Determining differences between critical closing pressure and resistance-area product: responses of the healthy young and old to hypocapnia

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16 **Abbreviations list**

17

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19 ARI Autoregulation index

20 BP Blood pressure

21 CA Cerebral autoregulation

22 CBF Cerebral blood flow

23 CBFV Cerebral blood flow velocity

24 CO₂ Carbon dioxide

25 CrCP Critical closing pressure

26 CVR Cerebrovascular reactivity

27 dCA Dynamic cerebral autoregulation

28 ECG Electrocardiogram

29 EtCO₂ End-tidal CO₂

30 HR Heart rate

31 MABP Mean arterial blood pressure

32 MCA Middle cerebral artery

33 PaCO₂ Partial pressure carbon dioxide

34 RAP Resistance-area product

35 SD Standard deviation

36 TCD Transcranial Doppler

37 Abstract

38 Healthy ageing has been associated with lower cerebral blood flow velocities (CBFV),
39 however, the behaviour of haemodynamic parameters associated with cerebrovascular tone
40 (critical closing pressure, CrCP) and cerebrovascular resistance (resistance-area product,
41 RAP) remains unclear. Specifically, evidence supports ageing being associated with greater
42 cerebrovascular tone and resistance during exercise with elevated CrCP and RAP in older
43 individuals at rest and during exercise. Comprehensive haemodynamic assessment of CrCP
44 and RAP during hyperventilation-induced hypocapnia in two distinct age groups (young ≤ 49
45 and old > 50) has not been described. CBFV in the middle cerebral artery (CBFV, transcranial
46 Doppler), blood pressure (BP, Finometer), and end-tidal CO₂ (EtCO₂, capnography) were
47 recorded in 104 healthy individuals (43 young [age 33.8 (9.3) years], 61 old [age 64.1 (8.5)
48 years]) during a minimum of 60 seconds of metronome-driven hyperventilation-induced
49 hypocapnia. Autoregulation index (ARI) was calculated as a function of time, using a moving
50 window autoregressive moving average model. CBFV was reduced in response to age
51 ($p < 0.0001$) and hypocapnia ($p = 0.023$) (young 57.3 (14.4) vs. 44.9 (11.1) cm.s⁻¹, old 51.7
52 (12.9) vs. 37.8 (9.6) cm s⁻¹). Critical closing pressure (CrCP) increased significantly in
53 response to hypocapnia (young 37.6 (18.5) vs. 39.7 (16.0) mmHg, old 33.9 (13.5) vs. 39.3
54 (11.4) mmHg; $p < 0.0001$). Resistance-area product was increased in response to age
55 ($p = 0.001$) and hypocapnia ($p = 0.004$) (young 1.02 (0.40) vs. 1.09 (11.07) mmHg cm.s⁻¹, old
56 1.16 (0.34) vs. 1.34 (0.39) mmHg cm.s⁻¹). Resistance-area product mediates the response to
57 during hypocapnia between the healthy young and old.

58 **New & Noteworthy**

59 Despite convincing data supporting advancing age being associated with lower cerebral blood
60 flow velocity, the behaviour of haemodynamic parameters associated with cerebrovascular
61 tone (critical closing pressure, CrCP) and cerebrovascular resistance (resistance-area product,
62 RAP) remains unclear. In a large group of young and old healthy individuals, we show
63 resistance-area product (RAP) and not critical closing pressure (CrCP) differing between
64 young and old individuals during hypocapnia induced by hyperventilation. These novel
65 findings underline the importance of assessing the separate contributions of RAP and CrCP.
66 This study provides further support for cerebrovascular response to CO₂ change as a
67 biomarker of ageing and describes mechanistic changes associated with ageing in greater
68 detail than prior studies.

Introduction

Carbon dioxide (CO₂) induced changes in vasomotor tone are influenced by several interactors including blood pressure, hypoxia, neuronal and autonomic activity (15). Age, another potential factor, has been associated with larger cerebral blood flow velocity (CBFV) responses to cognitive challenge (3). However, the literature surrounding cerebrovascular reactivity (CVR) changes during healthy ageing remains an area of controversy (12, 13). Cerebral haemodynamic parameter behaviours described over the physiological range of arterial CO₂ (PaCO₂) and improvements in cerebral autoregulation (CA) during hyperventilation-induced hypocapnia are apparent (1, 16, 17). During disease states, namely carotid stenosis and stroke, impaired CVR or CA are associated with increased risk for ischaemic events or worse stroke outcome, respectively (23, 24). The lack of capability of cerebral vessels to modify their calibre in response to CO₂ stimulus represents a crucial biomarker to differentiate health and disease (24). Unfortunately, in order to progress our understanding, factors influencing CVR and CA including age and sex need to be taken into consideration. Zhu et al. (33) demonstrated advanced age is associated with lower resting CBFV and CVR during hypocapnia. Interestingly they found increased CVR during hypercapnia, which contravenes findings from several other studies (6, 29). The association of ageing with lower CBFV appears to be well accepted (6). Though others have found no age-associated differences in CVR, with suggestions that arterial stiffness explains age-related differences in cerebrovascular conductance (11). Importantly, the behaviour of haemodynamic parameters associated with cerebrovascular tone (critical closing pressure, CrCP) and cerebrovascular resistance (resistance-area product, RAP) with respect to ageing remains unclear. Specifically, evidence supports ageing being associated with greater cerebrovascular tone and resistance during exercise with elevated CrCP and RAP in older individuals at rest and during exercise. In 2019, a large study demonstrated once more that advancing age is associated with decreases in CBFV, increases in CVR, and reduced vasoconstriction during hypocapnia, though no reference to standard haemodynamic parameters was provided (31). This aligns with the lack of data on the separate effects of the interaction of age with hypocapnia using RAP and CrCP, instead of CVR.

There is a distinct lack of studies assessing vasoconstrictive stimuli as compared to vasodilative studies (14). McKetton et al. (14) described the limitations of using blood oxygenation level dependent (BOLD) magnetic resonance imaging (MRI) to assess hypocapnic conditions, including inability to hyperventilate without motion artefacts and

elevated inter-subject variability. Longitudinal assessment of CVR using Transcranial Doppler over a time course of 1 to 3 years has shown no significant differences from baseline CVR, demonstrating robust reproducibility and acceptable inter-subject variability (29). Importantly, Galvin et al. (10) demonstrated elevated CVR during hypocapnia in those with coronary artery disease and age matched controls, suggesting the improved CVR and lower CBFV may provide insight into important mechanisms underlying neurological risk with ageing. Changes in CVR are considered to occur beyond the young-old age category (>50 years), with very-old (>80 years) exhibiting similar responses to the young-old (11).

Objective

To identify if healthy ageing is associated with differences in response to CO₂ change. Specifically, to understand the relationship between key CA metrics; the autoregulation index (ARI) calculated using the linear autoregressive-moving average (ARMA) model (17) to quantify the influence of CA or EtCO₂ on CBFV, as well as assessing resistance-area product (RAP, primary determinant of cerebrovascular resistance) and critical closing pressure (CrCP, measure of cerebral arterial tonus and intracranial pressure). We hypothesise that no differences exist in key haemodynamic parameters (CBFV, HR (heart rate), ABP (arterial blood pressure), CrCP, RAP and ARI) between the young (<50 years) and old (>50 years) during hypocapnic challenge. By understanding the differences and interactions between these central and peripheral parameters, there exists an opportunity to determine the mechanisms governing the regulation of cerebrovascular tone and dynamic CA processes during healthy ageing.

Methods

Subjects and measurements

The study was conducted in accordance with the Declaration of Helsinki (2000). Ethical approval was obtained from the University of Leicester Ethics Committee (Reference: jm591-c033) and the Northampton Research Ethics Committee (11/EM/0369). Healthy volunteers were recruited from a variety of settings including the local community, University departmental staff, students and their relatives. Participants aged above 18 years were included. Exclusion criteria were physical disease in the upper limb, poor insonation of both temporal bone windows and any significant history of cardiovascular, neurological or respiratory disease. Smokers were excluded. All participants provided written, informed

consent. The dataset generated from the younger individuals has been used to inform related publications (16-18).

Experimental protocol

The research was undertaken in the University of Leicester's Cerebral Haemodynamics in Ageing and Stroke Medicine (CHiASM) research laboratory, maintained at a constant ambient temperature of approximately 24°C and free of distraction. For the purposes of the study, participants were asked to refrain from caffeine and alcohol for a minimum period of four hours prior to measurements being undertaken. Beat-to-beat BP was recorded continuously using the Finometer® device (FMS, Finapres Measurement Systems, Arnhem, Netherlands), which was attached to the middle finger. The servo-correcting mechanism of the Finometer® was switched on and then off prior to measurements. The hand bearing the finger cuff was at the level of the heart to negate any hydrostatic pressure bias. HR was recorded using a standard 3-lead electrocardiogram (ECG).

EtCO₂ was measured throughout using small nasal cannulae (Salter Labs) connected to a capnograph (Capnocheck Plus). Bilateral insonation of the middle cerebral arteries (MCAs) was performed using transcranial Doppler (TCD) ultrasound (Viasys Companion III; Viasys Healthcare) with a 2MHz probe. The probes were secured in place with a head-frame that was adjusted to ensure comfort at the outset. The MCAs were identified according to two main characteristics: signal depth and velocities.

Measurements were continuously recorded at a rate of 500 samples/s in the PHYSIDAS data acquisition system (Department of Medical Physics, University Hospitals of Leicester NHS Trust). Systolic and diastolic brachial BP readings (OMRON Model 705IT) were performed at each stage of the measurements (normocapnia and hypocapnia) with a minimum of three recordings per individual. These values were then used to calibrate the Finometer recordings.

Hyperventilation induction strategy

Following a 20-minute stabilisation period, a 5-minute baseline recording was taken of the subject breathing spontaneously at rest. Hyperventilation strategies were conducted at least once, with repeated assessments conducted where possible, with 5-minute intervals between each to allow stabilisation of all parameters and return to normocapnia. The hyperventilation induction strategies involved 60 seconds of rest, with hyperventilation being maintained for a minimum of 90 seconds. Use of a continuous metronome (KORG Metronome MA-30),

started at a rate analogous to that of the subject's baseline resting rate. After 30 seconds to one minute of baseline recording, the rate was increased gradually over a period of 60 seconds to reach a hyperventilation rate 40% greater than baseline (around 25 breaths per minute). This was maintained for a further minimum of 60 seconds and followed by a minimum of 90 seconds rest.

Data Analysis

Data collected corresponded to individual recordings for each participant at baseline and during hypocapnia. First, recordings were inspected visually and calibrated to measured systolic and diastolic OMRON BP. Narrow spikes ($<100\text{ms}$) were removed using linear interpolation and the CBFV recording was then passed through a median filter. All signals were then low pass filtered with a zero-phase Butterworth filter with cut-off frequency of 20Hz. Automatic detection of the QRS complex of the ECG, to mark the R-R interval was used, but visual inspection was also undertaken with manual correction whenever necessary. This allowed HR, mean ABP and mean CBFV to be calculated for each cardiac cycle. The peak of the EtCO_2 signal was detected and breath-by-breath values were linearly interpolated and resampled in synchrony with the cardiac cycle.

Baseline files were analysed using a moving window autoregressive-moving average (MW-ARMA) model as described by Dineen et al. (8). Initially, an ARMA model is adopted to estimate the CBFV response to a step change in BP and the autoregulation index (ARI) was estimated by comparison with the 10 template CBFV step responses proposed by Tiecks et al. (30) using the first 60 s of data. The 60 s window was then shifted by 0.6 s and a new estimate of ARI was calculated. This process was repeated until the end of the signal was reached thus generating ARI estimates at each 0.6 s intervals (8). This produced multiple estimates of ARI, which were then averaged to produce a single baseline ARI value for each file. Having estimates of ARI every 0.6 s is sufficient to represent changes that can take place due to hypocapnia, even at very high respiratory rates caused by hyperventilation, which produce estimates of EtCO_2 with time intervals always longer than 2 s. The critical closing pressure (CrCP) and resistance-area product (RAP) were estimated using the first harmonic method (25).

For the hyperventilation strategies, continuous estimates of ARI were produced for each file using the same MW-ARMA model. These were then digitally marked at the point of EtCO_2 increase (signifying the end of hyperventilation) as this proved to be the most recognisable

and reproducible point between strategies. Marked files were synchronised at 90 seconds and these data used to produce coherent averages of ARI for each strategy, allowing comparison between the different conditions.

Statistical analysis

Data normality was confirmed with the Kolmogorov-Smirnov test. Baseline measurements were assessed for differences between values derived for right and left hemispheres using a paired Student's t-test. These were averaged when no significant differences were found. All peripheral and cerebral haemodynamic values were compared between the two strategies using multiple independent t-tests with Bonferroni correction to counteract multiple comparisons. Values of $p < 0.05$ were considered significant. Pearson's correlation coefficient analysis was used to assess for specific relationships between variables with positive interactions and differences between age groups.

Results

One-hundred and four healthy individuals, 43 young (≤ 49) and 61 old (> 50) were studied. The mean age of the younger group was 33.8 (9.3) and older group 64.1 (8.5) years (Table 1). The younger group had 24 females (56%) and the older group had 28 (46%) females. Figure 1 shows representative recordings of the CBFV response to hyperventilation in a single subject.

Cerebral and peripheral haemodynamic parameters at baseline

Baseline MAP was not significantly different between the age groups (90.6 (11.9) vs. 88.3 (11.1) mmHg, $p = 0.122$). HR was lower in the older age group (62.6 (9.8) vs. 67.6 (11.0) bpm, $p = 0.010$) (Table 1). Baseline CBFV was significantly lower in older participants (51.7 (12.9) vs. 57.3 (14.4) cm.s^{-1} , $p = 0.0001$). With respect to other cerebral haemodynamic parameters at baseline, RAP was significantly higher in older subjects (1.16 (0.34) vs. 1.02 (0.40) mmHg.cm s^{-1} , $p = 0.001$), though no differences were seen in CrCP or ARI (Table 1).

Effect of hypocapnia on cerebral and peripheral haemodynamics

As anticipated, there was a significant reduction in EtCO_2 with hyperventilation which was not age dependent ($p < 0.0001$). In addition, this was associated with increased HR ($p = 0.016$) and MAP ($p = 0.026$) (Table 1). The population response to the hypocapnic challenge is demonstrated in Figure 2. CBFV was reduced in response to hypocapnia (young 57.35 (14.38) vs. 44.89 (11.07) cm.s^{-1} , old 51.73 (12.89) vs. 37.80 (9.65) cm.s^{-1} , $p = 0.023$). Critical

227 closing pressure (CrCP) increased significantly in response to hypocapnia (young 37.65
228 (18.54) vs. 39.72 (15.96) mmHg, old 33.94 (13.52) vs. 39.32 (11.42) mmHg; $p<0.0001$).
229 Resistance-area product was increased in response hypocapnia (young 1.02 (0.40) vs. 1.09
230 (11.07) mmHg.scm⁻¹, old 1.16 (0.34) vs. 1.34 (0.39) mmHg.scm⁻¹, $p=0.004$).

231 *Interaction of age and hypocapnia*

232 Heart rate (HR) was the only parameter to demonstrate an interaction effect ($p=0.012$) with
233 an augmented response in older ($p=0.010$) individuals to hypocapnia (young 69.13 (11.94)
234 vs. 69.74 (12.94) beats/min, old 62.63 (9.79) vs. 67.62 (10.96) mmHg, $p=0.016$). Figure 3
235 demonstrates changes in CBFV, HR, MAP, CrCP, RAP and ARI between old and young
236 individuals from baseline to hypocapnic conditions. The correlation between RAP and HR
237 was assessed was correlation in young and old during hypocapnia with no non-significant
238 relationship demonstrated (Figure 3). However, a positive trend was noted in the older
239 population ($r=0.058$) with higher HR during hypocapnia being associated with higher RAP
240 values (Figure 3).

Discussion

Main findings

The four important findings of this study are: 1) demonstration for the first time that young and old healthy individuals have different cerebrovascular resistance mechanisms during hypocapnia; 2) older individuals mount a significantly greater HR response to hypocapnia 3) demonstration that dynamic response to hypocapnia, as expressed by the ARI, is CO₂-dependent; and 4) this study supports previous work demonstrating increases in CrCP and RAP in response to hypocapnia. Importantly, this study distinguishes differences between RAP and CrCP with reference to importance during healthy ageing.

Physiological Considerations

Effect of age and hypocapnia on cerebral haemodynamics

This study affirms prior work demonstrating an age related elevation in cerebrovascular response to hypocapnic challenge (10). CrCP has previously been shown to increase with hypocapnia though age was not considered within this particular study (1). Importantly in this study, ARI improved with CO₂ change ($p=0.008$) but did not vary across age groups, demonstrating comparable ability in older and younger individuals to autoregulate during hypocapnia. This finding concurs with previous work demonstrating a lack of an association between increasing age and ARI change during normocapnia (26). However, most marked is the dramatic rise in RAP in the older individuals beyond a non-significant rise in CrCP between the two age groups. RAP characterises vascular resistance according to the slope of the linear ABP-CBF relationship and CrCP refers to the theoretical pressure at which CBF falls to zero and is thought to be a marker of cerebrovascular transmural wall tension (therefore incorporating intracranial pressure effects) (27). The rise of RAP in older individuals has been demonstrated at baseline as shown in this study, though not during hypocapnia (27). This study provides evidence that older normotensive individuals have greater RAP to younger individuals during normocapnia and hypocapnia (25). Crucially, as has been shown during hypotension, the responses of CrCP and RAP are similar, as was demonstrated in this study, albeit with very mild hypotension induced during hypocapnia. Ogoh et al. (2011) previously showed older normotensive adults had an elevated RAP but not CrCP and reassuringly, this has been confirmed within this study (22). Prior work has demonstrated a greater relative change in CrCP in younger adults during upright posture with a suggestion CrCP response in younger adults maybe more sensitive (27). This study

provides for the first time an arterial gas paradigm to refute inferences from prior studies assessing metabolic demand, that highlight that CrCP is crucial in healthy ageing. Overall, the study provides a more detailed perspective on the influence of CrCP and RAP on governance of mechanisms involved in vasomotor control during healthy ageing and hypertensive states (22). The results highlight RAP as a key differentiator which accentuates cerebrovascular resistance as individuals age.

Effect of age and hypocapnia on peripheral haemodynamics

Nowak et al. (20, 21) demonstrated that hyperventilation-induced cerebral vasoconstriction led to a greater HR increment in those with orthostatic intolerance. Cerebral vasomotor response to hypocapnia was increased during this hypocapnia period with an expected lower CBFV. Despite a lack of data supporting this phenomenon in older as opposed to younger individuals, this study confirms the presence of a greater HR response in circumstances of hypocapnia and improved CVR. Brown et al. demonstrated that hypercapnia increases minute ventilation with little effect on HR, and prior work has shown that mild hyperventilation does not affect HR variability (HRV) indices (4). However, this study, in a large cohort of individuals across a wide spectrum of ages, provides a different perspective to previously accepted HRV literature. At rest, during normocapnia, older age individuals have consistently lower HRV than younger people, however, under experimental hypocapnic challenge, there is a paradoxical response. This paradoxical response has been documented during hypoxaemia with older men having smaller percentage increases in sympathetic nervous system activity from their elevated baselines, though demonstrating an attenuated tachycardia during acute hypoxaemia (7). This study provides novel data highlighting the presence of a similar attenuated tachycardia during hypocapnia in older adults.

The behaviour of RAP with reference to HR during hypocapnia is of interest. This relationship between RAP and posture change has been previously assessed in the young and old (27). This study showed that during change from supine to sit to stand (likely precipitating a HR response) was not associated with a significant change in RAP or indeed CrCP (27). However, the older had a greater RAP during changes in posture with a positive trend. Our study supports the findings for RAP, though refutes the importance of CrCP demonstrated during posture change, for hypocapnia conditions. However, our study does support the lack of significant differences in the behaviour of CrCP between age groups, as shown with posture change. In addition, our study affirms recent work demonstrating an

attenuated HR response in older individuals during hypocapnia, though we cannot draw comparisons as to the behaviour of RAP and CrCP with this work as this study is the first study to assess such variables across the adult lifespan during hypocapnic conditions (31).

Clinical Considerations

The presence of raised RAP and similar CrCP in older adults as compared to younger adults raises the importance of cerebrovascular resistance as rest and during hypocapnia as an important marker of ageing vascularity. In addition, the importance of standardising PaCO₂ operating points for clinical studies comparing data with patient data with healthy controls is paramount (17, 28). This study supports a HR response being associated with the cerebrovascular resistance index, RAP. This therefore highlights the potential relationship between cerebrovascular disease and cardiac disease with worsening cerebrovascular resistance possibly propagating effects on HR and overall cardiac demand. Importantly, with prior work demonstrating raised RAP and similar CrCP during hypertensive states, there is a suggestion that chronic hypertension may affect the ability of vessels to respond to CO₂ stimulus as opposed to alterations in tone. A number of previous studies assessing CrCP in health and disease states have shown that CrCP is influenced by structural and physiological parameters and responds to changes in pressure within the cranial cavity (25). This phenomenon was highlighted by Robertson et al. (2014) during their reference to “structural vs functional”, with the former being more closely related to adaptation to chronic hypertension (27). The key question is the potential for hypocapnic challenge to be used as a biomarker of healthy ageing and feasibility of such assessments. With a well-tolerated response across a wide age range, there is scope to extend this assessment to those with disease states for which a vasoconstrictive response would not be considered adverse (i.e. not in acute ischaemic stroke but perhaps in vascular dementia syndromes for which a spectrum of small vessel disease severity exists).

Limitations

Several limitations are to be considered within this study setup. Firstly, when considering TCD studies, in order to estimate CBF from CBFV, it is presumed the MCA diameter must remain constant. Prior work has shown changes in MCA diameter occur at extreme hypercapnia, however, changes during hypocapnia are less understood (5, 32). Secondly, the authors have previously demonstrated sex differences across the physiological range of PaCO₂, with a downward parallel shift of the RAP dependency on EtCO₂. This demonstrated

males have higher RAP values than females across the range of PaCO₂. Therefore, with a similar male and female split in both groups, these effects are unlikely to have been significant, though should be acknowledged. Importantly, recent work has shown that autoregulation is able to withstand the effects of female sex hormones and therefore the pre- and post-menopausal effects influences are less likely to have affected the results (9). Thirdly, sampling expired CO₂ with nasal prongs during hyperventilation can underestimate EtCO₂, however, unlike the face mask, they do not precipitate a sympathetic response and therefore it is considered an acceptable pragmatic methodological setup for this cerebral haemodynamic study (19). Lastly, decline in hypercapnic reactivity was shown in the Rotterdam cohort in individuals aged between 75-90 years (2). Although they did not include hypocapnia, this physiological phenomenon, and different behaviour exhibited by the real extremes of age is beyond the considered age groups within this study.

Future directions

This study provides further support for vascular responses to CO₂ as a biomarker of ageing and describes mechanistic changes associated with ageing in greater detail than prior studies. However, further work is required to elucidate this phenomenon in disease states and to characterise variation between different cerebrovascular pathologies. Specifically, assessing RAP response during exercise whereby HRV and response is broader and reliable correlations between the heart-brain axis can be investigated.

Conclusions

In a large group of young and old healthy individuals, we show resistance-area product (RAP) and not critical closing pressure (CrCP) mediates differences in cerebrovascular resistance responses to hypocapnia between the healthy young and old individuals. These novel findings underline the importance of assessing the separate contributions of RAP and CrCP. In addition, our findings support previous studies demonstrating exacerbated heart rate responses in the old.

Competing interests

No competing interests

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367 Department of Cardiovascular Sciences, University of Leicester. T.G.R., is an NIHR Senior
368 Investigator.

369 **Author contributions**

370 J.S.M., R.B.P., and T.G.R., conceived and designed the research. J.S.M., and V.J.H.,
371 conducted experiments. J.S.M., V.J.H., and R.B.P., analysed the data. J.S.M., R.B.P., and
372 T.G.R., wrote the manuscript. All authors read and approved the manuscript.

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References

1. **Ainslie PN, Celi L, McGrattan K, Peebles K and Ogoh S.** Dynamic cerebral autoregulation and baroreflex sensitivity during modest and severe step changes in arterial PCO₂. *Brain Res* 1230: 115-124, 2008.
2. **Bakker SL, de Leeuw FE, den Heijer T, Koudstaal PJ, Hofman A and Breteler MM.** Cerebral haemodynamics in the elderly: the rotterdam study. *Neuroepidemiology* 23: 4: 178-184, 2004.
3. **Beishon L, Minhas JS, Patrick K, Shanmugam I, Williams CAL, Panerai RB, Robinson TG and Haunton VJ.** The Effects of Healthy Ageing on Cerebral Blood Flow Responses to Cognitive Testing. *Curr.Aging Sci.* 2019.
4. **Brown SJ, Barnes MJ and Mundel T.** Effects of hypoxia and hypercapnia on human HRV and respiratory sinus arrhythmia. *Acta Physiol Hung* 101: 3: 263-272, 2014.
5. **Coverdale NS, Gati JS, Opalevych O, Perrotta A and Shoemaker JK.** Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J Appl Physiol (1985)* 117: 10: 1090-1096, 2014.
6. **Davis SM, Ackerman RH, Correia JA, Alpert NM, Chang J, Buonanno F, Kelley RE, Rosner B and Taveras JM.** Cerebral blood flow and cerebrovascular CO₂ reactivity in stroke-age normal controls. *Neurology* 33: 4: 391-399, 1983.
7. **Davy KP, Jones PP and Seals DR.** Influence of age on the sympathetic neural adjustments to alterations in systemic oxygen levels in humans. *Am J Physiol* 273: 2 Pt 2: R690-5, 1997.

- 398 8. **Dineen NE, Brodie FG, Robinson TG and Panerai RB.** Continuous estimates of
399 dynamic cerebral autoregulation during transient hypocapnia and hypercapnia. *J Appl Physiol*
400 108: 3: 604-613, 2010.
- 401 9. **Favre ME and Serrador JM.** Sex Differences in Cerebral Autoregulation is Unaffected
402 by Menstrual Cycle Phase in Young, Healthy Women. *Am J Physiol Heart Circ Physiol*
403 2019.
- 404 10. **Galvin SD, Celi LA, Thomas KN, Clendon TR, Galvin IF, Bunton RW and Ainslie**
405 **PN.** Effects of age and coronary artery disease on cerebrovascular reactivity to carbon
406 dioxide in humans. *Anaesth Intensive Care* 38: 4: 710-717, 2010.
- 407 11. **Kastrup A, Dichgans J, Niemeier M and Schabet M.** Changes of cerebrovascular CO₂
408 reactivity during normal aging. *Stroke* 29: 7: 1311-1314, 1998.
- 409 12. **Kastrup A, Kruger G, Neumann-Haefelin T and Moseley ME.** Assessment of
410 cerebrovascular reactivity with functional magnetic resonance imaging: comparison of CO₂(2)
411 and breath holding. *Magn Reson Imaging* 19: 1: 13-20, 2001.
- 412 13. **Kastrup A, Thomas C, Hartmann C and Schabet M.** Sex dependency of
413 cerebrovascular CO₂ reactivity in normal subjects. *Stroke* 28: 12: 2353-2356, 1997.
- 414 14. **McKetton L, Sobczyk O, Duffin J, Poublanc J, Sam K, Crawley AP,**
415 **Venkatraghavan L, Fisher JA and Mikulis DJ.** The aging brain and cerebrovascular
416 reactivity. *Neuroimage* 181: 132-141, 2018.
- 417 15. **Meng L and Gelb AW.** Regulation of cerebral autoregulation by carbon dioxide.
418 *Anesthesiology* 122: 1: 196-205, 2015.

- 419 16. **Minhas JS, Kennedy C, Robinson TG and Panerai RB.** Different strategies to initiate
420 and maintain hyperventilation: their effect on continuous estimates of dynamic cerebral
421 autoregulation. *Physiol Meas* 40: 015003, 2019.
- 422 17. **Minhas JS, Panerai RB and Robinson TG.** Modelling the cerebral haemodynamic
423 response in the physiological range of PaCO₂. *Physiol Meas* 39: 6: 065001-6579/aac76b,
424 2018.
- 425 18. **Minhas JS, Panerai RB and Robinson TG.** Sex differences in cerebral haemodynamics
426 across the physiological range of PaCO₂. *Physiol Meas* 39: 105009, 2018.
- 427 19. **Minhas JS, Robinson T and Panerai R.** PaCO₂ measurement in cerebral
428 haemodynamics: face mask or nasal cannulae? *Physiol.Meas.* 38: 7: N101-N106, 2017.
- 429 20. **Novak P.** Hypocapnic cerebral hypoperfusion: A biomarker of orthostatic intolerance.
430 *PLoS One* 13: 9: e0204419, 2018.
- 431 21. **Novak V, Novak P, Spies JM and Low PA.** Autoregulation of cerebral blood flow in
432 orthostatic hypotension. *Stroke* 29: 1: 104-111, 1998.
- 433 22. **Ogoh S, Fisher JP, Young CN and Fadel PJ.** Impact of age on critical closing pressure
434 of the cerebral circulation during dynamic exercise in humans. *Exp.Physiol.* 96: 4: 417-425,
435 2011.
- 436 23. **Palazzo P.** Evaluation of cerebral vasomotor reactivity and flow mediated dilation in
437 subjects without cerebrovascular disease. *Clin Neurophysiol* 122, 2011.

- 438 24. **Palazzo P, Maggio P, Passarelli F, Altavilla R, Altamura C, Pasqualetti P and**
439 **Vernieri F.** Lack of correlation between cerebral vasomotor reactivity and flow-mediated
440 dilation in subjects without vascular disease. *Ultrasound Med Biol* 39: 1: 10-15, 2013.
- 441 25. **Panerai RB.** The critical closing pressure of the cerebral circulation. *Med Eng Phys* 25:
442 8: 621-632, 2003.
- 443 26. **Patel N, Panerai RB, Haunton V, Katsogridakis E, Saeed NP, Salinet A, Brodie F,**
444 **Syed N, D'Sa S and Robinson TG.** The Leicester cerebral haemodynamics database:
445 normative values and the influence of age and sex. *Physiol Meas* 37: 9: 1485-1498, 2016.
- 446 27. **Robertson AD, Edgell H and Hughson RL.** Assessing cerebrovascular autoregulation
447 from critical closing pressure and resistance area product during upright posture in aging and
448 hypertension. *Am J Physiol Heart Circ Physiol* 307: 2: H124-33, 2014.
- 449 28. **Salinet AS, Robinson TG and Panerai RB.** Effects of cerebral ischemia on human
450 neurovascular coupling, CO₂ reactivity, and dynamic cerebral autoregulation. *J Appl Physiol*
451 (1985) 118: 2: 170-177, 2015.
- 452 29. **Schwertfeger N, Neu P, Schlattmann P, Lemke H, Heuser I and Bajbouj M.**
453 Cerebrovascular reactivity over time course in healthy subjects. *J Neurol Sci* 249: 2: 135-
454 139, 2006.
- 455 30. **Tiecks FP, Lam AM, Aaslid R and Newell DW.** Comparison of static and dynamic
456 cerebral autoregulation measurements. *Stroke* 26: 6: 1014-1019, 1995.
- 457 31. **Tomoto T, Riley J, Turner M, Zhang R and Tarumi T.** Cerebral vasomotor reactivity
458 during hypo- and hypercapnia across the adult lifespan. *J Cereb Blood Flow Metab.*
459 271678X19828327, 2019.

- 460 32. **Verbree J, Bronzwaer AS, Ghariq E, Versluis MJ, Daemen MJ, van Buchem MA,**
461 **Dahan A, van Lieshout JJ and van Osch MJ.** Assessment of middle cerebral artery
462 diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI.
463 *J.Appl.Physiol.(1985)* 117: 10: 1084-1089, 2014.
- 464 33. **Zhu YS, Tarumi T, Tseng BY, Palmer DM, Levine BD and Zhang R.** Cerebral
465 vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes.
466 *J Cereb Blood Flow Metab.* 33: 8: 1190-1196, 2013.

467

468 **Table 1.** Population distribution values for CBFV, HR, MAP, EtCO₂, CrCP, RAP and ARI for young and old cohorts at baseline and during
469 hypocapnic conditions

	Young (n=43)		Old (n=61)		Independent t-test <i>p</i> -values		
	Baseline	Hypocapnia	Baseline	Hypocapnia	Age	CO ₂ Change	Interaction
EtCO ₂ (mmHg)	38.00 (3.14)	30.67 (5.54)	38.11 (5.04)	29.87 (3.88)	0.288	0.0001	0.576
CBFV (cm s ⁻¹)	57.35 (14.38)	44.89 (11.07)	51.73 (12.89)	37.80 (9.65)	0.0001	0.023	0.568
HR (beats/min)	69.13 (11.94)	69.74 (12.94)	62.63 (9.79)	67.62 (10.96)	0.010	0.016	0.012
MAP (mmHg)	86.76 (12.26)	85.45 (13.09)	90.60 (11.90)	88.30 (11.11)	0.122	0.026	0.666
CrCP (mmHg)	37.65 (18.54)	39.72 (15.96)	33.94 (13.52)	39.32 (11.42)	0.830	0.0001	0.626
RAP (mmHg cm s ⁻¹)	1.02 (0.40)	1.09 (0.44)	1.16 (0.34)	1.34 (0.39)	0.001	0.004	0.852
ARI	5.52 (1.89)	7.63 (1.74)	5.54 (2.17)	7.23 (1.66)	0.133	0.008	0.100

470 Values are mean (SD). CBFV, cerebral blood velocity; HR, heart rate; MAP, mean arterial blood pressure; EtCO₂, end-tidal carbon dioxide CrCP, critical closing pressure;
471 RAP, resistance area product; ARI, Autoregulation Index; M, male; F, female. CBFV, CrCP, RAP and ARI were averaged for the right and left MCAs.

472

473 **Figure legends**

474 **Figure 1.** Representative recordings of CBFV, ABP and EtCO₂ from a 60 year old female study participant. Dotted vertical lines represent onset
475 of hyperventilatory manoeuvre.

476 **Figure 2.** Population average curves for the dependence of CBFV, HR, MAP, CrCP and RAP on EtCO₂

477 Corresponding shaded areas represent the $\pm$ SD boundaries

478 **Figure 3.** Changes in CBFV, HR, MAP, CrCP, RAP and ARI between old and young individuals from baseline to hypocapnic conditions

479 Error bars show $\pm$ one SD

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481 Figure 1: Representative recordings of CBFV, ABP and EtCO₂ from a 60 year old female
482 study participant. Dotted vertical lines represent onset of hyperventilatory manoeuvre.
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484

Figure 2: Population mean $\pm$ SD curves for the dependence of CBFV, HR, MAP, CrCP and RAP on EtCO₂

Figure 2: Changes in CBFV, HR, MAP, CrCP, RAP and ARI between old and young individuals from baseline to hypocapnic conditions. NB: * = $p < 0.05$; *# = interaction $p < 0.05$.

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Figure 3: Correlation coefficient for dependence of RAP on HR during hypocapnia for young (A) and old (B). 95% confidence intervals presented.