Presence of High Concentration Oxygen and Carbon Dioxide?

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## Abstract

Reliability of CBF velocity (CBFV) and dynamic CA estimates (expressed as autoregulation index: ARI) using spontaneous fluctuations in blood pressure (BP) has been demonstrated. However, reliability during co-administration of O<sub>2</sub> and CO<sub>2</sub> is unknown. Bilateral CBFV (using transcranial Doppler), BP and RR interval recordings were performed in healthy volunteers (7 males, 4 females, age: 54 ± 10 years) on two occasions over 9 ± 4 days. Four 5-minute recordings were made whilst breathing air (A), then 5%CO<sub>2</sub> (C), 80%O<sub>2</sub> (O) and mixed O<sub>2</sub> + CO<sub>2</sub> (M) in random order. CBFV was recorded; ARI was calculated using transfer function analysis. Precision was quantified as within-visit standard error of measurement (SEM) and the coefficient of variation (CV). CBFV and ARI estimates with A (SEM: 3.85 & 0.87; CV: 7.5% & 17.8%, respectively) were comparable to a previous reproducibility study. The SEM and CV with C and O were similar, though higher values were noted with M. Bland-Altman plots indicated no significant bias across all gases for CBFV and ARI (bias < 0.06 cm.s<sup>-1</sup> and < 0.05, respectively). Thus, TCD-estimated CBFV and ARI during inhalation of O<sub>2</sub> and CO<sub>2</sub> have acceptable levels of reproducibility and can be used to study the effect of these gases on cerebral haemodynamics.

**Key words:** blood pressure cerebral blood flow, cerebral haemodynamics, high flow oxygen, reliability, reproducibility, carbon dioxide

## Introduction

Cerebral autoregulation (CA) is the inherent ability of the cerebral arterioles to maintain cerebral blood flow (CBF), despite changes in cerebral perfusion pressure, over the usual range of mean blood pressure 60-150mmHg (Aaslid, Lindegaard et al. 1989, Paulson, Strandgaard et al. 1990). With intact CA, physiological or pathological changes in blood pressure (BP) do not significantly alter CBF. CA is mediated by changes in resistance vessels e.g. vasodilation during reduced cerebral perfusion pressure (CPP) and vasoconstriction in response to increased CPP, with resulting stable CBF and avoidance of damage to arterioles from increased CPP.

CA can be measured in a static (sCA) or dynamic fashion (dCA). The former is the measurement of CA using a steady-state approach, where arterial BP is slowly increased using drugs such as phenylephrine (Tiecks, Lam et al. 1995, Panerai 1998). dCA measures the transient response of CBF to a sudden change in ABP; indicating the recovery of CBF following an abrupt change in BP. More recently, physiological spontaneous BP fluctuations have been used to measure dCA using transcranial Doppler ultrasound (TCD) and a beat-to-beat BP device (Panerai 1998, Zhang, Zuckerman et al. 1998). TCD provides a quick, relatively easy method of measuring CBF velocity (CBFV), with good temporal resolution (Panerai 1998).

This approach uses spontaneous BP changes as the autoregulatory stimulus (input) and compares it to CBFV (output) using Transfer Function Analysis (TFA). CBFV is used as a surrogate for CBF and is assumed to be proportional to it since the middle cerebral artery (MCA) diameter does not vary significantly (variation <3.0%) (Zhang, Zuckerman et al. 1998). However, a recent MRI study by Verbree et al. (2014) showed that although MCA diameter remains constant during small incremental changes of end-tidal CO<sub>2</sub> (PETCO<sub>2</sub>) from baseline normocapnia, larger changes to higher PETCO<sub>2</sub> values do change diameter (Verbree, Bronzwaer et al. 2014). This effect was seen with high resolution MRI at a PETCO<sub>2</sub> level of +2kPa above baseline (Verbree, Bronzwaer et al. 2014). TFA usually obtains estimates of gain and phase over a range of frequencies, as well as estimates of the coherence function, which indicates the reliability of the TFA measures (Blaber, Bondar et al. 1997, Zhang, Zuckerman et al. 1998). The final value given for the effectiveness of CA using the TFA method is expressed using the autoregulatory index (ARI), proposed by Tiecks et al (Tiecks, Lam et al. 1995). ARI values range from 0 to 9, where 0 is the absence of CA

and 9 is the best observed CA. Panerai et al. (1998) showed that the Tieck's model (Tiecks, Lam et al. 1995) can be used to grade autoregulation based on spontaneous fluctuations of ABP and CBFV. The Tieck's model provides ten templates for reference of CBFV response curves to a step change in mean BP (Tiecks, Lam et al. 1995).

Inhalation of 5% CO<sub>2</sub> is commonly used with TCD to estimate CVR, an important cerebral haemodynamic parameter. Although cerebral autoregulation can be affected by a number of changes in physiological conditions (CO<sub>2</sub>, intracranial pressure, etc), CVR provides a measure primarily of vessel response (constriction or dilatation) (Chen, Liu et al. 2014). A sitting and squatting autoregulation study examined the hypothesis that the phase difference that occurs between an induced oscillation in blood pressure and the resultant oscillation in middle cerebral artery (MCA) flow velocity predominantly reflect the competence of cerebral autoregulation (Birch, Dirnhuber et al. 1995). The study involved the exercise described above alongside first breathing normally, secondly hyperventilating (hypocapnia), and finally while breathing air containing 5% carbon dioxide (hypercapnia) (Birch, Dirnhuber et al. 1995). High concentration oxygen is commonly used in pre-hospital and emergency facilities for patients with acute stroke, despite the lack of any conclusive evidence regarding benefits (Singhal 2006). The National Institute for Health and Care Excellence (NICE) recommend supplemental oxygen only be used if saturations drop below 95%, and do not recommend "routine use" in acute stroke patients who are not hypoxic (National Collaborating Centre for Chronic Conditions 2008). Given the paucity of studies reporting the inherent reliability or variability of TCD-estimated CBFV and ARI during concomitant inhalation of oxygen or carbon dioxide, this study aimed to assess the reliability of these parameters during inhalation of these gases.

## **Materials and Methods**

The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the East Midlands Research Ethics Committee (Reference: 11/EM/0287). Eleven volunteers were recruited from departmental staff via e-mail and by inviting relatives of subjects used in another study being carried out in the department. Based on previous studies carried out in the department, a sample size of 11 in each group was estimated to detect an ARI difference of >2, with a power of 80%, and 5% probability of Type I error (Brodie, Atkins et al. 2009).

All participants had no history of stroke or transient ischaemic attack and had given written informed consent. Each subject underwent two periods of measurement with a mean of  $9 \pm 4$  days between each recording; recordings being undertaken at approximately the same time of day for each subject. Subjects were asked to avoid excessive exertion before the measurements, as well as caffeinated beverages for at least 2 hours prior to assessment. During testing, subjects laid supine in a quiet, temperature controlled ( $20-24^{\circ}\text{C}$ ) cardiovascular research laboratory. Subjects were free from distraction for the duration of recordings.

Heart rate was measured using a continuous three-lead ECG, BP was measured using servo-controlled photoplethysmography arterial volume clamping of the digital artery (Finapres), and both MCAs were insonated using two 2 MHz probes secured at the temporal window using a head frame. MCAs were identified according to the characteristics described by Aaslid et al. (i.e. the signal depth and velocity and characteristic shape of the waveform) (Aaslid, Lindegaard et al. 1989).

After attaching the measurement equipment onto the subject, 4 readings were taken. The first reading was carried out during inhalation of room air (A), and the other three were carried out, in random order, during inhalation of high concentration oxygen (80%  $\text{O}_2$  and 20% nitrogen) (O), 5%  $\text{CO}_2$  (in air) (C) and  $\text{O}_2$  with  $\text{CO}_2$  (80%  $\text{O}_2$ , 5%  $\text{CO}_2$  and 15% nitrogen) (M). The latter three were administered using a continuous positive airway pressure (CPAP) mask with passive breathing of gas mixture collected in a Douglas bag. For standardization, a CPAP mask was also affixed to the subject during A. Absence of any inward air leak was confirmed by documenting a rise in end tidal  $\text{CO}_2$  ( $\text{etCO}_2$ ). The recording in A was for 5 minutes and was 6 minutes for O, C and M, where gas was administered after 1 minute, maintained for 4 minutes and was switched off for the final one minute. A one-minute break between readings allowed washout of gases and the physiological calibration of the servo-adjust mechanism of the Finapres. Readings were repeated in an identical fashion in the event of equipment difficulties.

To ensure the same segment of the MCA was insonated during the repeat measurement, the location of the temporal window, the depth of insonation and the velocity of the MCA blood flow were noted during the first measurement. Efforts were made during the repeat measurement to find this location again, to minimise a possible source of variability. All recordings were taken by the same researcher.

Data were collated onto customised software (PHYSIDAS, physiological data acquisition system) created by the Medical Physics Group, University of Leicester. The data were transferred to an MS-DOS computer for offline data editing and analysis. The BP was first calibrated and the data were visually inspected to remove obvious data spikes using linear interpolation. BP drift or excessive signal noise led to data rejection. CBFV channels were passed through a median filter of appropriate width to minimise the effect of noise. The R-R interval was marked using the ECG trace and used to extract the mean CBFV and ABP for each cardiac cycle. All beat-to-beat parameters (mean CBFV, mean BP, heart rate, end-tidal CO<sub>2</sub>) were then re-sampled at 0.2 Hz. Under visual inspection, any persisting spikes were removed using linear interpolation.

Transfer function analysis (TFA) of spontaneous fluctuations of BP (input) and CBFV (output) were performed using the fast Fourier transform in conjunction with the Welch method using data segments with 512 samples (102.4 s) and 50% superposition. The CBFV response to a hypothetical step change in BP was derived from the inverse Fourier transform of the gain and phase frequency responses. ARI was estimated using the ten template CBFV response curves to a step change in mean BP (Tiecks, Lam et al. 1995, Brodie, Atkins et al. 2009). The template with the best fit was selected by least squares providing the corresponding ARI value (Tiecks, Lam et al. 1995, Brodie, Atkins et al. 2009).

### *Statistical analysis*

Data analysis was commenced by testing for Gaussian distribution using the D'Agostino & Pearson omnibus normality test. Paired t-tests were used to identify inter-hemispheric differences. Inter-hemispheric values were averaged in the absence of significant differences. Subjects with only unilateral CBFV/ARI values (due to poor TCD windows, for example) had this single value used. A repeated measures ANOVA was performed to test for differences in CBFV and ARI values due to the four different conditions. This was followed by a Tukey's multiple comparison post hoc test. A value of  $p < 0.05$  was adopted to indicate statistical significance.

The standard error of mean (SEM) was calculated to obtain an index of absolute reliability. The coefficient of variation (CV) was subsequently calculated as a measure of relative reliability, to enable comparison between parameters. Bland-Altman plots were constructed

to quantify the bias and limits of agreement between the two measurement sessions (Euser, Dekker et al. 2008, Pinna, Maestri et al. 2007).

## Results

Eleven volunteers (7 male), were recruited with a mean age of  $54 \pm 10$  (range 31-67) years. Subjects had no past history of stroke and were non-smokers. Four subjects were ex-smokers (stopped smoking on average 29 years ago with an average history of 28 pack-years). One subject had diet controlled type 2 diabetes but was otherwise healthy. Subject characteristics are displayed in [Table 1](#).

Paired t-tests between the CBFV values for each hemisphere revealed no significant difference; hence these values were averaged. No data had to be rejected due to artefactual errors. One subject did not have bilateral ARI estimates, so the unilateral reading was used. Average CBFV and ARI values were normally distributed. No significant differences were seen in CBFV (Table 2) and ARI (Table 3) values between visits 1 and 2 for all manoeuvres. CV (%) values ranged from 7.11-8.24% for CBFV (Table 2) and 17.83-32% for ARI (Table 3), respectively. Oxygen saturations were assessed at key time points to confirm a rise in saturations during oxygen inhalation as compared to baseline ( $99.2 \pm 1.0$  vs.  $97.1 \pm 1.3$ ,  $p < 0.0001$ ). Bland-Altman plots for the ARI and the CBFV values were plotted for each manoeuvre and are shown in the Figure 1.

Furthermore, data quality was assessed using: fitting correlation coefficient, rate of data rejection and rate of insonation failure is depicted by a high fitting correlation coefficient (a numerical value representing the extent to which the data fits Tieck's step response model) average value ( $0.84 \pm 0.08$  under A and  $0.85 \pm 0.08$  in O), a low rate of data rejection and a low insonation failure rate (13%, comparable to the expected 15%) (Alexandrov, Demchuk et al. 1999).

## Discussion

The intrinsic reliability of measuring dCA using spontaneous BP fluctuations has been demonstrated by Brodie et al. (Brodie, Atkins et al. 2009). The reliability of this method during inhalation of oxygen and carbon dioxide has not yet been established. This holds importance in the context of assessment of the impact of gases on cerebral haemodynamics.

Further work is then possible on diseased states following accurate confirmation of acceptable levels of reproducibility. This study therefore assessed reproducibility of this methodology during inhalation of oxygen and/or carbon dioxide.

In order to assess the reliability of this method, our group previously undertook a study to estimate the extent of variability in dCA measured using TCD and spontaneous BP fluctuations in the same subjects on 4 separate measurement sessions (i.e. intra-subject variability) (Brodie, Atkins et al. 2009). We demonstrated excellent reliability of CBFV and reported that ARI had comparable reliability with that of the measurement of heart rate. This was the first study to provide an assessment of cerebral autoregulation during spontaneous fluctuations of BP, and importantly addressed the longstanding sample size requirement to reinforce reproducibility in future studies. A limitation of this study though, was the lack of PETCO<sub>2</sub> measurement.

#### **ARI & CBFV (air)**

The CV for ARI under normal gas conditions was ~13% in the study by Brodie et al, compared to ~18% obtained in the same conditions (A) in this study (Brodie, Atkins et al. 2009). The higher CV for ARI in this study can be explained by appreciating the method used to calculate the values ( $SEM = SD/\sqrt{n}$  and  $CV = SEM/\mu ARI$ ). A lower number of repeat measurements (n) and a lower mean ARI would result in higher CV. Thus, the difference in CV ARI is explained by the differences in these parameters (n=2 vs. n=4; mean ARI ~5 vs. ~6), and indicates that the reproducibility of ARI (A) is similar in both studies.

The CV for CBFV under normal gas conditions was ~1.7% in the study by Brodie et al, compared to ~7.5% obtained in the same conditions (air) in this study. The higher CV for CBFV in the current study is likely to be due to the lower mean CBFV (52 vs. 66 cm.s<sup>-1</sup>). Thus, the difference in CV CBFV is explained by the difference in mean CBFV and the lower number of repeat measurements (as indicated for ARI), indicating that the reproducibility of CBFV (air) is similar in both studies.

#### **ARI & CBFV (gas manoeuvres)**

The CV for ARI tended to be higher with the other manoeuvres (Table 3), particularly with mixed gas inhalation. The reasons for this are unclear, but it may be that variability is increased with increase in concentration of inhaled oxygen or carbon dioxide, with a potential



additive effect of both gases. However, the CV for CBFV was similar with all manoeuvres, with slightly higher CV during inhalation of both gases (mixed).

The current study utilised well-validated methodology, with standardised criteria for rejection of poor readings (Panerai 1998). TCD measurements provide a good temporal resolution and are commonly used for estimation of CBFV and ARI. Furthermore, dCA estimated from spontaneous transient BP fluctuations is comparable to classical dCA using induced BP change and excludes any effects from inflation and deflation of thigh cuffs in the traditional manoeuvre (Aries, Elting et al. 2010, Panerai 2009, Dineen, Brodie et al. 2010, Atkins, Brodie et al. 2010). We also ensured standard administration of gases via a sealed CPAP mask system, and confirmed seal by documenting rise in PETCO<sub>2</sub>).

As previously mentioned, data were considered to be of high quality with a strong fitting correlation coefficient, average value, low rate of data rejection and low rate of insonation failure.

### **Relevance for clinical population**

This study demonstrates the reproducibility of TCD-estimated CBFV and derived ARI (using TFA) reported in a previous study [Brodie, Atkins et al, 2009) confirming the robustness of this technique. In addition, reproducibility of these parameters has now been demonstrated during inhalation of high concentration oxygen (for applications either in a clinical scenario or for research purposes) or 5% CO<sub>2</sub> (commonly used for CVR estimation). We conclude that the technique can be employed for future clinical studies using TCD in the context of air, 5% CO<sub>2</sub> or high concentration oxygen. The reported properties of the variables (SEM CBFV and SEM ARI) can be used for estimation of sample size for future studies, for example, a study of the effect of high concentration oxygen in acute stroke or other disease states.

Moreover, estimates of variability of CBFV and/or ARI may have prognostic value in certain disease conditions e.g. cerebrovascular disease or dementia, similar to recent findings of the prognostic value of BP variability (Rothwell, Howard et al. 2010). Rothwell et al. (2010) showed that visit-to-visit BP variability, particularly maximum systolic blood pressure values, correlate with future stroke risk (Rothwell, Howard et al. 2010).

### **Limitations**

Firstly, the complex nature of the assessments and subsequent data processing requires strict standardised procedures as was followed in this study. However, despite precautions, it is difficult to state that insonation was carried out in identical manner at both assessments and may have contributed to some variation. Secondly, the studied TCD technique measures CBFV as a surrogate for CBF, with the assumption that MCA diameter remains relatively constant throughout the procedure. Whilst traditional data indicate that blood pressure changes (Newell, Aaslid et al. 1994, Serrador, Picot et al. 2000), stimulated orthostasis using lower body negative pressure (Serrador et al) and changes in  $\text{etCO}_2$  (Serrador, Picot et al. 2000) do not significantly alter MCA diameter, more recent data challenge this concept. Giller and colleagues reported an increase MCA diameter by less than 4% with increase in  $\text{PETCO}_2$  (Giller, Bowman et al. 1993). Coverdale and colleagues reported a relative increase or decrease in MCA cross-sectional area (CSA) with hypercapnia and hypocapnia, respectively using phase contrast MRI (Coverdale, Gati et al. 2014). It is likely that there is a dose-dependent effect of change in  $\text{PETCO}_2$  on MCA size (Verbree, Bronzwaer et al. 2014), and reasonable to conclude that any changes due to inhalation of 5%  $\text{CO}_2$  are small and have little if any impact on CBFV estimates using TCD. Thus, the demonstrated reproducibility in this study with 5%  $\text{CO}_2$  is likely to be acceptable, and no comment can be made about TCD estimated variables with higher concentrations of  $\text{CO}_2$ .

Thirdly, continuous oxygen saturation monitoring would have allowed a more detailed assessment of the association between oxygen levels and cerebral haemodynamic parameters. Nonetheless, oxygen saturations were assessed at key time points to confirm a rise in saturations during oxygen inhalation as compared to baseline ( $99.2 \pm 1.0$  vs.  $97.1 \pm 1.3$ ,  $p < 0.0001$ ).

Fourthly, the study participants were healthy volunteers with a mean age of  $54 \pm 10$  years. Thus, the demonstrated reproducibility of measurements in this study may not apply in older individuals or in disease states like cerebrovascular disease, where CBFV / ARI variability in the presence of oxygen or carbon dioxide may differ.

## **Conclusions**

The present study is the first to look at the reliability of CBFV and derived ARI using spontaneous BP fluctuations, in the presence of oxygen, carbon dioxide and mixed carbon dioxide and oxygen.

We have shown that TCD-estimated CBFV and ARI (using TFA) during inhalation of O<sub>2</sub> and CO<sub>2</sub> have acceptable levels of reproducibility. This method is thus appropriate to study the effect of these gases on cerebral haemodynamic parameters. However, further assessments of these parameters are warranted in diseased states.

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### **Disclosures**

The authors have no conflict of interest.

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<b>Number of subjects</b>	11	
<b>Age (years)</b>	54 ± 10 [31,67]	
<b>Gender (Males: Females)</b>	7:4	
<b>V1 and V2 gap (days)</b>	9 ± 4 [5, 19]	
<b>Alcohol intake (units/ week)</b>	9 ± 9	
<b>Caffeine intake* (hours)</b>	<b>V1</b>	<b>V2</b>
	4 ± 6	3 ± 1
<b>HR (bpm)</b>	68 ± 14 [55, 103]	68 ± 11 [54, 92]
<b>Omron SBP (mmHg)</b>	133 ± 15 [104, 155]	132 ± 12 [103, 149]
<b>Omron DBP (mmHg)</b>	80 ± 8 [68, 94]	79 ± 8 [64, 89]

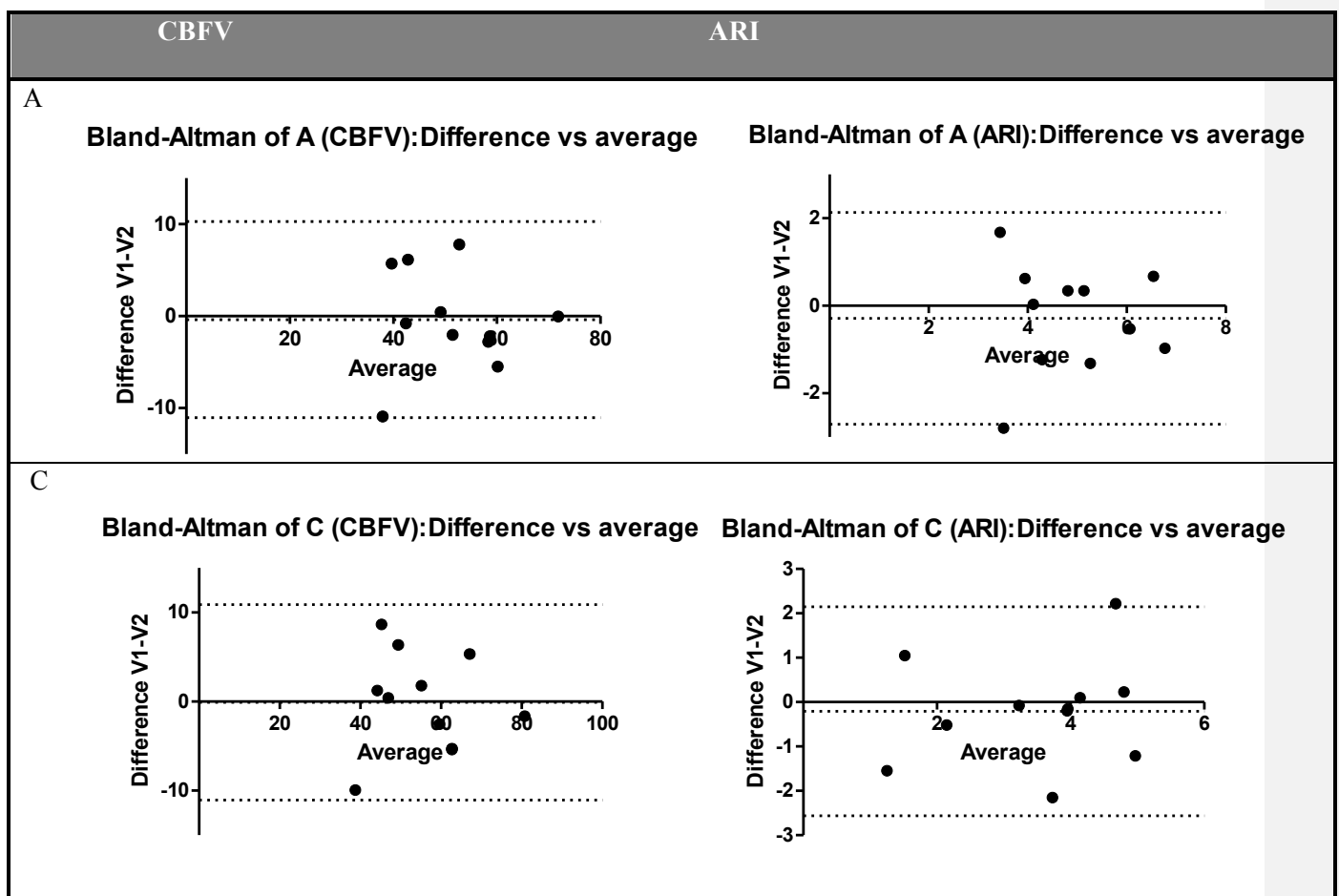
Table 1 Subject baseline demographics. Values are mean ± SD and ranges are denoted in brackets, \*prior to measurement, V1= visit 1 and V2= visit 2

	<b>Mean CBFV</b>		<b>CBFV intra-subject reproducibility</b>	
	<i>Visit 1</i>	<i>Visit 2</i>	<i>SEM</i>	<i>CV (%)</i>
A	51.5 ± 10.3	51.6 ± 10.9	3.85	7.49
C	56.1 ± 12.4	55.7 ± 13.0	3.96	7.11
O	50.0 ± 10.2	50.0 ± 10.0	3.62	7.27
M	57.4 ± 12.8	57.8 ± 14.4	4.74	8.24

Table 2 CBFV values (mean ± SD) for visit 1 and visit 2, with corresponding SEM and CV values, for all gas manoeuvres. With the exception of CV, all values are in units of cm.s<sup>-1</sup>.

	<i>Mean ARI</i>		<i>ARI intra-subject reproducibility</i>	
	<i>Visit 1</i>	<i>Visit 2</i>	<i>SEM</i>	<i>CV (%)</i>
A	4.7 ± 1.4	5.0 ± 1.3	0.87	17.83
C	3.6 ± 1.9	3.9 ± 1.1	0.85	24.38
O	5.6 ± 1.1	5.4 ± 1.3	1.25	23.60
M	3.3 ± 1.4	3.3 ± 1.9	1.05	32.03

Table 3 ARI Values (mean ± SD) for visit 1 and visit 2, with corresponding SEM and CV values, for all gas manoeuvres



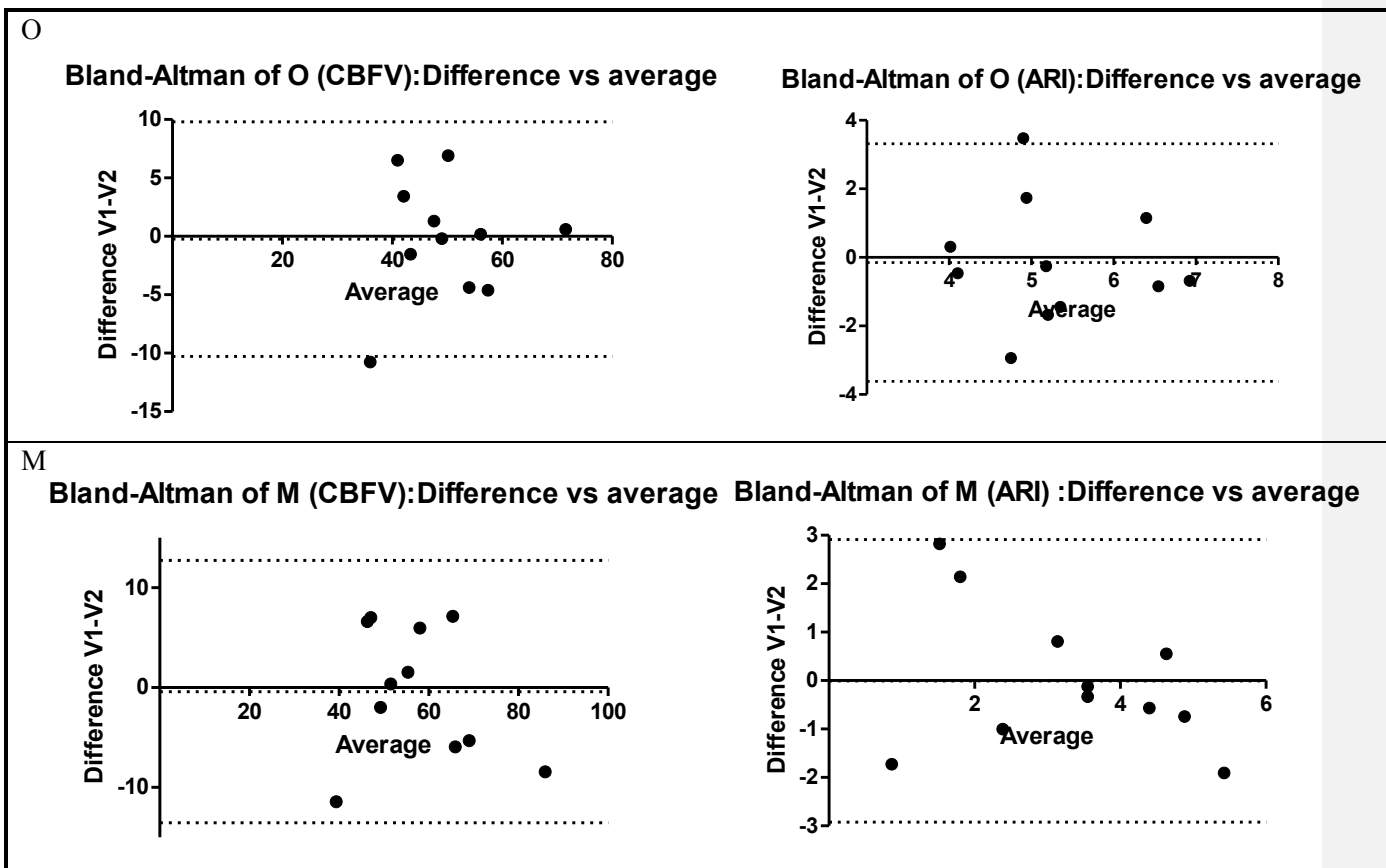


Figure Bland-Altman plots between visit one and two for each of the manoeuvres for CBFV and ARI values