Bilateral regional extracranial blood flow regulation to hypoxia and unilateral duplex ultrasound measurement error

Friend, Alex; Rogan, Matthew; Rossetti, Gabriella; Lawley, Justin ; Mullins, Paul; Sandoo, Aamer; Macdonald, Jamie; Oliver, Sam

Experimental Physiology

DOI:
10.1113/EP089196

Published: 01/07/2021

Peer reviewed version

This article was accepted in its current form to Experimental Physiology on 14th April 2021.

DOI: 10.1113/EP089196

Bilateral regional extracranial blood flow regulation to hypoxia and unilateral duplex ultrasound measurement error

A.T. Friend[1], M. Rogan[2], G.M.K. Rossetti[1,3], J.S. Lawley[4], P.G. Mullins[2], A. Sandoo[1], J.H. Macdonald[1], and S.J. Oliver[1]

Affiliations:

[1]Extremes Research Group, School of Sport, Health and Exercise Sciences, College of Human Sciences, Bangor University, Bangor, Gwynedd, United Kingdom

[2]Bangor Imaging Unit, School of Psychology, College of Human Sciences, Bangor University, Bangor, Gwynedd, United Kingdom

[3]Centre for Integrative Neuroscience and Neurodynamics, School of Psychology and Clinical Language Sciences, University of Reading, Reading, Berkshire, United Kingdom

[4]Department of Sport Science, Division of Physiology, University of Innsbruck, Innsbruck, Austria

Running title: Bilateral extracranial blood flow regulation to hypoxia

Keywords: hypoxia, cerebrovascular, internal carotid artery, vertebral artery, Doppler, ultrasonography, bias

Word count: 6715

Reference count: 40

Corresponding author: Samuel J. Oliver, Bangor University, George Building, Normal Site, Gwynedd, LL57 2PZ, s.j.oliver@bangor.ac.uk, ORCID: 0000-0002-9971-9546

Subject area: Environmental and exercise physiology

What is the central question of this study?

Is blood flow regulation to hypoxia different between the internal carotid arteries (ICA) and vertebral arteries (VA), and what is the measurement error in unilateral extracranial artery assessments compared to bilateral?

What is the main finding and its importance?

ICA and VA blood flow regulation to hypoxia is comparable when factoring for vessel type and vessel side. Compared to bilateral assessment, vessels assessed unilaterally had individual measurement errors of up to 37%. Assessing the vessel with the larger resting blood flow, not the left or right vessel, reduces unilateral measurement error.

Abstract

Whether blood flow regulation to hypoxia is similar between left and right internal carotid arteries (ICA) and vertebral arteries (VA) is unclear. Extracranial blood flow is regularly calculated by doubling a unilateral assessment; however, lateral artery differences may lead to measurement error. This study aimed to determine extracranial blood flow regulation to hypoxia when factoring for vessel type (ICA or VA) and vessel side (left or right) effects, and investigate unilateral assessment measurement error compared to bilateral assessment. In a repeated-measures crossover design, extracranial arteries of 44 participants were assessed bilaterally by duplex ultrasound during 90 minutes of normoxic and poikilocapnic hypoxic (12.0% fraction of inspired oxygen) conditions. Linear mixed model analyses revealed no 'Condition' × 'Vessel Type' × 'Vessel Side' interaction for blood flow, vessel diameter, and flow velocity (all $P > 0.05$) indicating left and right ICA and VA blood flow regulation to hypoxia was similar. Bilateral hypoxic reactivity was comparable [ICA, 1.4 (1.0) vs VA, 1.7 (1.1) $\Delta\% \cdot \Delta SpO_2^{-1}$; $P = 0.12$]. Compared to bilateral assessment, unilateral mean measurement error of the relative blood flow response to hypoxia was up to 5%, but individual errors reached 37% and were greatest in ICA and VA with the smaller resting blood flow due to a ratio-scaling problem. In conclusion, left and right ICA and VA regulation to hypoxia is comparable when factoring for vessel type and vessel side. Assessing the ICA and VA vessels with the larger resting blood flow, not the left or right vessel, reduces unilateral measurement error.

Introduction

Cerebral blood flow regulation is critical to support oxygen delivery to match the high metabolic demand of the brain and to maintain normal neurovascular function (Ogoh, 2017; Willie, Tzeng, Fisher, & Ainslie, 2014). During acute hypoxia when arterial oxygen content is reduced, global cerebral blood flow increases (Hoiland, Howe, Coombs, & Ainslie, 2018). However, hypoxia-induced changes in blood flow are not uniform across the brain (Lawley, Macdonald, Oliver, & Mullins, 2017; Rossetti et al., 2020), which may in part relate to different regulation of blood flow at the extracranial arteries (Lewis, Messinger, Monteleone, & Ainslie, 2014; Ogoh et al., 2013; Subudhi et al., 2014; Willie et al., 2012).

Regional extracranial blood flow regulation can be measured non-invasively by duplex ultrasound of the vertebral arteries (VA) that feed the homeostatic posterior brain regions, and the internal carotid arteries (ICA) that feed the more functional anterior brain regions. In response to hypoxia, absolute increases in blood flow within the ICA are greater than the VA (Lafave et al., 2019). When indexed as a relative response some evidence indicates that the blood flow increase to hypoxia in the VA is greater than in the ICA (Lewis et al., 2014; Ogoh et al., 2013; Subudhi et al., 2014; Willie et al., 2012). Although the majority of literature reporting differences between ICA and VA blood flow regulation has been in response to hypoxia, the greater relative response in the VA has also been observed in response to other stressors including carbon dioxide, orthostasis, and exercise, and is proposed as a mechanism to preferentially maintain posterior blood flow to the homeostatic brain regions (Sato, Fisher, et al., 2012; Sato, Oue, Yoneya, Sadamoto, & Ogoh, 2016; Sato, Sadamoto, et al., 2012). Despite this compelling argument, a disparity exists within the literature with a similar number of studies failing to report differences between the ICA and VA blood flow regulation to hypoxia (Hoiland et al., 2017; Lafave et al., 2019; Willie, Smith, et al., 2014). It is also

contentious whether the increase in blood flow to hypoxia at the extracranial arteries is regulated by a change in vessel diameter (Lewis et al., 2014) or not (Ogoh et al., 2013).

In contrast to the study of ICA (anterior) versus VA (posterior) extracranial regional blood flow response to hypoxia, the effect of vessel side has yet to be considered in studies using duplex ultrasound. Regulation of extracranial blood flow to hypoxia is typically assessed by doubling unilateral measurements of the ICA and VA. Reports suggest that at rest the left and right ICA have equal blood flow, whereas the right VA has 20–30% less blood flow than the left VA as a consequence of its smaller resting vessel diameter (Khan et al., 2017; Schöning, Walter, & Scheel, 1994). Anatomical variations in the aortic branching that alter shear stress and vascular resistance between arteries have been proposed as a possible mechanism for the difference in lateral extracranial blood flow (Hu et al., 2013; van Campen, Verheugt, & Visser, 2018). Moreover, intra- and extracranial cerebrovasculature have the capacity for compensatory collateral flow as demonstrated during and after vessel occlusion (Romero et al., 2010; Wang et al., 2019), and immediately after endarterectomy (Aleksic & Brunkwall, 2009; Wang et al., 2019). Therefore, the interplay between extracranial artery vessel type (ICA or VA) *and* vessel side (left or right) should be considered when investigating the global haemodynamic response to a stressor.

Another possible explanation for the equivocal findings in regional extracranial blood flow regulation to hypoxia is the method by which extracranial blood flow data is acquired and expressed. In assessments of brachial artery vascular function by flow-mediated dilation (FMD), a negative correlation between resting brachial artery diameter and the percentage change in diameter suggests that the calculation of diameter percentage change in smaller brachial arteries overestimates the relative FMD response (Atkinson & Batterham, 2013a, 2013b; Atkinson, Batterham, Thijssen, & Green, 2013). Further, brachial arteries with smaller diameters displayed more varied responses than those with large diameters. Consequently, the

relatively larger blood flow increase to hypoxia in the VA compared to ICA previously reported (Lewis et al., 2014; Ogoh et al., 2013; Subudhi et al., 2014; Willie et al., 2012) may be a product of a ratio-scaling problem arising from random intra-individual lateral anatomical and resting blood flow differences, more prominent in the VA than the ICA, when unilateral rather than bilateral measurements are used.

The primary aim of this study was to determine the extracranial blood flow regulation to acute poikilocapnic hypoxia when factoring for vessel type (ICA or VA) and vessel side (left or right) effects. In this study, we assessed left and right ICA and VA blood flow, vessel diameter, and flow velocity by duplex ultrasound. We hypothesised that when factoring for vessel type and vessel side extracranial blood flow regulation to hypoxia would be similar in left and right ICA and VA. The secondary aim of this study was to investigate the measurement error in unilateral compared to bilateral calculations of extracranial blood flow. Although other stimuli might be used (e.g. carbon dioxide, exercise), we chose to examine the extracranial measurement error to acute poikilocapnic hypoxia as it has previously been shown to increase cerebral blood flow by approximately 30% for a 1–2 h period (Lewis et al., 2014; Morris, Flück, Ainslie, & McManus, 2017). Based on the previously identified ratio-scaling problem with FMD, we hypothesised that a negative relationship would exist between extracranial artery resting normoxic blood flow and the relative blood flow response to hypoxia. We also expected that extracranial arteries with the smaller resting blood flow would have a more varied relative blood flow response to hypoxia than those arteries with larger resting blood flows. Consequently, the measurement error to the relative blood flow response to hypoxia was hypothesised to be greater when calculated from doubling a unilateral measurement of the lateral extracranial artery with the smaller resting blood flow.

Methods

Ethical Approval

Ethical approval for this study was obtained from Bangor University (proposal number 2019-16489, accepted 11/03/2019) and was conducted following the standards of the *Declaration of Helsinki 2013,* except for registration in a database, with written informed consent obtained from all study volunteers.

Participants

Forty-four young healthy participants were recruited in this study [17 female, 24 (5) years, 177 (9) cm, 72 (9) kg, haemoglobin 15 (1) $g \cdot dL^{-1}$, haematocrit 44 (4) %]. Participants were non-smokers, and free from cardiovascular, haematological, and neurological disease. Participants had not resided overnight at an altitude of >2500 m within the last six months. Participants were screened for vascular abnormalities to ensure reliable ICA and VA ultrasound images could be acquired. To minimise the impact of fluctuations in sex hormones on blood flow measurements (Krejza, Mariak, Huba, Wolczynski, & Lewko, 2001) female participants were included if they had contraceptive-induced amenorrhea or a regular menstruating cycle. Participants with a regular menstrual cycle were tested during the early follicular phase (day 1 to 7) or the placebo phase of the oral contraceptive. Participants were instructed to refrain from consuming alcohol and from undertaking exhaustive exercise within 24 hours of experimental trials. Experimental trials were completed at the same time of day and participants were encouraged to match their diet and supplement intake, including caffeinated beverages, before arrival at the laboratory.

Study Design

A repeated-measures, crossover design was used where each participant completed two experimental trials separated by a minimum of 48 hours. Experimental trials consisted of a 90 min exposure to either normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) or poikilocapnic

hypoxia (FiO_2 = 12.0%) in a temperature [26 (2) °C] and humidity [30 (4) %] controlled environmental chamber (Hypoxico Inc, New York, USA).

Experimental procedures

On entry to the chamber, participants were instrumented with a 3-lead electrocardiogram, pulse oximeter, and a blood-pressure cuff to measure heart rate, peripheral arterial oxygen saturation (SpO_2; Model 9590 Oximeter; Nonin Medical Inc. Minnesota, USA), and mean arterial blood pressure (MAP; Model M6 AC ME, Omron Healthcare Co., Ltd, Kyoto, Japan), respectively. After 20 min participants lay supine for 10 min before a facemask was attached to measure the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) and minute ventilation ($\dot{V}E$) for 5 min (Metalyzer 3B, CORTEX Biophysik, GmbH; Leipzig; Germany). Following this, blood flow measurements of the left and right ICA and VA were completed by duplex ultrasound. Cardiovascular measurements were obtained at 30 min intervals and respiratory measurements were obtained at minute 30 and 90.

Duplex ultrasound acquisition and analysis

All extracranial blood flow measurements were collected by the same operator (ATF), using duplex ultrasound with a 12 MHz linear transducer (Acuson X300, Siemens Healthcare, GMbH; Erlanden: Germany) at 30 frames per second, and per recommended technical guidelines (Thomas, Lewis, Hill, & Ainslie, 2015). Bilateral ICA and VA blood flow was calculated from consecutive left and right measurements. To improve the accuracy of the blood flow measurements and minimise the trade-off between B-mode and pulsed-wave Doppler mode (Thomas et al., 2015), vessel diameter and flow velocity measurements were collected in consecutive 30 s recordings with care taken to maintain the same position within the vessel. High-resolution B-mode images were used to measure vessel diameter. Flow velocity was measured using Doppler velocity spectrum with the cursor set in the centre of the vessel with

a 60º angle of insonation with the Doppler gate adjusted to fill the size of the vessel. ICA were measured 1.0–1.5 cm distal to the carotid bifurcation and VA were measured between C3 and the subclavian artery. The order of imaging was (1) VA right, (2) ICA right, (3) VA left, and (4) ICA left. In a separate day-to-day reproducibility study (N = 10) completed by the same operator (ATF), the coefficient of variation (CV) of this technique for blood flow, vessel diameter, and flow velocity of the ICA (11%, 4%, and 7%) and VA (9%, 2%, and 7%) were comparable with recommended guidelines (Thomas et al., 2015).

All data was captured and stored for subsequent offline analysis by an investigator blinded to the condition of the experimental trials. Following a conservative image quality check, data and statistical analysis were completed on 33 ICA pairs (24 male, 9 female) and 43 VA pairs (26 male, 17 female). The 11 ICA exclusions were due to lack of clear insonation and poor image quality whilst the 1 VA exclusion was due to the presence of an unidentified branching vessel. Offline analysis was adapted from standardised procedures described elsewhere (Hoiland et al., 2017; Ogoh et al., 2013). Specifically, mean flow velocity was calculated using half the time-averaged maximum velocity (TAMx) and was averaged from 10 cardiac cycles to minimise the impact of respiration. Mean vessel diameter was measured using an automated edge-detection tracking software (Brachial Analyser, Medical Imaging Applications, Iowa, USA) and was calculated from a weighted average of the peak systolic and diastolic diameters across 10 cardiac cycles [(systolic diameter x ⅓) + (diastolic diameter x ⅔)]. Subsequently, blood flow was calculated using the following equation:

$$Blood\ flow\ (ml \cdot min^{-1}) = [TAMx\ (cm \cdot s^{-1})/2] \times [\pi \times (mean\ artery\ diameter\ (mm)/2)^2] \times 60$$

Absolute and relative change in blood flow was calculated as the change in blood flow from normoxia to hypoxia at the same time point using the following equations:

$$Absolute\ change\ in\ blood\ flow\ to\ hypoxia\ (ml \cdot min^{-1}) = hypoxic\ blood\ flow\ (ml \cdot min^{-1}) - normoxic\ blood\ flow\ (ml \cdot min^{-1})$$

$$Relative\ change\ in\ blood\ flow\ to\ hypoxia\ (\%) = [(hypoxic\ blood\ flow\ (ml \cdot min^{-1}) - normoxic\ blood\ flow\ (ml \cdot min^{-1}))/normoxic\ blood\ flow\ (ml \cdot min^{-1})] \times 100$$

To control for differences between individual responses to poikilocapnic hypoxia, an index of absolute and relative hypoxic blood flow reactivity was calculated by normalising these values to the absolute change in SpO_2 (ΔSpO_2).

To calculate the difference in resting normoxic blood flow between lateral arteries of the ICA and VA, the following equation was used:

$$Lateral\ artery\ difference\ in\ resting\ normoxic\ blood\ flow\ (\%) = [(Larger\ blood\ flow\ (ml \cdot min^{-1}) - smaller\ blood\ flow\ (ml \cdot min^{-1}))/smaller\ blood\ flow\ (ml \cdot min^{-1})] \times 100$$

Extracranial arteries were also identified and grouped by the lateral vessel (left or right) with the larger resting normoxic blood flow.

Statistical Analysis

Statistical analysis was conducted using SPSS Statistics v25 (IBM Corp., Armonk, NY, USA). Values are mean (SD) unless otherwise stated and statistical significance was set at $P < 0.05$.

To determine any differences in cardiorespiratory variables during normoxia and hypoxia a linear mixed model (LMM) was used. Fixed effects of interest were 'Condition' (normoxia or hypoxia), 'Time' (30, 60, or 90 min), as well as the interaction ('Condition' × 'Time'), with 'Participant ID' added as a random effect. Baseline data were not included as these measurements were collected during seated rest before entry to the environmental chamber.

To determine whether there are resting normoxic blood flow differences between left and right ICA and VA, a LMM was used with fixed effects of interest 'Vessel Type' (ICA or VA), and 'Vessel Side' (left or right), as well as the interaction ('Vessel Type' × 'Vessel Side'), adding 'Participant ID' added as a random effect.

To determine whether there are differences in blood flow regulation to hypoxia between the four extracranial arteries, a LMM was used to examine left and right ICA and VA absolute blood flow, vessel diameter, and flow velocity in normoxia and hypoxia. Specifically, fixed effects of interest were 'Condition', 'Vessel Type', and 'Vessel Side', with 'Participant ID' as added as a random effect. The primary outcome of interest was the interaction ('Condition' × 'Vessel Type' × 'Vessel Side'). In addition, for conventional purposes, a LMM was used to examine the absolute change and relative change in blood flow, vessel diameter, and flow velocity regulation to hypoxia (i.e. change from normoxia) between the ICA and VA with 'Vessel Type', and 'Vessel Side' and their interaction ('Vessel Type' × 'Vessel Side') as fixed effects of interest, adding 'Participant ID' as a random effect. Values from LMM analysis are reported as estimated marginal means and an estimated SD which was derived from the standard error (SE), where n is the sample size [$Estimated\ SD = SE\ \times \left(\sqrt{n}\right)$] (Shenouda, Gillen, Gibala, & MacDonald, 2017).

To determine the measurement error in unilateral compared to bilateral calculations of relative extracranial blood flow response to hypoxia, we investigated whether a ratio-scaling problem

exists and quantified the measurement error by Bland-Altman analysis (Bland & Altman, 1986). Disproportionate ratio-scaling in the calculation of relative change ratios has been described extensively elsewhere (Atkinson & Batterham, 2013a, 2013b; Atkinson et al., 2013). For clarity, the analysis used in the present study is described. Pearson's correlation was used to determine whether a negative relationship between resting normoxic blood flow and the relative blood flow response to hypoxia existed in the left and right arteries of the ICA and VA. To confirm that the relationships between resting normoxic blood flow and the relative blood flow response to hypoxia were statistically different from the relationships between resting normoxic blood flow and the absolute blood flow response to hypoxia, correlation coefficients were compared using Fisher's Z transformation via the cocor online software (Diedenhofen & Musch, 2015). Then, appropriate ratio-scaling was applied to the ICA and VA to calculate the 'corrected' ICA and VA blood flow response to hypoxia. The calculation of the regression slope between logarithmically-transformed normoxic blood flow and hypoxic blood flow was used to determine whether hypoxic blood flow scales disproportionately for the range of values of normoxic blood flow, with an upper confidence limit [95%CI] being less than 1.0 indicating this to be true. An analysis of covariance analysis (ANCOVA) model was used to determine group differences between unilateral and bilateral calculations of logged-scale change in blood flow (Δblood flow), with logarithmically-transformed normoxic blood flow as a covariate. Back-transformation of covariate-adjusted Δblood flow were converted to Δ%blood flow as the final corrected, and more conventional, calculation of the relative blood flow response to hypoxia. Bland-Altman analysis was used to determine the level of agreement between unilateral and bilateral calculations of the relative blood flow response to hypoxia for the ICA and VA. Unilateral measurements were determined by resting normoxic blood flow (smaller or larger) or vessel side (left or right) and doubled before calculating the relative blood flow response to hypoxia. The mean difference between unilateral and bilateral calculations of the

relative blood flow response to hypoxia was determined as the measurement bias (error), with the respective 95% confidence intervals, and the 95% limits of agreement.

Results

Cardiorespiratory responses to hypoxia

There were no interactions for cardiorespiratory responses during the period of supine rest (Table 1, all $P > 0.05$). Compared to normoxia, acute poikilocapnic hypoxia increased heart rate [Main effect of 'Condition'; +11 (6) bpm; $P < 0.001$], MAP [+1 (4) mmHg; $P < 0.05$] and $\dot{V}E$ [+0.9 (1.4) $L \cdot min^{-1}$; $P < 0.001$], and decreased $P_{ET}CO_2$ [-3.9 (2.1) mmHg; $P < 0.001$] and SpO_2 [-20 (3) %; $P < 0.001$] during supine rest. Cardiorespiratory responses were stable between 30–90 min with the exception of an increase in MAP [Main effect of 'Time'; +2 (4) mmHg, 90 vs 60 min; $P < 0.01$] and a decline in $\dot{V}E$ [-0.5 (1.5) $L \cdot min^{-1}$, 90 vs 30 min; $P < 0.05$] in both conditions.

Resting extracranial artery characteristics

There was no 'Vessel Type' × 'Vessel Side' interaction ($P = 0.17$), nor a main effect of 'Vessel Side' ($P = 0.74$), in resting normoxic blood flow between the left and right ICA [299 (43) and 288 (43) $mL \cdot min^{-1}$, 4%] and left and right VA [103 (43) and 95 (43) $mL \cdot min^{-1}$, 8%], but there was a main effect of 'Vessel Type' ($P < 0.001$). The difference in resting normoxic blood flow between lateral arteries ranged from 1 to 91% for the ICA and 0 to 400% for the VA. More participants had a larger resting normoxic blood flow in the right ICA (14 left, 19 right) and VA (21 left, 22 right) than left.

Extracranial artery blood flow regulation to hypoxia

LMM analyses revealed no 'Condition' × 'Vessel Type' × 'Vessel Side' interaction for blood flow (Figure 1a; $P = 0.62$), vessel diameter (Figure 1b; $P = 0.70$), and flow velocity (Figure 1c;

P = 0.64), indicating that ICA and VA blood flow regulation to acute poikilocapnic hypoxia did not differ as a function of vessel type and vessel side. There was also no 'Condition' × 'Vessel Side' interaction for vessel diameter (P = 0.32), flow velocity (P = 0.18), blood flow (P = 0.86), and the volume of left and right extracranial blood flow were similar in hypoxia [left ICA + VA, 495 (93) $mL \cdot min^{-1}$ vs right ICA + VA, 501 (93) $mL \cdot min^{-1}$; P = 0.89].

Due to the ICA and VA differences at normoxic baseline, there was a 'Condition' × 'Vessel Type' interaction for blood flow (P < 0.001). Subsequently, to account for the large discrepancy between ICA and VA blood flow at normoxic baseline, this variable was analysed using the conventionally reported change scores from normoxia. LMM analysis revealed no 'Vessel Type' × 'Vessel Side' interaction for the absolute and relative blood flow response to hypoxia between the left and right ICA and VA (Figure 2a, 2c, 2e, 2g, all P > 0.05) and reaffirmed that there was no main effect of 'Vessel Side' for these blood flow variables (all P > 0.05). As expected, a main effect of 'Vessel Type' (i.e. when data was pooled as the bilateral value) revealed that the ICA had a greater absolute blood flow response to hypoxia [Figure 2b; 155 (63) vs 57 (65) $\Delta mL \cdot min^{-1}$, P < 0.001], and absolute hypoxic reactivity [Figure 2d, 8.0 (3.3) vs 3.0 (3.4) $\Delta mL \cdot min^{-1} \cdot \Delta SpO_2^{-1}$; P < 0.001] than the VA. Whereas, there was no main effect of 'Vessel Type' for the relative blood flow response to hypoxia [Figure 2f, 26.6 (21.6) vs 33.2 (22.6) Δ%; P = 0.053] and relative hypoxic reactivity [Figure 2h, 1.4 (1.0) vs 1.7 (1.1) $\Delta\% \cdot \Delta SpO_2^{-1}$; P = 0.12]. When calculated bilaterally, acute poikilocapnic hypoxia increased global blood flow by 29.1 (18.1) % [776 (124) vs 995 (124) $mL \cdot min^{-1}$; P < 0.001]. There was no 'Condition' × 'Vessel Type' interaction for vessel diameter (P = 0.29) and flow velocity (P = 0.37), which had a similar relative vessel diameter response to hypoxia [ICA 6.9 (3.7) vs VA 7.0 (3.8) %; P = 0.83] and relative flow velocity response to hypoxia [ICA 11.2 (15.7) vs VA 15.6 (16.1) %; P = 0.09] between bilateral calculations of the ICA and VA.

The relationship between resting normoxic blood flow and the blood flow response to hypoxia

No relationship was observed between resting normoxic blood flow and the absolute blood flow response to hypoxia (Figures 3a and 3b). However, negative relationships were identified between resting normoxic blood flow and the relative ICA and VA blood flow response to hypoxia (Figures 3c and 3d, r = -0.33 and -0.37, respectively; $P < 0.001$). These relationships were statistically different from the relationships observed between resting normoxic blood flow and the absolute blood flow response to hypoxia (both $P < 0.001$). These relationships indicated that vessels with smaller resting blood flow were associated with a greater and more varied relative blood flow change to hypoxia. As negative relationships were identified between normoxic blood flow and the relative blood flow response to hypoxia, ratio-scaling was conducted. The regression slopes between logarithmically-transformed resting normoxic blood flow and hypoxic blood flow were 0.70 (95%CI [0.49 to 0.91]) for the ICA and 0.79 (95%CI [0.67 to 0.91]) for the VA with each upper confidence limit of less than 1.0, indicating that vessels with smaller resting blood flow were associated with a disproportionately large relative change in hypoxic blood flow. The covariate-adjusted group means for the relative blood flow response to hypoxia after ANCOVA analysis indicated smaller differences between left and right calculations of the ICA [27.5 (16.8) and 25.1 (16.8) %] and VA [28.3 (22.5) and 32.4 (22.5) %] compared with non-corrected values of the relative blood flow response to hypoxia (Supplementary Table 1).

Measurement bias between unilateral and bilateral calculations of the relative blood flow response to hypoxia

Bland-Altman analysis revealed doubling unilateral ICA measurements from the vessel with the smaller resting normoxic blood flow overestimated the relative ICA blood flow response to hypoxia by 5% (95%CI [1 to 9], limits of agreement: -19 to +29%) compared to bilateral calculations (Figure 4a). Doubling unilateral ICA measurements from the vessel with the larger resting normoxic blood flow underestimated the relative ICA blood flow response to hypoxia

by 4% (95%CI [-7 to 0], limits of agreement: -23 to +16%) compared to bilateral calculations (Figure 4b). There was no significant bias in the relative VA blood flow response to hypoxia from doubling unilateral VA measurements from the vessel with the smaller or larger resting normoxic blood flow compared to bilateral calculations (Figure 4c and 4d). However, the vessel with the larger resting normoxic blood flow had the lowest measurement bias and narrowest limits of agreement to the relative VA blood flow response to hypoxia compared to doubling bilateral calculations (Figure 4d).

Bland-Altman analysis revealed no significant bias in the relative ICA blood flow response to hypoxia from doubling left or right unilateral ICA measurements compared to bilateral calculations (Figures 5a and 5b). Bland-Altman analysis revealed doubling unilateral right VA measurements overestimated the relative VA blood flow response to hypoxia by 4% (95%CI [0 to 7], limits of agreement: -21 to +28%) compared to bilateral calculations (Figure 5d). In contrast, there was no significant bias in the relative VA blood flow response to hypoxia from doubling unilateral left VA measurements compared to bilateral calculations (Figure 5c).

Discussion

Main findings

The principal finding of this study was that extracranial blood flow regulation to hypoxia is comparable (Figure 1) when factoring for vessel type (ICA or VA) and vessel side (left or right). The increase in blood flow to hypoxia was regulated by an increase in vessel diameter and flow velocity in all extracranial vessels. Global extracranial blood flow to hypoxia increased from 776 (124) to 995 (124) $mL \cdot min^{-1}$ (29.1%, $P < 0.001$) that was equally distributed between the left and right sides [left ICA + VA, 495 (93) vs right ICA + VA, 501 (93); $P = 0.89$]. When conventionally reported as the change score from normoxia, the bilateral absolute blood flow response to hypoxia was greater in the ICA than the VA (Figure 2b and

2d), whereas the bilateral relative blood flow response to hypoxia was comparable between the ICA and VA (Figure 2f and 2h). We are unaware of previous duplex ultrasound investigations that have assessed bilateral extracranial blood flow regulation to hypoxia, nor considered the effect of vessel type and vessel side.

This study also identified negative relationships between extracranial artery resting normoxic blood flow and the relative blood flow response to hypoxia for the ICA and VA (Figure 3c and 3d), which illustrated a ratio-scaling problem akin to that previously described with FMD assessment of brachial artery vascular function (Atkinson & Batterham, 2013a, 2013b; Atkinson et al., 2013). Compared with bilateral measurement of relative blood flow change to hypoxia, the common practice of doubling unilateral measurements led to average errors of up to 5%, and individual errors of up to 37%, which were greatest and more varied in the extracranial arteries with smaller resting normoxic blood flow (Figure 4a – 5d).

Bilateral extracranial blood flow regulation to hypoxia

When assessed bilaterally, acute poikilocapnic hypoxia caused the same vasodilation [ICA 6.9 (3.7) vs VA 7.0 (3.8) %; $P = 0.87$]) and comparable relative increases in blood flow and blood flow reactivity in the ICA and VA (Figure 2f and 2h). These regional blood flow responses to acute hypoxia are similar to those previously reported from studies employing the typical method of doubling unilateral measurements (Lewis et al., 2014; Morris et al., 2017; Willie et al., 2012). There are as many studies reporting that the increase in blood flow to hypoxia is mediated by vasodilation in both ICA and VA to extreme (<80% SpO_2) poikilocapnic hypoxia (Lewis et al., 2014; Morris et al., 2017) or isocapnic hypoxia (Fernandes et al., 2018; Hoiland et al., 2017) as there are reporting no vasodilation (Lafave et al., 2019; Ogoh et al., 2013; Willie et al., 2012; Willie, Smith, et al., 2014), with others suggesting regionally-specific vasodilation (Kellawan, Harrell, Roldan-Alzate, Wieben, & Schrage, 2017; Subudhi et al., 2014).

Notwithstanding the methodological differences of inducing hypoxia that is known to affect the cerebrovascular response, such as the clamping of carbon dioxide (Kellawan et al., 2017; Ogoh et al., 2013; Willie et al., 2012), exposure to high-altitude hypobaric hypoxia (Hoiland et al., 2017; Lafave et al., 2019; Subudhi et al., 2014; Willie, Smith, et al., 2014) and length of exposure (Lewis et al., 2014), the aforementioned studies are often limited by their sample size and therefore sensitivity to detect small differences where high inter-individual variability with exposure to acute severe hypoxia is notable (Willie, Smith, et al., 2014). Compared to previous literature, the present study was conducted in a relatively large cohort and is strengthened by bilateral measurement of the blood flow response to hypoxia which provides more certainty that blood flow is similarly regulated in ICA and VA in response to acute poikilocapnic hypoxia.

Extracranial artery blood flow measurement error

The absolute increase in blood flow to hypoxia was comparable within ICA (Figure 3a) and VA (Figure 3b) irrespective of the resting blood flow. In contrast, significant negative relationships were identified between resting blood flow and the relative blood flow response to hypoxia in both the ICA and VA, where vessels with smaller resting blood flow had greater relative blood flow responses to hypoxia (Figure 3c and 3d). This indicated the same ratio-scaling problem in extracranial arteries as has previously been described with FMD assessment of the brachial artery vascular function (Atkinson & Batterham, 2013a, 2013b; Atkinson et al., 2013). In brief, Atkinson and Batterham describe this relationship to be a fundamental ratio-scaling problem when using relative change ratios (i.e. Δ%FMD = [peak diameter – resting diameter]/resting diameter x100) where the numerator (i.e. difference in diameter) does not scale proportionately for the range of denominator values (i.e. resting diameter). The negative relationships also indicated that the relative change in blood flow to hypoxia were more varied in vessels with smaller resting normoxic blood flow. This skewness towards the group with the

smaller scores (i.e. smaller resting normoxic blood flow) is common with ratio indices since ratios cause the outcome data to be non-normally distributed even when the two ratios are normally distributed (Atkinson & Batterham, 2013b; Vickers, 2001). This relationship highlights a mathematical, rather than physiological, source of measurement error when adopting a unilateral rather than bilateral assessment.

When compared to the bilateral calculation, doubling of a unilateral extracranial measurement of the relative blood flow response to hypoxia from the vessel with the smaller resting blood flow led to a greater mean measurement bias (5%) and wider limits of agreement (up to 31%) than from the vessel with the larger resting blood flow (Figure 4). Despite the mean bias of a unilateral measurement compared to the bilateral measurement being small (3 to 5%), it is misleading to judge the measurement error of a unilateral assessment from this metric alone. To fully examine measurement error, mean bias, the width of the limits of agreement, and visual inspection of the Bland-Altman plots for a constant or proportional bias should be completed (Giavarina, 2015). Here, the limits of agreement of a unilateral measurement were -26 to 31% (Figure 4), which can be considered significant when considering the magnitude is similar to the mean extracranial relative blood flow response to hypoxia. These wide limits of agreement indicate a low level of precision in unilateral measurements, compared to bilateral measurements, which may lead to erroneous interpretation of data particularly in small sample cohorts. Moreover, the Bland-Altman analysis revealed a proportional measurement bias that was more prominent in the vessels with the smaller blood flow (i.e. Figure 4a). Therefore, doubling a unilateral extracranial measurement from the vessel with the smaller resting normoxic blood flow is the least comparable, and causes the greatest measurement error, to the true bilateral relative blood flow response to hypoxia.

In investigations of extracranial blood flow regulation to hypoxia, a unilateral measurement of the right VA is overwhelmingly favoured (Fernandes et al., 2018; Hoiland et al., 2017; Lafave

et al., 2019; Lewis et al., 2014; Morris et al., 2017; Ogoh et al., 2013; Willie et al., 2012) compared to the left VA (Subudhi et al., 2014; Willie, Smith, et al., 2014). The rationale often stated for the right side being chosen is to account for the 20–30% smaller blood flow in the right VA compared to the left VA such that absolute calculations of regional and global blood flow are an underestimation (Lewis et al., 2014; Ogoh et al., 2013). However, due to the stark differences in resting blood flow between the ICA and VA, regional blood flow response to stressors such as hypoxia are commonly reported relative to resting blood flow (Willie et al., 2012). Disproportionate scaling in the calculation of blood flow relative change may, in part, have contributed to conclusions of preferential blood flow regulation to the posterior circulation compared to anterior circulation in previous research (Lewis et al., 2014; Ogoh et al., 2013; Subudhi et al., 2014; Willie et al., 2012). In these studies, the relative blood flow response to hypoxia was greater in the VA (posterior) than ICA (anterior) based on unilateral measures from the right VA (Lewis et al., 2014; Ogoh et al., 2013; Willie et al., 2012). In the current study, when unilateral extracranial measurements were selected on the vessel side, right VA measurements overestimated the relative blood flow response to the greatest degree (4%; Figure 4d) and had the widest limits of agreement (-21 to 28%) compared to the bilateral calculation. This finding is particularly noteworthy given that many investigators choose to scan the right rather than the left VA presuming that the right VA is the conservative option when doubling a unilateral measurement. But, as detailed in this study, vessels with the smaller resting blood flow are more susceptible to greater and more varied measurement errors due to the ratio-scaling problem when describing a relative blood flow response.

Perspectives and application

In this study, there was no statistical difference in resting blood flow between the left and right vessels of the ICA (4%) and VA (8%). Therefore, we may have underestimated the group mean measurement error of unilateral compared to bilateral assessment that may be found in future

studies. This is particularly likely for the VA as the left-to-right blood flow difference in the VA is typically reported in the range of 20–30% (Khan et al., 2017; Schöning et al., 1994). The heterogeneity between individuals in the magnitude of difference between left and right extracranial arteries blood flow (ICA: 1 to 91% and VA: 0 to 400%) means that without examining the contralateral vessel there is an increased likelihood of substantial measurement error in the calculation of the relative blood flow response to hypoxia.

To eliminate measurement error in the relative blood flow response to hypoxia, bilateral measurements should be used. However, if this is infeasible, we advise the ICA and VA vessel with the larger resting normoxic blood flow be measured for each participant following pre-screening of both the left and right vessels based on two inferences. Firstly, vessels with the smaller resting normoxic blood flow were associated with a greater and more varied relative blood flow response to hypoxia (Figure 2). This is also supported by the Bland-Altman analysis that identified the greatest measurement bias and limits of agreement, and the presence of a proportional bias is caused by unilateral measurements of the extracranial vessel with the smaller blood flow (Figure 4). Secondly from a practical aspect, successfully imaging a vessel, maintaining consistent flow velocity with a centrally-positioned Doppler gate, and accurately measuring vessel diameter (whether manual or automated) are all easier in the vessel containing the larger blood flow. Moreover, to improve efficiency and feasibility of identifying the vessel with the larger resting normoxic blood flow before experimental trials, the left and right vessel diameter could be measured using the standard built-in caliper method available in ultrasound devices as a strong index of vessel blood flow (Cipolla, 2009). Where simultaneous insonation of two extracranial arteries is necessary this is normally achieved by contralateral measurements due to ultrasound interference and physical probe space limitations (Sato et al., 2016). In this instance, the extracranial artery (ICA or VA) with the widest difference in resting normoxic blood flow between the left and right vessels should be prioritised in imaging to

minimise measurement error. We advise these methods to be applied when measuring extracranial blood flow to other vasoactive stimuli such as carbon dioxide, orthostasis, and exercise.

Methodological considerations

The interaction of oxygen and carbon dioxide tensions are key factors in the overall change in cerebral blood flow during exposure to hypoxia (Bruce et al., 2016; Friend, Balanos, & Lucas, 2019; Lucas et al., 2011). The single bout of poikilocapnic hypoxia used here resulted in a range of SpO_2, $\dot{V}E$ and, $P_{ET}CO_2$ between participants, therefore not all individuals had similar systemic hypoxia. However, when the relative blood flow response to hypoxia was corrected for the differences in SpO_2 as an index of relative hypoxic reactivity the ICA and VA were found to remain similar (Figure 2h). Future research should use stepwise gas manipulations to investigate the regulation of extracranial arteries through the range of hypoxic severities typically experienced. Bilateral calculations of blood flow were derived from consecutive rather than simultaneous measurements of the left and right arteries as we only had access to a single ultrasound. However, the short time difference introduced by using consecutive left and right measurements likely had limited influence on the interpretation of our findings as the ICA and VA measurements were obtained whilst participants were rested. A wide range of methodological techniques are currently employed to measure cerebral blood flow at rest and in response to stressors, each with its advantages and disadvantages (Tymko, Ainslie, & Smith, 2018). Duplex ultrasound offers a non-invasive, volumetric measurement of intravascular blood flow with excellent temporal resolution important for the assessment of cerebral blood flow to dynamic stressors (e.g. hypoxia, carbon dioxide, orthostasis, and exercise). However, to obtain accurate and reliable (~10% day-to-day CV) measurements considerable ultrasound training is required (Thomas et al., 2015). Notwithstanding this, the results presented here reveal a source of previously under-recognised measurement error in the assessments of

unilateral extracranial relative blood flow response to vasoactive stimuli, and provides a systematically approached consensus for the selection of unilateral extracranial measurements to minimise this measurement error when bilateral measurement is infeasible.

Conclusions

ICA and VA blood flow regulation to hypoxia is comparable when factoring for vessel type (ICA or VA) and vessel side (left or right) effects. Bilateral calculations of the ICA and VA indicated the same degree of vasodilation and comparable increases in relative blood flow to acute poikilocapnic hypoxia. Compared to bilateral assessment of the relative blood flow response to hypoxia, individual unilateral measurement error reached 37%, and were greatest in ICA and VA with the smaller resting blood flow due to a ratio-scaling problem. Where bilateral assessment is infeasible assessing the ICA and VA vessels with the larger resting blood flow, not the left or right vessel, reduces unilateral measurement error.

References

Aleksic, M., & Brunkwall, J. (2009). Extracranial Blood Flow Distribution During Carotid Surgery. *Journal of Vascular Surgery*, *50*(5), 1244. https://doi.org/10.1016/j.jvs.2009.09.014

Atkinson, G., & Batterham, A. M. (2013a). Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis*, *226*(2), 425–427. https://doi.org/10.1016/j.atherosclerosis.2012.11.027

Atkinson, G., & Batterham, A. M. (2013b). The percentage flow-mediated dilation index: A large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vascular Medicine (United Kingdom)*, *18*(6), 354–365. https://doi.org/10.1177/1358863X13508446

Atkinson, G., Batterham, A. M., Thijssen, D. H. J., & Green, D. J. (2013). A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *Journal of Hypertension*, *31*(2), 287–291. https://doi.org/10.1097/HJH.0b013e32835b8164

Bland, J. M., & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet*, *327*(8476), 307–310. https://doi.org/10.1016/S0140-6736(86)90837-8

Bruce, C. D., Steinback, C. D., Chauhan, U. V., Pfoh, J. R., Abrosimova, M., Vanden Berg, E. R., … Day, T. A. (2016). Quantifying cerebrovascular reactivity in anterior and posterior cerebral circulations during voluntary breath holding. *Experimental Physiology*, *101*(12), 1517–1527. https://doi.org/10.1113/EP085764

Cipolla, M. J. (2009). The cerebral circulation. In *Integrated systems physiology: From molecule to function* (Vol. 1, pp. 1–59). Morgan & Claypool Publishers.

Diedenhofen, B., & Musch, J. (2015). cocor: A Comprehensive Solution for the Statistical Comparison of Correlations. *PLOS ONE*, *10*(4), e0121945. https://doi.org/10.1371/journal.pone.0121945

Fernandes, I. A., Rocha, M. P., Campos, M. O., Mattos, J. D., Mansur, D. E., Rocha, H. N. M., … Nóbrega, A. C. L. (2018). Reduced arterial vasodilatation in response to hypoxia impairs cerebral and peripheral oxygen delivery in hypertensive men. *Journal of Physiology*, *596*(7), 1167–1179. https://doi.org/10.1113/JP275545

Friend, A. T., Balanos, G. M., & Lucas, S. J. E. (2019). Isolating the independent effects of hypoxia and hyperventilation-induced hypocapnia on cerebral haemodynamics and cognitive function. *Experimental Physiology*, *104*(10), 1482–1493. https://doi.org/10.1113/EP087602

561 Giavarina, D. (2015). Understanding Bland Altman analysis. *Biochemia Medica*, *25*(2), 141–
562 151. https://doi.org/10.11613/BM.2015.015

563 Hoiland, R. L., Bain, A. R., Tymko, M. M., Rieger, M. G., Howe, C. A., Willie, C. K., …
564 Ainslie, P. N. (2017). Adenosine receptor-dependent signaling is not obligatory for
565 normobaric and hypobaric hypoxia-induced cerebral vasodilation in humans. *Journal of*
566 *Applied Physiology*, *122*(4), 795–808. https://doi.org/10.1152/japplphysiol.00840.2016

567 Hoiland, R. L., Howe, C. A., Coombs, G. B., & Ainslie, P. N. (2018). Ventilatory and
568 cerebrovascular regulation and integration at high-altitude. *Clinical Autonomic*
569 *Research*, *28*(4), 423–435. https://doi.org/10.1007/s10286-018-0522-2

570 Hu, X. Y., Li, Z. X., Liu, H. Q., Zhang, M., Wei, M. L., Fang, S., … Liu, J. R. (2013).
571 Relationship between vertebral artery hypoplasia and posterior circulation stroke in
572 Chinese patients. *Neuroradiology*, *55*(3), 291–295. https://doi.org/10.1007/s00234-012-
573 1112-y

574 Kellawan, J. M., Harrell, J. W., Roldan-Alzate, A., Wieben, O., & Schrage, W. G. (2017).
575 Regional hypoxic cerebral vasodilation facilitated by diameter changes primarily in
576 anterior versus posterior circulation. *Journal of Cerebral Blood Flow and Metabolism*,
577 *37*(6), 2025–2034. https://doi.org/10.1177/0271678X16659497

578 Khan, M. A., Liu, J., Tarumi, T., Lawley, J. S., Liu, P., Zhu, D. C., … Zhang, R. (2017).
579 Measurement of cerebral blood flow using phase contrast magnetic resonance imaging
580 and duplex ultrasonography. *Journal of Cerebral Blood Flow and Metabolism*, *37*(2),
581 541–549. https://doi.org/10.1177/0271678X16631149

582 Krejza, J., Mariak, Z., Huba, M., Wolczynski, S., & Lewko, J. (2001). Effect of Endogenous
583 Estrogen on Blood Flow Through Carotid Arteries. *Stroke*, *32*(1), 30–36.
584 https://doi.org/10.1161/01.str.32.1.30

585 Lafave, H. C., Zouboules, S. M., James, M. A., Purdy, G. M., Rees, J. L., Steinback, C. D.,
586 … Day, T. A. (2019). Steady-state cerebral blood flow regulation at altitude: interaction
587 between oxygen and carbon dioxide. *European Journal of Applied Physiology*, *119*(11–
588 12), 2529–2544. https://doi.org/10.1007/s00421-019-04206-6

589 Lawley, J. S., Macdonald, J. H., Oliver, S. J., & Mullins, P. G. (2017). Unexpected reductions
590 in regional cerebral perfusion during prolonged hypoxia. *Journal of Physiology*, *595*(3),
591 935–947. https://doi.org/10.1113/JP272557

592 Lewis, N. C. S., Messinger, L., Monteleone, B., & Ainslie, P. N. (2014). Effect of acute
593 hypoxia on regional cerebral blood flow: effect of sympathetic nerve activity. *Journal of*
594 *Applied Physiology*, *116*(9), 1189–1196.
595 https://doi.org/10.1152/japplphysiol.00114.2014

596 Lucas, S. J. E., Burgess, K. R., Thomas, K. N., Donnelly, J., Peebles, K. C., Lucas, R. A. I.,
597 … Ainslie, P. N. (2011). Alterations in cerebral blood flow and cerebrovascular
598 reactivity during 14 days at 5050 m. *The Journal of Physiology*, *589*(3), 741–753.
599 https://doi.org/10.1113/jphysiol.2010.192534

600 Morris, L. E., Flück, D., Ainslie, P. N., & McManus, A. M. (2017). Cerebrovascular and
601 ventilatory responses to acute normobaric hypoxia in girls and women. *Physiological*
602 *Reports*, *5*(15). https://doi.org/10.14814/phy2.13372

603 Ogoh, S. (2017). Relationship between cognitive function and regulation of cerebral blood
604 flow. *Journal of Physiological Sciences*, *67*(3), 345–351.
605 https://doi.org/10.1007/s12576-017-0525-0

606 Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi, A. W., & Miyamoto, T. (2013).
607 Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries.
608 *Experimental Physiology*, *98*(3), 692–698.

https://doi.org/10.1113/expphysiol.2012.068015

Romero, J. R., Pikula, A., Nguyen, T. N., Nien, Y. L., Norbash, A., & Babikian, V. L. (2010). Cerebral Collateral Circulation in Carotid Artery Disease. *Current Cardiology Reviews*, *5*(4), 279–288. https://doi.org/10.2174/157340309789317887

Rossetti, G. M., d'Avossa, G., Rogan, M., Macdonald, J. H., Oliver, S. J., & Mullins, P. G. (2020). Reversal of neurovascular coupling in the default mode network: Evidence from hypoxia. *Journal of Cerebral Blood Flow & Metabolism*, 0271678X2093082. https://doi.org/10.1177/0271678X20930827

Sato, K., Fisher, J. P., Seifert, T., Overgaard, M., Secher, N. H., & Ogoh, S. (2012). Blood flow in internal carotid and vertebral arteries during orthostatic stress. *Experimental Physiology*, *97*(12), 1272–1280. https://doi.org/10.1113/expphysiol.2012.064774

Sato, K., Oue, A., Yoneya, M., Sadamoto, T., & Ogoh, S. (2016). Heat stress redistributes blood flow in arteries of the brain during dynamic exercise. *Journal of Applied Physiology*, *120*(7), 766–773. https://doi.org/10.1152/japplphysiol.00353.2015

Sato, K., Sadamoto, T., Hirasawa, A., Oue, A., Subudhi, A. W., Miyazawa, T., & Ogoh, S. (2012). Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. *Journal of Physiology*, *590*(14), 3277–3290. https://doi.org/10.1113/jphysiol.2012.230425

Schöning, M., Walter, J., & Scheel, P. (1994). Estimation of cerebral blood flow through color duplex sonography of the carotid and vertebral arteries in healthy adults. *Stroke*, *25*(1), 17–22. https://doi.org/10.1161/01.str.25.1.17

Shenouda, N., Gillen, J. B., Gibala, M. J., & MacDonald, M. J. (2017). Changes in brachial artery endothelial function and resting diameter with moderate-intensity continuous but

not sprint interval training in sedentary men. *Journal of Applied Physiology*, *123*(4), 773–780. https://doi.org/10.1152/japplphysiol.00058.2017

Subudhi, A. W., Fan, J. L., Evero, O., Bourdillon, N., Kayser, B., Julian, C. G., … Roach, R. C. (2014). AltitudeOmics: Effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery. *Experimental Physiology*, *99*(5), 772–781. https://doi.org/10.1113/expphysiol.2013.075184

Thomas, K. N., Lewis, N. C. S., Hill, B. G., & Ainslie, P. N. (2015). Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *309*(7), R707–R720. https://doi.org/10.1152/ajpregu.00211.2015

Tymko, M. M., Ainslie, P. N., & Smith, K. J. (2018). Evaluating the methods used for measuring cerebral blood flow at rest and during exercise in humans. *European Journal of Applied Physiology*, *118*(8), 1527–1538. https://doi.org/10.1007/s00421-018-3887-y

van Campen, C. (Linda) M. C., Verheugt, F. W. A., & Visser, F. C. (2018). Cerebral blood flow changes during tilt table testing in healthy volunteers, as assessed by Doppler imaging of the carotid and vertebral arteries. *Clinical Neurophysiology Practice*, *3*, 91–95. https://doi.org/10.1016/j.cnp.2018.02.004

Vickers, A. J. (2001). The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: A simulation study. *BMC Medical Research Methodology*, *1*, 1–4. https://doi.org/10.1186/1471-2288-1-6

Wang, J., Zheng, C., Hou, B., Huang, A., Zhang, X., & Du, B. (2019). Four collateral circulation pathways were observed after common carotid artery occlusion. *BMC Neurology*, *19*(1), 201. https://doi.org/10.1186/s12883-019-1425-0

656 Willie, C. K., Macleod, D. B., Shaw, A. D., Smith, K. J., Tzeng, Y. C., Eves, N. D., …
657 Ainslie, P. N. (2012). Regional brain blood flow in man during acute changes in arterial
658 blood gases. *Journal of Physiology*, *590*(14), 3261–3275.
659 https://doi.org/10.1113/jphysiol.2012.228551

660 Willie, C. K., Smith, K. J., Day, T. A., Ray, L. A., Lewis, N. C. S., Bakker, A., … Ainslie, P.
661 N. (2014). Regional cerebral blood flow in humans at high altitude: Gradual ascent and
662 2 wk at 5,050 m. *Journal of Applied Physiology*, *116*(7), 905–910.
663 https://doi.org/10.1152/japplphysiol.00594.2013

664 Willie, C. K., Tzeng, Y. C., Fisher, J. A., & Ainslie, P. N. (2014). Integrative regulation of
665 human brain blood flow. *Journal of Physiology*, *592*(5), 841–859.
666 https://doi.org/10.1113/jphysiol.2013.268953

667

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest: There authors declare that they have no conflicts of interest.

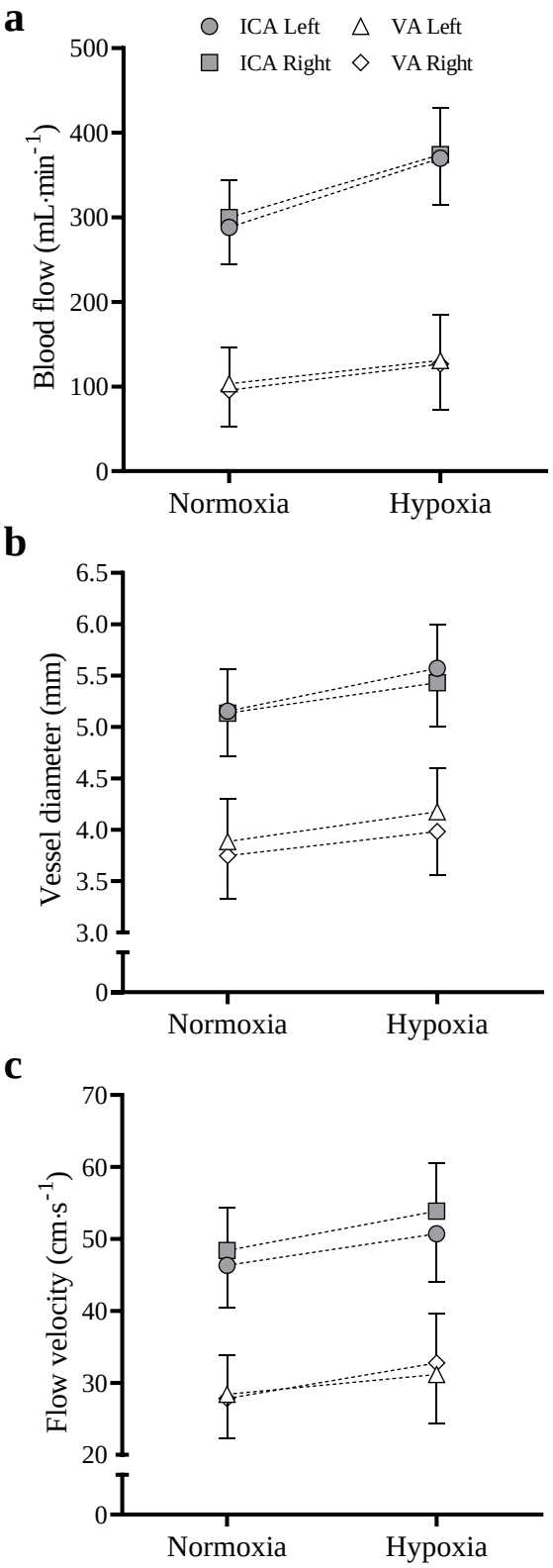
Author contributions: AF and SO conceived and designed the study. All authors contributed to the acquisition, analysis, or interpretation of data for the work. AF and SO drafted the manuscript, with all remaining authors reviewing and providing critical feedback important for intellectual content. All authors have approved the final version of the paper and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding: No funding was received.

Acknowledgements: We would like to thank the participants for their time and effort in this study. We also thank Poppy Barsby, Liam Joyce, and Harry Nicholson for their contribution to data collection.

	Normoxia								Hypoxia								*P*		
	Baseline		30 min		60 min		90 min		Baseline		30 min		60 min		90 min		Condition	Time	Interaction
SpO_2 (%)	98	(1)	98	(1)	99	(1)	99	(1)	99	(1)	80	(5)	78	(6)	78	(6)	<0.001	0.70	0.13
Heart Rate (bpm)	71	(14)	63	(10)	60	(9)	60	(10)	68	(12)	73	(14)	71	(12)	72	(12)	<0.001	0.30	0.84
MAP (mmHg)	90	(7)	84	(7)	83	(7)	85	(7)	91	(6)	85	(9)	85	(8)	86	(8)	<0.05	<0.01	0.71
$P_{ET}CO_2$ (mmHg)	-		37.5	(3.6)	-		37.4	(3.6)	-		34.2	(3.2)	-		32.6	(3.7)	<0.001	0.12	0.06
$\dot{V}E$ ($L \cdot min^{-1}$)	-		9.5	(1.7)	-		8.6	(1.3)	-		10.3	(1.9)	-		9.6	(1.9)	<0.001	<0.05	0.45

Table 1. Cardiorespiratory responses to normoxia and acute poikilocapnic hypoxia. Data were collected during a seated baseline and during supine rest between 30–90 min in a temperature [26 (2) °C] and humidity [30 (4) %] controlled environmental chamber during normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) and acute poikilocapnic hypoxia (FiO_2 = 12.0%). Linear mixed model analysis was completed for the period of supine rest. Values are mean (SD). Abbreviations: SpO_2, peripheral arterial oxygen saturation; MAP, mean arterial pressure; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $\dot{V}E$, minute ventilation.

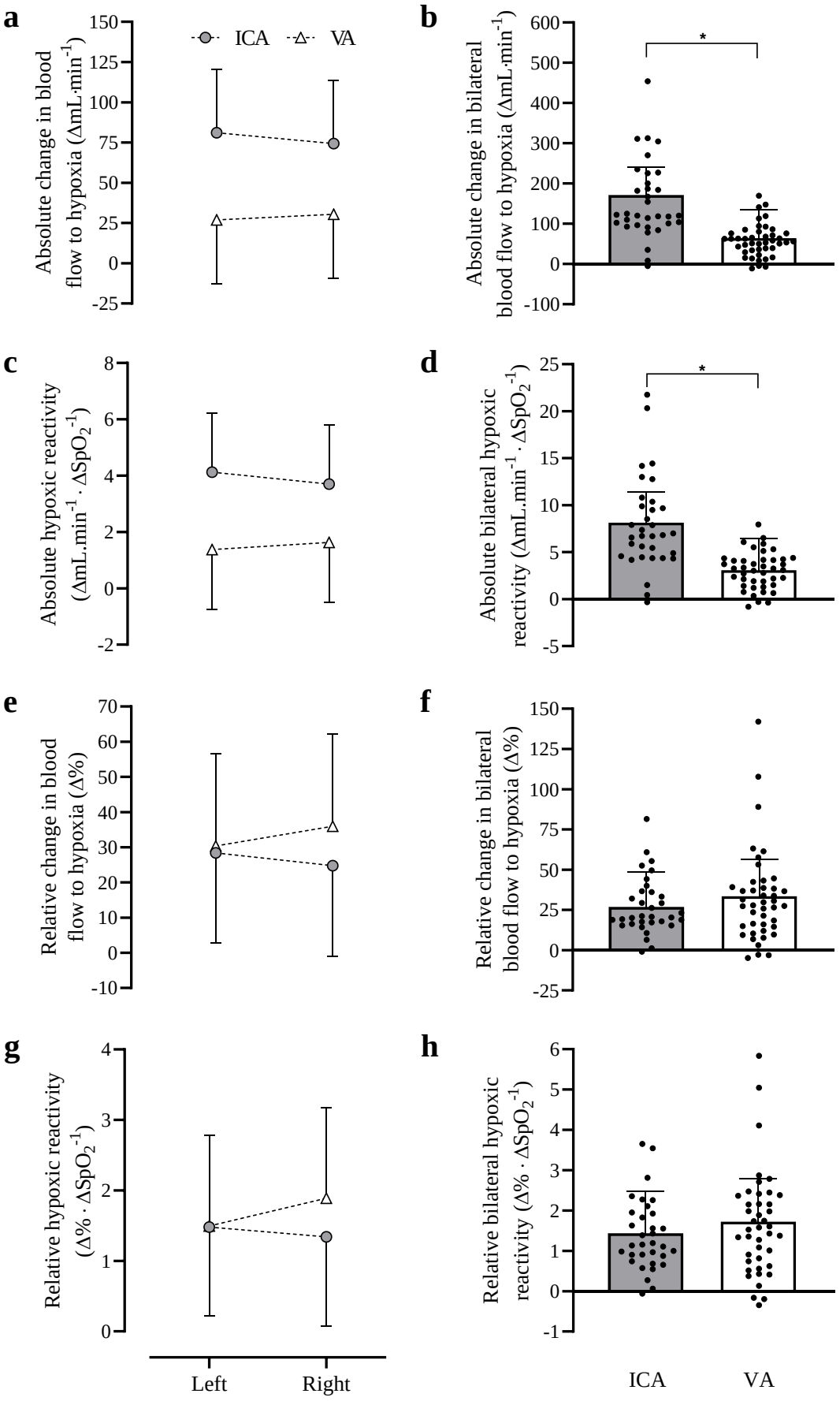


735

736 **Figure 1. Extracranial artery blood flow regulation in normoxia and acute**

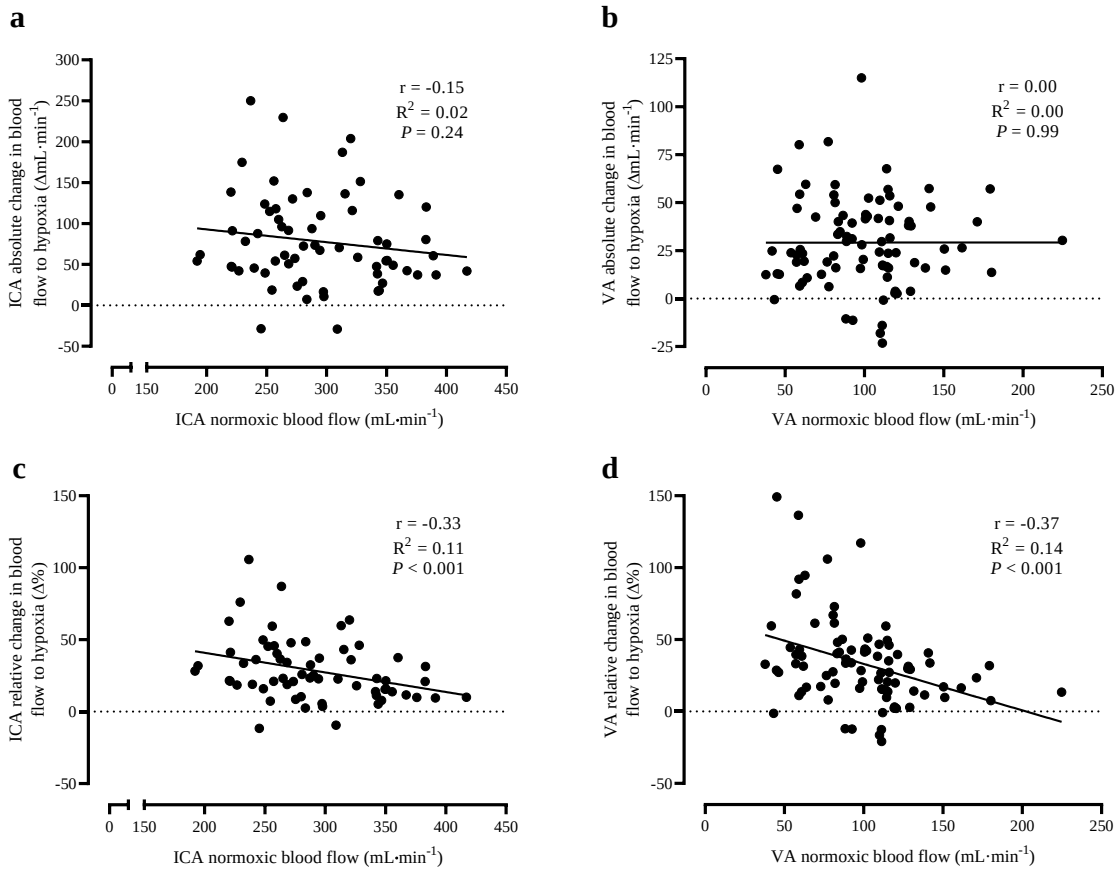
737 **poikilocapnic hypoxia.** Left and right internal carotid artery (ICA) and vertebral artery (VA)

738 blood flow regulation was measured from normoxia (fraction of inspired oxygen [FiO_2] =
739 20.9%) to acute poikilocapnic hypoxia (FiO_2 = 12.0%). Linear mixed model analysis
740 revealed no 'Condition' (normoxia or hypoxia) × 'Vessel Type' (ICA or VA) × 'Vessel
741 Side' (left or right) interaction for blood flow (a, $mL \cdot min^{-1}$; P = 0.62), vessel diameter (b,
742 mm; P = 0.70), and flow velocity (c, $cm \cdot s^{-1}$; P = 0.64), adding 'Participant ID' as a random
743 effect. Data points are estimated marginal means (estimated SD) from LMM analysis. Raw
744 mean (SD) data are presented in Supplementary Table 1.


a
Absolute change in blood flow to hypoxia (ΔmL·min-1)
ICA
VA
b
Absolute change in bilateral blood flow to hypoxia (ΔmL·min-1)
*
c
Absolute hypoxic reactivity (ΔmL.min-1 · ΔSpO2-1)
d
Absolute bilateral hypoxic reactivity (ΔmL.min-1 · ΔSpO2-1)
*
e
Relative change in blood flow to hypoxia (Δ%)
f
Relative change in bilateral blood flow to hypoxia (Δ%)
g
Relative hypoxic reactivity (Δ% · ΔSpO2-1)
Left
Right
h
Relative bilateral hypoxic reactivity (Δ% · ΔSpO2-1)
ICA
VA

745

Figure 2. Extracranial artery blood flow response and reactivity to acute poikilocapnic hypoxia. Left and right internal carotid artery (ICA; grey circle or bars) and vertebral artery (VA; white triangle or bars) blood flow response to acute poikilocapnic hypoxia (fraction of inspired oxygen [FiO_2] = 12.0%). Linear mixed model (LMM) analysis revealed no 'Vessel Type' (ICA or VA) × 'Vessel Side' (left or right) interaction for the absolute change in blood flow to hypoxia (a, $\Delta mL \cdot min^{-1}$; $P = 0.32$), absolute hypoxic reactivity (c, $\Delta mL \cdot min^{-1} \cdot \Delta SpO_2^{-1}$; $P = 0.37$), the relative change in blood flow to hypoxia (e, Δ%; $P = 0.15$), or relative hypoxic reactivity (g, $\Delta\% \cdot \Delta SpO_2^{-1}$; $P = 0.13$). There were no main effects of 'Vessel Side' for these blood flow variables (all $P > 0.05$). Main effects of 'Vessel Type' were revealed for the absolute change in bilateral blood flow to hypoxia (b; $P < 0.001$), absolute bilateral hypoxic reactivity (d; $P < 0.001$), but not for the relative change in bilateral blood flow to hypoxia (f; $P = 0.053$), or relative bilateral hypoxic reactivity (h; $P = 0.12$). * $P < 0.001$ between ICA and VA. Data points represent individuals' ICA and VA blood flow responses to acute hypoxia. Bars are estimated marginal means (estimated SD) from LMM analysis.



760

761 **Figure 3. Relationships between resting normoxic blood flow and the absolute or relative**
762 **blood flow response to acute poikilocapnic hypoxia in the extracranial arteries.** Internal
763 carotid arteries (ICA) and vertebral arteries (VA) blood flow response were assessed from
764 normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) to acute poikilocapnic hypoxia (FiO_2
765 = 12.0%). The blood flow response to hypoxia is presented as the absolute change ($\Delta mL \cdot min^{-1}$
766 ; a and b) and the relative change (Δ%; c and d) from resting normoxic blood flow ($mL \cdot min^{-1}$
767). Data plots include the left and right vessels of the ICA and the VA.

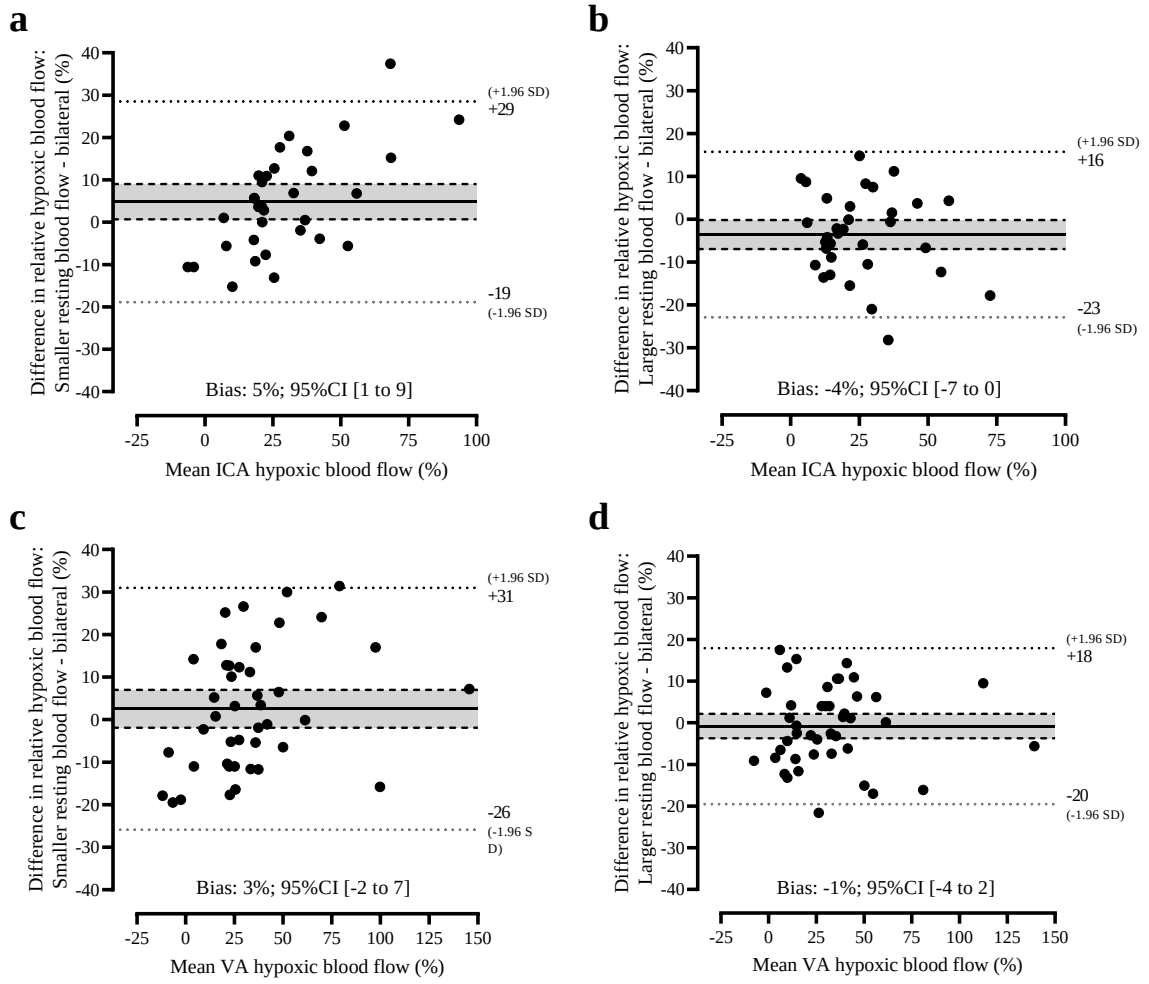


Figure 4. Bland-Altman plots of the measurement bias between a unilateral assessment of the vessel with the smaller or larger resting normoxic blood flow and the bilateral calculation of the relative change in blood flow from normoxia to acute poikilocapnic hypoxia of the extracranial arteries. Internal carotid arteries (ICA) and vertebral arteries (VA) relative blood flow response from normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) to acute poikilocapnic hypoxia (FiO_2 = 12.0%) were calculated from doubling unilateral measurements of the vessel with the smaller (a and c) or larger (b and d) resting normoxic blood flow and compared to the bilateral calculation of the relative blood flow response to hypoxia. Average bias (solid black line) is reported with respective 95% confidence intervals (dashed black lines), and ±1.96SD limits of agreement (dotted black lines).

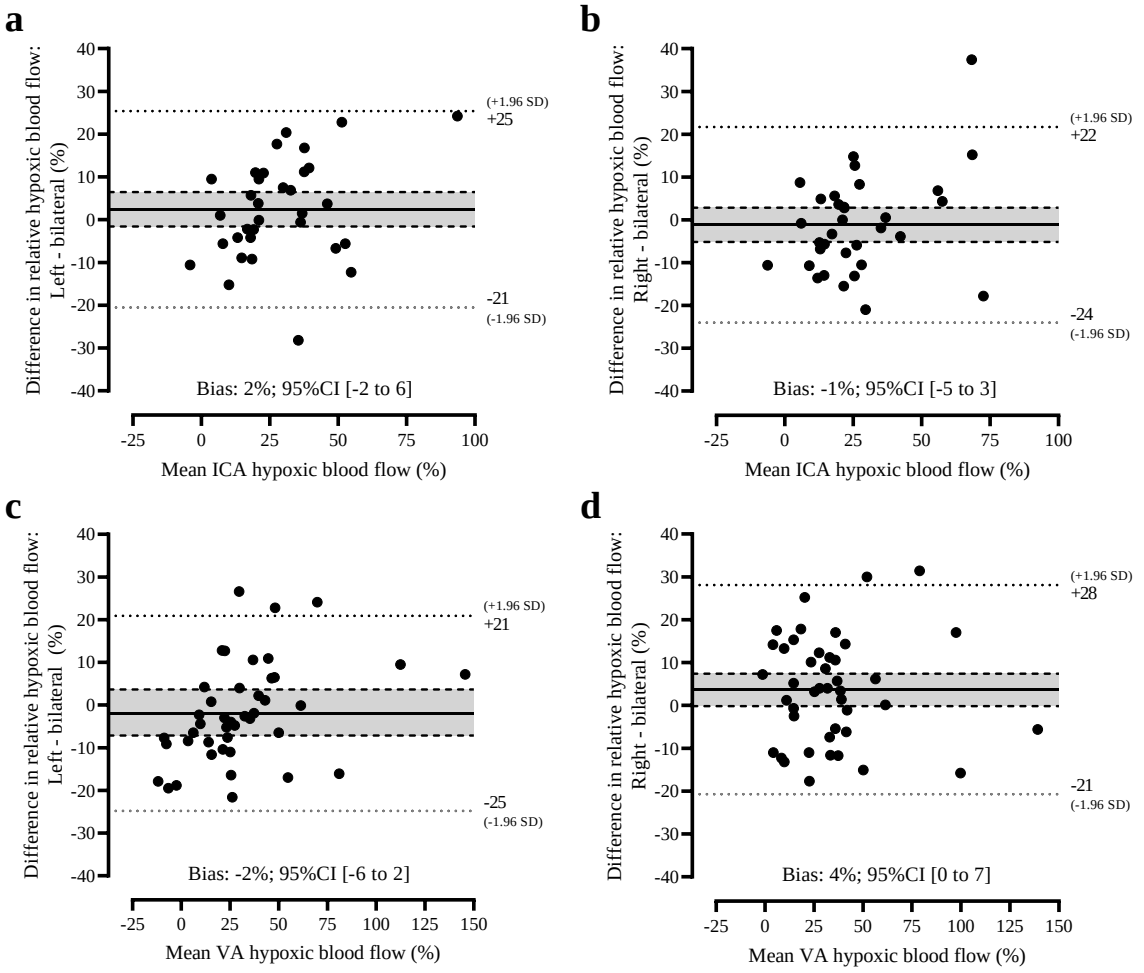


Figure 5. Bland-Altman plots of the measurement bias between a unilateral assessment of the left or right vessel and the bilateral calculation of the relative change in blood flow from normoxia to acute poikilocapnic hypoxia of the extracranial arteries. Internal carotid arteries (ICA) and vertebral arteries (VA) relative blood flow response from normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) to acute poikilocapnic hypoxia (FiO_2 = 12.0%) were calculated from doubling unilateral measurements of the left (a and c) or right (b and d) side and compared to the bilateral calculation of the relative blood flow response to hypoxia. Average bias (solid black line) is reported with respective 95% confidence intervals (dashed black lines), and ±1.96SD limits of agreement (dotted black lines).

	Normoxia		Hypoxia		Δ		Δ%	
Blood flow (mL·min^{-1}) *								
Internal carotid artery								
Left only	575	(94)	738	(130)	163	(108)	29.7	(21.6)
Right only	598	(117)	747	(155)	149	(117)	26.1	(21.7)
Bilateral	587	(79)	743	(117)	156	(97)	27.3	(17.8)
Vertebral artery								
Left only	206	(64)	261	(83)	55	(53)	30.6	(33.0)
Right only	190	(76)	252	(86)	62	(40)	36.2	(28.3)
Bilateral	198	(44)	256	(50)	58	(40)	32.5	(28.4)
Vessel diameter (mm)								
Internal carotid artery								
Left only	5.16	(0.37)	5.58	(0.39)	0.42	(0.26)	8.24	(5.24)
Right only	5.14	(0.40)	5.43	(0.43)	0.30	(0.23)	5.86	(4.62)
Mean average	5.15	(0.31)	5.50	(0.34)	0.36	(0.21)	7.00	(4.14)
Vertebral artery								
Left only	3.88	(0.42)	4.17	(0.44)	0.29	(0.17)	7.57	(4.38)
Right only	3.75	(0.49)	3.98	(0.47)	0.23	(0.16)	6.50	(4.69)
Mean average	3.82	(0.28)	4.08	(0.27)	0.26	(0.13)	6.98	(3.66)
Flow velocity (cm·s^{-1})								
Internal carotid artery								
Left only	46.0	(7.2)	50.4	(7.3)	4.4	(7.3)	10.8	(17.9)
Right only	48.1	(7.9)	53.5	(8.6)	5.4	(8.5)	12.7	(18.9)
Mean average	47.1	(7.0)	52.0	(6.9)	4.9	(6.8)	11.6	(15.5)
Vertebral artery								
Left only	28.3	(5.4)	31.1	(5.6)	2.8	(5.5)	11.9	(21.8)
Right only	27.7	(5.6)	32.7	(5.9)	4.9	(4.8)	19.7	(20.2)
Mean average	28.0	(4.6)	31.9	(4.6)	3.8	(4.5)	15.4	(19.0)

Supplementary Table 1. Extracranial artery blood flow, vessel diameter, and flow velocity in normoxia and acute poikilocapnic hypoxia. Blood flow (mL·min^{-1}), vessel diameter (mm), and flow velocity (time-averaged maximum velocity; cm·s^{-1}) were assessed

794 in the left and right internal carotid arteries (ICA) and vertebral arteries (VA) during
795 normoxia (fraction of inspired oxygen [FiO_2] = 20.9%) and acute poikilocapnic hypoxia
796 (FiO_2 = 12.0%). Left and right vessel blood flows were calculated by doubling unilateral
797 measurements, whereas bilateral blood flow was calculated as the sum of left and right
798 unilateral measurements. Data are mean (SD) of the raw values, with the absolute (Δ) and
799 relative change (Δ%) from normoxia. Linear mixed model analysis revealed no 'Condition'
800 (normoxia or hypoxia) × 'Vessel Type' (ICA or VA) × 'Vessel Side' (left or right)
801 interaction for blood flow (a, $mL \cdot min^{-1}$; $P = 0.62$), vessel diameter (b, mm; $P = 0.70$), and
802 flow velocity (c, $cm \cdot s^{-1}$; $P = 0.64$), with 'Participant ID' as a random effect. LMM revealed
803 no 'Condition' × 'Vessel Side' interactions. * Interaction effect of 'Condition' × 'Vessel
804 Type' ($P < 0.001$).