# Cerebrovascular carbon dioxide reactivity and flow mediated dilation in young healthy South Asian and Caucasian European men

**Authors**: Rehan T Junejo[1,2], Sophie May[1], Sultan Alsalahi[1], Mohammad Alali[1], Shigehiko Ogoh[3] & James P Fisher[4]

**Institutions:** [1]School of Sport, Exercise & Rehabilitation Sciences, College of Life & Environmental Sciences, University of Birmingham, UK; [2]Liverpool Centre for Cardiovascular Science, Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK; [3]Department of Biomedical Engineering, Toyo University, Japan; [4]Faculty of Medical & Health Sciences, Department of Physiology, University of Auckland, New Zealand.

**Running Title**: Ethnic differences in cerebrovascular reactivity

**Address for correspondence**: James P. Fisher, PhD, Faculty of Medical & Health Sciences, Department of Physiology, University of Auckland, 85 Park Road, Grafton, Auckland 1023, New Zealand. Tel (+649) 3737599 x86320. Email: jp.fisher@auckland.ac.nz

**ABSTRACT**

South Asians living in the UK have a 1.5-fold greater risk of ischemic stroke than the general population. Impaired cerebrovascular carbon dioxide ($CO_2$) reactivity is an independent predictor of ischemic stroke and cardiovascular mortality. We sought to test the hypothesis that cerebrovascular $CO_2$ reactivity is reduced in South Asians. Middle cerebral artery blood velocity (MCA $V_m$) was measured at rest and during stepwise changes in partial pressure of end-tidal $CO_2$ ($P_{ET}CO_2$) in South Asian (n=16) and Caucasian European (n=18) men that were, young (~20 years), healthy and living in the UK. Incremental hypercapnia was delivered via the open circuit steady-state method, with stages of 4% and 7% $CO_2$ (≈21% Oxygen, Nitrogen balanced). Cerebrovascular $CO_2$ reactivity was calculated as the change in MCA $V_m$ per mmHg change in $P_{ET}CO_2$. MCA $V_m$ was not different in South Asian (59 (9) cm/s; mean (standard deviation)) and Caucasian Europeans (61 (12) cm/s; $P>0.05$). Similarly, cerebrovascular $CO_2$ reactivity was not different between the groups (South Asian, 2.53 (0.76) cm/s/mmHg vs. Caucasian European, 2.61 (0.81) cm/s/mmHg; $P>0.05$). Brachial artery flow-mediated dilatation was lower in South Asian (5.48 (2.94) %) compared to Caucasian European (7.41 (2.28) %; $P<0.05$); however when corrected for shear rate, no between group differences in flow-mediated dilatation were observed ($P>0.05$). Flow-mediated dilation was not correlated with cerebrovascular $CO_2$ reactivity measures. In summary, cerebrovascular $CO_2$ reactivity and flow-mediated dilation when corrected for shear rate are preserved in young healthy South Asian men living in the UK.

**Keywords**: brain, cerebral circulation, flow-mediated dilatation.

## NEW AND NOTEWORTHY

Previous reports have identified an increased risk of ischemic stroke and peripheral endothelial dysfunction in South Asians compared to Caucasian Europeans. The main finding of this study is that cerebrovascular carbon dioxide reactivity (an independent predictor of ischemic stroke) is not different in healthy young South Asian and Caucasian European adult men.

## ABBREVIATIONS

BP, blood pressure; $CO_2$, carbon dioxide; CVCi, cerebrovascular conductance index; ECG, electrocardiograph; FMD, flow-mediated dilation; FMDc, covariate corrected flow-mediated dilation; HR, heart rate; MAP, mean arterial pressure; $MCA_V$, middle cerebral artery mean blood velocity; $N_2$, nitrogen; $O_2$, oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; $SR_{AUC}$, shear rate area under the curve; TCD, transcranial Doppler

## INTRODUCTION

South Asian migrants from the Indian sub-continent in the United Kingdom have an ischemic stroke mortality that is ~1.5 times greater than the general population (44), while ischemic stroke onset typically occurs at a younger age in South Asians than ethnically White Caucasian Europeans (20). Although broadly attributable to cultural and socioeconomic factors (12), there is a paucity of information about the underlying physiological mechanisms for such ethnic differences in cerebrovascular health (37). The brain has a high metabolic demand and possesses multiple interactive regulatory mechanisms. The latter ensure that cerebral blood flow remains relatively stable independent of changes in perfusion pressure (cerebral autoregulation), that local perfusion is closely matched to neuronal activation and metabolism (neurovascular coupling), and that cerebrovascular responses to changes in carbon dioxide (cerebrovascular $CO_2$ reactivity) are adequate to assist the maintenance of central [H+]. Bathula et al. (4) observed that cerebral autoregulation is poorer and cerebrovascular resistance is higher in South Asians (of Punjabi Sikh origin) compared to people with "European origins". However, it remains to be determined whether cerebrovascular $CO_2$ reactivity is blunted (i.e., diminished cerebral vasodilatory reserve) in South Asians.

It has long been established that the cerebral vasculature is highly sensitive to changes in the partial pressure of arterial $CO_2$ (19), and since this time an impaired cerebrovascular $CO_2$ reactivity has been established as an independent predictor of ischemic stroke (23) and identified in several cardiovascular, cerebrovascular and neurological disorders (13, 18, 23, 41). Cerebrovascular dysfunction may lead to neuronal dysfunction and neurodegeneration since neurons depend on arterial vasodilatation for adequate perfusion to ensure oxygen/$CO_2$ homeostasis, nutrient delivery and elimination of potentially toxic metabolites (46). The mechanism whereby $CO_2$ modifies cerebral blood vessel tone is complex. Among the various

contributory factors, endothelium-derived nitric oxide is considered to be an important local regulator of cerebral blood flow that plays a role in hypercapnia-induced vasodilatation (14, 40, 43). Indeed, acute infusion of L-arginine (the substrate for endothelial nitric oxide synthase) restores impairments in cerebrovascular $CO_2$ reactivity manifest in patients with cardiovascular risk factors (45), while hypercapnia-induced increases in cerebral blood flow are attenuated by inhibition of nitric oxide synthase activity with N-nitro-L-arginine methyl ester (L-NAME) in rats (5). Moreover, individuals or groups in whom impaired peripheral vascular nitric oxide signaling has been identified are reported to demonstrate a reduced cerebrovascular $CO_2$ reactivity (21). Therefore, the observation that brachial artery flow-mediated dilation, indicative of attenuated endothelium-derived nitric oxide mediated vasodilation, is reduced in South Asians compared to Caucasian Europeans (6, 30) may also point to a reduced cerebrovascular $CO_2$ reactivity.

The aim of this study was to investigate whether cerebrovascular $CO_2$ reactivity is impaired in young healthy South Asians compared to Caucasian Europeans. Based on prior reports identifying the greater incidence of cerebrovascular events in South Asians and peripheral endothelial dysfunction, we hypothesized that cerebrovascular $CO_2$ reactivity would be lower in healthy young South Asian adults when compared to age-matched Caucasian Europeans. Brachial artery flow-mediated dilation, a well-established marker of peripheral vascular (endothelial) function, was also determined in accordance with established guidelines (32, 42). Lastly, we assessed whether an association between brachial artery flow-mediated dilation and cerebrovascular $CO_2$ reactivity existed in the population studied.

## METHODS

*Ethical Approval.*

The experiments were undertaken in accordance with the Declaration of Helsinki, except for registration in a database, and were approved by the University of Birmingham, Science, Technology, Engineering and Mathematics Ethical Review (approval number ERN_17-1161). Written informed consent was obtained from all participants after each had received a detailed verbal and written explanation of the study procedures.

*Participant characteristics.*

Sixteen South Asians with ethnic roots in Indian-Subcontinent (Bangladesh, India, Maldives, Nepal, Pakistan and Sri Lanka) and eighteen Caucasian Europeans living in the UK volunteered for this study. Accordingly, each participant confirmed the ethnic origins of all four of their grandparents. South Asian participants were first or second-generation migrants. No participant had a known history of pulmonary, cardiovascular, metabolic or neurological diseases and were not taking prescription or over-the-counter medication. One participant in each group was found to have raised blood pressure and recommended to have an appointment with their general practitioner. Upon follow-up both were confirmed as being normotensive. All participants were accustomed to recreational exercise, but none was a competitive athlete.

*Experimental measures.*

Height and weight, along with waist (level of the umbilicus) and hip (level of the femoral trochanter) circumference were measured. Heart rate (HR) was monitored using a lead II electrocardiogram (ECG) (Morgan 509 Cardiac Monitor, Kent, UK). Arterial blood pressure (BP) was measured continuously using finger photoplethysmography (Portpress,

Finapres Medical Systems BV, Amsterdam, The Netherlands) and corrected with automatic brachial sphygmomanometer readings (Omorn 750IT, Milton Keynes, UK). Middle cerebral artery mean blood velocity (MCA $V_m$) was continuously monitored using transcranial Dopper ultrasonography (Doppler Box X, DWL, Sipplingen, Germany). A 2 MHz probe, mounted on an adjustable headband, was fixed at the temporal window to insonate the right MCA at a depth of 40-65 mm. Participants wore a mouthpiece and nose-clip, and the partial pressure of end-tidal $CO_2$ ($P_{ET}CO_2$) was provided by a capnograph connected to the mouthpiece by an anesthetic sample line (Gas Analyzer, ADInstruments, Dunedin, New Zealand). Breath-by-breath fluctuations in $P_{ET}CO_2$ were used to calculate respiratory rate. Analogue signals were digitized at 1 kHz (Powerlab, ADInstruments) and recorded using multi-channel data acquisition software (LabChart 7, ADInstruments). Simultaneous recordings of the left brachial artery diameter and flow velocity were obtained with the arm at heart level using Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA). The artery was insonated 10–15 cm proximal to the medial epicondyle at 60°. Duplex imaging was used to obtain a B-mode image of vessel diameter and pulse-wave mode of peak blood velocity using a 4-15 Hz multi-frequency linear-array transducer (Terason uSmart 15L4) held in place with an adjustable probe holder. Ultrasound measurements were made in accordance with technical recommendations (32, 42). Recordings were screen captured, stored as video files and offline analysis carried out using automated edge detection and wall tracking software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy) (11).

***Experimental Protocol***

This cross-sectional study included a screening/familiarization visit prior to the experimental session. Participants were instructed to abstain from food for 2 h, caffeinated beverages for 12 h, strenuous exercise for 24 h and multi-vitamin use for 7 days before

154 experimental sessions. The study was conducted in a temperature controlled cardiovascular
155 laboratory (21–24 ℃). Participants were asked to lie supine comfortably for ~10 min on a
156 medical examination couch. A narrow inflatable cuff (5 cm width, Hokanson, Bellevue, WA,
157 USA) was placed 5-7 cm distal to the medial epicondyle. The flow-mediated dilatation
158 protocol was then conducted with the brachial artery insonnated for the simultaneous
159 measurement of diameter and flow velocity. The flow-mediated dilatation protocol comprised
160 of a 2 min baseline, a 5 min cuff inflation to a supra-systolic pressure of > 240 mmHg and a 3
161 min recovery period with the cuff deflated.

162 To assess cerebrovascular $CO_2$ reactivity a 10-min baseline was acquired while
163 participants breathed room air. During this period, a minimum of 3 brachial artery blood
164 pressure readings were obtained using the automated sphygmomanometer. Participants then
165 breathed gas mixtures from a Douglas bag containing air enriched with $CO_2$ (hypercapnia),
166 via a two-way non-rebreathing valve. Specifically, participants received 4 % $CO_2$ (≈21 % $O_2$,
167 $N_2$ balanced) for 4-min, followed by 7 % $CO_2$ (≈21 % $O_2$, $N_2$ balanced) for 4 min, then were
168 switched back to room air (18, 33). Hemodynamic and respiratory parameters were recorded
169 throughout and once these had returned to baseline, participants were asked to increase their
170 respiratory depth and rate in order to achieve an equal but opposite change in their $P_{ET}CO_2$ as
171 during the hypercapnic challenge, with each step lasting 2 min (hypocapnia).
172
173 *Data analysis*
174 Body mass index (BMI) was expressed as the ratio of the participants' weight and the
175 height squared. Digitally recorded data were extracted in an anonymized manner. Mean
176 arterial pressure (MAP) was the mean blood pressure over each cardiac cycle. Brachial artery
177 blood flow was calculated as:

$$\text{Brachial artery blood flow} = [\frac{\text{Peak Envelope Velocity}}{2} \cdot (\pi\ (0.5\ \cdot \text{Diameter})^2)] \cdot 60$$

178 Brachial artery flow-mediated dilatation was taken as the maximal change in brachial
179 artery diameter following cuff deflation. The time to peak diameter was obtained between the
180 cuff deflation and the maximal artery dilation, and time to peak blood flow (reactive
181 hyperemia) was obtained between cuff deflation and maximal flow velocity. Shear rate was
182 calculated as brachial artery blood velocity multiplied by 4 and divided by brachial artery
183 diameter. Shear rate area under the curve ($SR_{AUC}$) was calculated as an integral between the
184 cuff deflation and the maximal artery dilation. Flow-mediated dilatation was expressed as
185 absolute and relative change in diameter. A ratio between flow-mediated dilatation and
186 $SR_{AUC}$ (FMD-to-$SR_{AUC}$ ratio) was also calculated and multiplied by 1000 (32, 42). Further,
187 based on recent guidelines (2), baseline and maximal brachial artery diameters were log-
188 transformed and the difference between them calculated. Logged difference in diameter was
189 entered in an analysis of covariance (ANCOVA) where ethnicity constituted a fixed factor
190 and log-transformed baseline diameter a covariate. The covariate adjusted means were then
191 back-transformed and expressed as percentage changes for covariate corrected flow-mediated
192 dilatation ($FMD_C$).
193 Cerebrovascular conductance index (CVCi) was calculated as MCA $V_m$ / MAP.
194 Baseline values are taken as mean of the whole 10-min baseline period. For cerebrovascular
195 $CO_2$ reactivity, values were acquired over the last minute of each hypercapnic and
196 hypocapnic step. Cerebrovascular $CO_2$ reactivity was assessed using linear and exponential
197 models (39). For exponential model, values of the exponent and $R^2$ and for linear model, the
198 values of slope and $R^2$ of % change in (Δ) MCA Vm and % Δ CVCi versus $P_{ET}CO_2$ (mmHg)
199 were calculated. Cerebrovascular $CO_2$ reactivity was separately expressed as the linear slope
200 of Δ MCA $V_m$ (cm/s) and Δ CVCi (cm/s/mmHg) versus the change in $P_{ET}CO_2$ in mmHg,
201 between the two hypercapnic steps and two hypocapnic steps (18, 33). Additional analyses of
202 cerebrovascular $CO_2$ reactivity were undertaken by calculating the slope of % Δ MCA Vm

and % Δ CVCi versus Δ $P_{ET}CO_2$ (in mmHg) with the hypercapnic and hypocapnic steps (9, 31).

*Statistical Analysis*

Data distribution was assessed by the Shapiro-Wilk test. Normally distributed data were analyzed using two-tailed Students t-test, while non-normally distributed data were analyzed using Mann-Whitney Rank Sum test. The correlation between cerebrovascular $CO_2$ reactivity and flow-mediated dilatation was assessed using Pearson's product moment correlation. Effect size (Cohen's *d*) was calculated as the difference between means of two groups divided by the averaged standard deviation (SD). Statistical analysis was performed using Sigmaplot 13.0 (Systat Software Inc, London, UK). Significance was set at $p < 0.05$. Normally distributed data are presented as mean (SD), unless stated, while non-normally distributed data are presented as median [interquartile range].

## RESULTS

*Participant characteristics and baseline haemodynamics*

Participant characteristics are presented in Table 1. Groups were closely matched for age, weight, BMI and waist-to-hip ratio. At baseline, no between-group differences in heart rate, systolic BP, diastolic BP and respiratory rate were observed. Similarly, MCA $V_m$, CVCi and MAP were not different between the South Asian and Caucasian European groups ($P>0.05$), however $P_{ET}CO_2$ was lower in South Asians ($P<0.05$; Figure 1).

*Cerebrovascular $CO_2$ reactivity*

Figure 2 shows the MCA $V_m$, CVCi and MAP response to both the hypercapnic and hypocapnic steps of the cerebrovascular $CO_2$ reactivity test in the South Asian and Caucasian European groups. As anticipated, hypercapnia produced pronounced increases in MCA $V_m$ and CVCi, while conversely both were reduced with hypocapnia. Of note, no between-group differences were observed in any index of cerebrovascular $CO_2$ reactivity (Figure 3, Table 2).

*Brachial artery flow-mediated dilatation*

Flow-mediated dilatation was lower in the South Asian than Caucasian European group ($P<0.05$, Figure 4). This between group difference persisted with correction for baseline diameter ($FMD_C$ $P<0.05$, Table 3). Peak reactive hyperemia was not different between groups ($P>0.05$, Table 3). However, $SR_{AUC}$ was lower in South Asians than Caucasian Europeans ($P<0.05$, Table 3) and when brachial artery flow-mediated dilatation was corrected for $SR_{AUC}$ (i.e., FMD-to-$SR_{AUC}$ ratio) the between group difference was no longer evident ($P>0.05$, Figure 4). No significant association between FMDc and hypercapnic cerebrovascular $CO_2$ reactivity (4% to 7%; Figure 3) was observed either for the whole group

240 (r = 0.08, P = 0.669), or individually for South Asians and Caucasian Europeans (r = -0.05, P
241 = 0.854 and r = 0.18, P = 0.475, respectively).

## DISCUSSION

The major novel finding of the present study is that cerebrovascular $CO_2$ reactivity is not different in young healthy South Asians and Caucasian Europeans. In addition, brachial artery flow-mediated dilatation was lower in South Asians when expressed as a percentage change from baseline. However, during flow-mediated dilation testing South Asians had a lower shear rate response ($SR_{AUC}$), which when accounted for (FMD-to-$SR_{AUC}$ ratio), flow-mediated dilatation was not different between groups. These findings suggest that: 1) contrary to our hypothesis, cerebrovascular $CO_2$ reactivity is not lower in healthy young South Asian adults than age-matched Caucasian Europeans, and 2) apparent reductions in brachial artery flow-mediated dilatation in South Asians (6, 30) may be attributable to a reduced ischemic stimulus rather than endothelial dysfunction *per se*.

Prior reports have identified a greater incidence of cerebrovascular events in South Asians (20, 44). Given the prognostic significance of impaired cerebrovascular $CO_2$ reactivity as an independent predictor of ischemic stroke (23) and its association with multiple cardiovascular, cerebrovascular and neurological disorders (13, 18, 23, 41), we anticipated that cerebrovascular $CO_2$ reactivity would be lower in South Asian adults than age-matched Caucasian Europeans. Moreover, Hurr et al. (15) identified that African Americans (23±4 years), a group at higher risk of cardiovascular and cerebrovascular disease, exhibited an attenuated cerebrovascular vasodilatation in response to hypercapnia compared to age-matched Caucasian Americans. Contrary to expectation, we did not observe a difference in cerebrovascular $CO_2$ reactivity between young healthy South Asian and Caucasian European men; neither did we observe between-group differences in MCA $V_m$ nor CVCi. In a population-based sample Bathula et al. (4) noted a higher MCA $V_m$ (38.0±0.7 vs. 41.4±0.7 cm/s) and cerebrovascular resistance (resistivity index), but poorer cerebral autoregulation (low frequency gain, 0.45±0.01 vs. 0.50±0.01 cm/s/mmHg) in South Asians of Punjabi Sikh

origin (n=127) compared to people with "European origins" (n=128). Interestingly, the elevated cerebrovascular resistance in South Asians was attributable to hyperglycaemia (e.g., blood glucose, glycated haemoglobin). The cohort studied by Bathula et al. (4) had a wide age range (35-75 years) and comorbidities, including hypertension, diabetes, coronary heart disease and metabolic syndrome, which perhaps is reflected in their comparatively low MCA $V_m$ values (7, 17, 18, 29). However, this is in contrast to the young and healthy participants recruited to the present study and may explain why we did not observe any differences in MCA $V_m$, CVCi and cerebrovascular $CO_2$ reactivity between the South Asian and Caucasian European groups studied.

Coronary heart disease risk is elevated in migrant South Asians to the UK (3, 25). Of note, according to the 1991 England and Wales Census data, the relative risk of death from coronary heart disease was 3 in Indian Asian men aged 20-29 years, compared to age-matched Caucasian Europeans (3). The excess coronary heart disease risk in South Asians is not explained by conventional risk factors (e.g., smoking, hypercholesterolemia, hypertension) (24), although an increased prevalence of insulin resistance and diabetes has been implicated (26). Endothelial dysfunction in South Asians (i.e., attenuated brachial artery flow-mediated dilatation and $N^G$-Monomethyl-L-arginine induced vasoconstriction) is also speculated to contribute to the elevated coronary heart disease risk, and has been identified in both young (30) and older (6) South Asian groups. In the present study when we expressed flow-meditated dilatation simply as the percentage change from baseline in brachial artery diameter, it was reduced in South Asians compared to Caucasian Europeans. This experimental approach and the associated findings are in agreement with previous reports (6, 30). It is noteworthy that despite no differences in baseline brachial artery diameter, velocity and blood flow, the $SR_{AUC}$ was attenuated in the South Asian group. Accordingly, when flow-mediated dilatation responses were adjusted to account for this (i.e., via the FMD-to-

292 $SR_{AUC}$ ratio), the between group difference was no longer observed. This is important
293 because the magnitude of the evoked shear stress is mechanistically coupled with the
294 dilatation observed, but no previous studies reporting a blunted flow-mediated dilatation in
295 South Asians versus European Caucasians have accounted for this (6, 20, 30, 44). In
296 accordance with recent guidelines (32, 42), it is deemed important to account for shear stress
297 when making between group comparisons. The reason for the lower $SR_{AUC}$ in South Asian
298 group is unclear, but may relate to a lower maximal vascular conductance and/or attenuated
299 metabolic vasodilation induced by ischemia. Indeed, as the hyperemia dynamics are coupled
300 with metabolism, it is a possible that the results of this study reflect a lower and/or altered
301 metabolic response to ischemia in South Asians; a possibility that requires further
302 investigation.

303 Brachial artery flow-mediated dilatation and hypercapnia-induced cerebral
304 vasodilatation share common mechanisms, with endothelial derived nitric oxide reported to
305 mediate both, at least partially (14, 16, 40, 43). In the population-based Rotterdam Study,
306 Portegies et al. (36) observed that lower cerebrovascular $CO_2$ reactivity was associated with
307 an increased risk of all-cause mortality (1.10, 95% confidence interval [CI] 1.01-1.19),
308 cardiovascular mortality (1.09 [95% CI 0.94-1.26]) and non-cardiovascular mortality (1.10
309 [95% CI 0.99-1.21]), which points towards cerebrovascular $CO_2$ reactivity being more
310 broadly associated with systemic vascular dysfunction. Moreover, brachial artery endothelial
311 dysfunction (i.e., attenuated forearm reactive hyperemia) and impaired cerebrovascular $CO_2$
312 reactivity coexist in patients with long standing diabetes and/or hypertension (21). Similarly,
313 both an impaired cerebrovascular responses to hypercapnia (15) and an attenuated brachial
314 artery flow-mediated dilatation (34) have been identified in African Americans, relative to
315 Caucasian Americans, albeit not in the same cohort. In contrast, we observed no association
316 between cerebrovascular $CO_2$ reactivity and brachial artery flow-mediated dilatation in our

study population, which possibly reflects the young and healthy cohort with a relatively narrow (i.e., normal) range of vascular responsiveness.

The results of this study should be viewed in the context of the following experimental limitations. Despite the widely acknowledged value of transcranial Doppler in the evaluation of cerebral vascular function, it is an inherent limitation of the method that MCA $V_m$ is only proportional to cerebral blood flow if the cross-sectional area of the MCA remains unchanged. Although, good correlations have been observed between MCA $V_m$ and cerebral blood flow when $P_{ET}CO_2$ is altered (8, 35), there is evidence to suggest MCA diameter increases with robust hypercapnia (i.e., $\Delta P_{ET}CO_2$ of greater than ~7-9 mmHg) (1, 22, 28). $P_{ET}CO_2$ has been employed as a non-invasive surrogate for the partial pressure of arterial $CO_2$ ($P_aCO_2$) in the present study because a strong positive linear correlation between $P_{ET}CO_2$ and $P_aCO_2$ has been identified (27); however, it is acknowledged that $P_{ET}CO_2$ may underestimate $P_aCO_2$ at rest (38). We also acknowledge the ongoing debate relating to the relative strengths and weaknesses of approaches developed to determine cerebrovascular $CO_2$ reactivity (10). The method used here has shown a good between-day test-retest reliability (intraclass correlation of 0.938 [95% CI 0.759-0.985] $P<0.001$; co-efficient of variation for the method error of 6.06%) (18). The extent to which our findings may be more broadly generalized is limited by the inclusion of only healthy young men. We also failed to collect diet and socioeconomic data for the participants and did not objectively assess their activity patterns in a detailed manner. Future studies should consider the important potential interaction between sex, aging, diet, socioeconomic levels, activity patterns and ethnicity in the regulation of peripheral vasculature and cerebrovascular function.

In summary, we report for the first time that cerebrovascular $CO_2$ reactivity is not different in young healthy South Asians and Caucasian Europeans. Furthermore, when the brachial artery flow-mediated dilatation response was expressed relative to the shear stress

342 stimulus (which was also lower in South Asians), no between group differences were
343 observed.

## ACKNOWLEDGMENTS

The time and effort expended by all the volunteer participants is greatly appreciated.

## GRANT AND DISCLOSURES

None.

## REFERENCES


1. **Al-Khazraji BK, Shoemaker LN, Gati JS, Szekeres T, and Shoemaker JK**. Reactivity of larger intracranial arteries using 7 T MRI in young adults. *J Cereb Blood Flow Metab* 39: 1204-1214, 2019.
2. **Atkinson G, and Batterham AM**. Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis* 226: 425-427, 2013.
3. **Balarajan R**. Ethnicity and variations in the nation's health. *Health Trends* 27: 114-119, 1995.
4. **Bathula R, Hughes AD, Panerai RB, Potter JF, Mc GTSA, Tillin T, Shore AC, Hale R, Chambers J, Kooner J, and Chaturvedi N**. South Asians have adverse cerebrovascular haemodynamics, despite equivalent blood pressure, compared with Europeans. This is due to their greater hyperglycaemia. *Int J Epidemiol* 40: 1490-1498, 2011.
5. **Buchanan JE, and Phillis JW**. The role of nitric oxide in the regulation of cerebral blood flow. *Brain Res* 610: 248-255, 1993.
6. **Chambers JC, McGregor A, Jean-Marie J, and Kooner JS**. Abnormalities of vascular endothelial function may contribute to increased coronary heart disease risk in UK Indian Asians. *Heart* 81: 501-504, 1999.
7. **Cho SJ, Sohn YH, Kim GW, and Kim J-S**. Blood flow velocity changes in the middle cerebral artery as an index of the chronicity of hypertension. *J Neurol Sci* 150: 77-80, 1997.
8. **Clark JM, Skolnick BE, Gelfand R, Farber RE, Stierheim M, Stevens WC, Beck Jr G, and Lambertsen CJ**. Relationship of 133Xe cerebral blood flow to middle cerebral arterial flow velocity in men at rest. *J Cereb Blood Flow Metab* 16: 1255-1262, 1996.
9. **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* 117: 1090-1096, 2014.
10. **Fierstra J, Sobczyk O, Battisti-Charbonney A, Mandell DM, Poublanc J, Crawley AP, Mikulis DJ, Duffin J, and Fisher JA**. Measuring cerebrovascular reactivity: what stimulus to use? *J Physiol* 591: 5809-5821, 2013.
11. **Gemignani V, Faita F, Ghiadoni L, Poggianti E, and Demi M**. A system for real-time measurement of the brachial artery diameter in B-mode ultrasound images. *IEEE Trans Med Imaging* 26: 393-404, 2007.
12. **Gunarathne A, Patel JV, Gammon B, Gill PS, Hughes EA, and Lip GY**. Ischemic stroke in South Asians: a review of the epidemiology, pathophysiology, and ethnicity-related clinical features. *Stroke* 40: e415-423, 2009.
13. **Haight TJ, Bryan RN, Erus G, Davatzikos C, Jacobs DR, D'Esposito M, Lewis CE, and Launer LJ**. Vascular risk factors, cerebrovascular reactivity, and the default-mode brain network. *Neuroimage* 115: 7-16, 2015.
14. **Hainsworth AH, Oommen AT, and Bridges LR**. Endothelial cells and human cerebral small vessel disease. *Brain Pathol* 25: 44-50, 2015.
15. **Hurr C, Kim K, Harrison ML, and Brothers RM**. Attenuated cerebral vasodilatory capacity in response to hypercapnia in college-aged African Americans. *Exp Physiol* 100: 35-43, 2015.
16. **Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, and Luscher TF**. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 91: 1314-1319, 1995.
17. **Junejo RT, Braz ID, Lucas SJ, van Lieshout JJ, Phillips AA, Lip GY, and Fisher JP**. Neurovascular coupling and cerebral autoregulation in atrial fibrillation. *J Cereb Blood Flow Metab* 0: 0271678X19870770, 2019.

18. **Junejo RT, Braz ID, Lucas SJE, van Lieshout JJ, Lip GYH, and Fisher JP**. Impaired Cerebrovascular Reactivity in Patients With Atrial Fibrillation. *J Am Coll Cardiol* 73: 1230-1232, 2019.
19. **Kety SS, and Schmidt CF**. The Effects of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption of Normal Young Men. *J Clin Invest* 27: 484-492, 1948.
20. **Khan NA, McAlister FA, Pilote L, Palepu A, Quan H, Hill MD, Fang J, and Kapral MK**. Temporal trends in stroke incidence in South Asian, Chinese and white patients: A population based analysis. *PLoS One* 12: e0175556, 2017.
21. **Lavi S, Gaitini D, Milloul V, and Jacob G**. Impaired cerebral CO2 vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 291: H1856-1861, 2006.
22. **Liu P, De Vis JB, and Lu H**. Cerebrovascular reactivity (CVR) MRI with CO2 challenge: A technical review. *Neuroimage* 187: 104-115, 2019.
23. **Markus H, and Cullinane M**. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. *Brain* 124: 457-467, 2001.
24. **McKeigue PM, Ferrie JE, Pierpoint T, and Marmot MG**. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 87: 152-161, 1993.
25. **McKeigue PM, Miller GJ, and Marmot MG**. Coronary heart disease in south Asians overseas: a review. *J Clin Epidemiol* 42: 597-609, 1989.
26. **McKeigue PM, Shah B, and Marmot MG**. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 337: 382-386, 1991.
27. **McSwain SD, Hamel DS, Smith PB, Gentile MA, Srinivasan S, Meliones JN, and Cheifetz IM**. End-tidal and arterial carbon dioxide measurements correlate across all levels of physiologic dead space. *Respir Care* 55: 288-293, 2010.
28. **Miller KB, Howery AJ, Rivera-Rivera LA, Johnson SC, Rowley HA, Wieben O, and Barnes JN**. Age-Related Reductions in Cerebrovascular Reactivity Using 4D Flow MRI. *Front Aging Neurosci* 11: 2019.
29. **Moghaddasi M, Mamarabadi M, and Habibi AH**. A comparison of cerebral vasomotor reactivity in diabetic and nondiabetic Iranian patients. *J Res Med Sci* 15: 50-53, 2010.
30. **Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M, Rahman S, Shah AM, Marber MS, and Kearney MT**. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arterioscler Thromb Vasc Biol* 27: 936-942, 2007.
31. **Nowak-Flück D, Ainslie PN, Bain AR, Ahmed A, Wildfong KW, Morris LE, Phillips AA, and Fisher JP**. Effect of healthy aging on cerebral blood flow, CO2 reactivity and neurovascular coupling during exercise. *J Appl Physiol* 125: 1917-1930, 2018.
32. **Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, Mather KJ, and Wallace JP**. Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. *Cardiovasc Ultrasound* 6: 44, 2008.
33. **Peebles K, Celi L, McGrattan K, Murrell C, Thomas K, and Ainslie PN**. Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein PCO2. *J Physiol* 584: 347-357, 2007.
34. **Perregaux D, Chaudhuri A, Rao S, Airen A, Wilson M, Sung BH, and Dandona P**. Brachial vascular reactivity in blacks. *Hypertension* 36: 866-871, 2000.

35. **Poeppel T, Terborg C, Hautzel H, Herzog H, Witte O, Mueller H-W, and Krause B**. Cerebral haemodynamics during hypo-and hypercapnia. *Nuklearmedizin* 46: 93-100, 2007.
36. **Portegies ML, de Bruijn RF, Hofman A, Koudstaal PJ, and Ikram MA**. Cerebral vasomotor reactivity and risk of mortality: the Rotterdam Study. *Stroke* 45: 42-47, 2014.
37. **Rambihar VS, Rambihar SP, and Rambihar VS**. Race, ethnicity, and heart disease: A challenge for cardiology for the 21st century. *Am Heart J* 159: 1-14, 2010.
38. **Robbins PA, Conway J, Cunningham DA, Khamnei S, and Paterson DJ**. A comparison of indirect methods for continuous estimation of arterial PCO2 in men. *J Appl Physiol (1985)* 68: 1727-1731, 1990.
39. **Sato K, Sadamoto T, Hirasawa A, Oue A, Subudhi AW, Miyazawa T, and Ogoh S**. Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. *J Physiol* 590: 3277-3290, 2012.
40. **Schmetterer L, Findl O, Strenn K, Graselli U, Kastner J, Eichler HG, and Wolzt M**. Role of NO in the O2 and CO2 responsiveness of cerebral and ocular circulation in humans. *Am J Physiol* 273: R2005-2012, 1997.
41. **Smolinski L, and Czlonkowska A**. Cerebral vasomotor reactivity in neurodegenerative diseases. *Neurol Neurochir Pol* 50: 455-462, 2016.
42. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 300: H2, 2011.
43. **Toda N, Ayajiki K, and Okamura T**. Cerebral blood flow regulation by nitric oxide: recent advances. *Pharmacol Rev* 61: 62-97, 2009.
44. **Wild SH, Fischbacher C, Brock A, Griffiths C, and Bhopal R**. Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *J Public Health (Oxf)* 29: 191-198, 2007.
45. **Zimmermann C, and Haberl RL**. L-arginine improves diminished cerebral CO2 reactivity in patients. *Stroke* 34: 643-647, 2003.
46. **Zlokovic BV**. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* 12: 723-738, 2011.

## TABLES

**Table 1.** Participant characteristics.

| | Caucasian European | South Asian | P value |
|---|---|---|---|
| **n** | 18 | 16 | |
| **Age** (years) | 21 [20-22] | 21 [20-25] | 0.505 |
| **Height** (cm) | 1.80 (0.07) | 1.76 (0.06) | 0.074 |
| **Weight** (kg) | 75.0 (8.5) | 76.1 (11.4) | 0.733 |
| **BMI** ($kg/m^2$) | 23.2 (2.4) | 24.7 (3.2) | 0.139 |
| **Waist circumference** (cm) | 78 [77-81] | 80 [75-89] | 0.387 |
| **Hip circumference** (cm) | 97 [95-98] | 99 [93-100] | 0.341 |
| **Waist / Height ratio** (au) | 0.44 (0.03) | 0.47 (0.06) | 0.050 |
| **Waist / Hip ratio** (au) | 0.80 [0.79-0.84] | 0.82 [0.78-0.86] | 0.557 |
| **Heart rate** ($b \cdot min^{-1}$) | 63 [58-66] | 67 [58-73] | 0.248 |
| **Systolic BP** (mmHg) | 124 (9) | 119 (9) | 0.070 |
| **Diastolic BP** (mmHg) | 67 [63-71] | 67 [64-72] | 0.972 |
| **Respiratory rate** ($b \cdot min^{-1}$) | 14 [13-15] | 15 [13-16] | 0.343 |

Values are displayed as mean (SD) when normally distributed or median [interquartile range] when non-normally distributed. BMI, body mass index; au, arbitrary units.

485 **Table 2.** Cerebrovascular $CO_2$ reactivity parameters.

| | | Caucasian European | South Asian | Effect Size | P value |
|---|---|---|---|---|---|
| **MCA $V_m$ %** (%·mmHg$^{-1}$) | Linear Slope | 3.06 [2.77-3.18] | 3.26 [2.83-3.45] | 0.064 | 0.221 |
| | $R^2$ | 0.96 (0.02) | 0.95 (0.04) | 0.316 | 0.397 |
| | Exponent | 0.029 [0.027-0.031] | 0.030 [0.028-0.032] | 0.126 | 0.691 |
| | $R^2$ | 0.99 [0.97-0.99] | 0.98 [0.96-0.99] | 0.500 | 0.221 |
| **CVCi %** (%·mmHg$^{-1}$) | Linear Slope | 2.62 [2.26-2.83] | 2.87 [2.66-3.29] | 0.234 | 0.076 |
| | $R^2$ | 0.96 [0.93-0.98] | 0.94 [0.92-0.98] | 0.250 | 0.458 |
| | Exponent | 0.027 [0.024-0.028] | 0.028 [0.025-0.029] | 0.105 | 0.629 |
| | $R^2$ | 0.96 [0.94-0.98] | 0.96 [0.93-0.97] | 0.123 | 0.605 |
| **Hypercapnic MCA $V_m$ Slope** (cm·s$^{-1}$·mmHg$^{-1}$) | BL to 4% | 1.49 [1.34-2.32] | 1.84 [1.23-2.24] | 0.083 | 0.931 |
| | 4% – 7% | 2.61 (0.81) | 2.53 (0.76) | 0.102 | 0.754 |
| **Hypocapnic MCA $V_m$ Slope** (cm·s$^{-1}$·mmHg$^{-1}$) | BL to -4% | 1.83 (1.10) | 1.97 (1.03) | 0.131 | 0.849 |
| | -4% – -7% | 1.00 (0.57) | 0.92 (0.53) | 0.145 | 0.656 |
| **Hypercapnic CVCi Slope** (cm·s$^{-1}$·mmHg$^{-2}$) | BL to 4% | 0.017 (0.014) | 0.022 (0.013) | 0.370 | 0.282 |
| | 4% to 7% | 0.023 (0.009) | 0.022 (0.011) | 0.099 | 0.769 |
| **Hypocapnic CVCi Slope** (cm·s$^{-1}$·mmHg$^{-2}$) | BL to -4% | 0.025 (0.017) | 0.024 (0.014) | 0.064 | 0.934 |
| | -4% to -7% | 0.008 [0.005-0.014] | 0.010 [0.002-0.014] | 0.124 | 0.617 |
| **Hypercapnic %Δ MCA $V_m$ /Δ $P_{ET}CO_2$** (%·mmHg$^{-1}$) | BL to 4% | 3.02 [2.07-3.74] | 3.06 [2.31-4.12] | 0.079 | 0.666 |
| | 4% to 7% | 3.57 [3.24-4.00] | 3.68 [3.22-4.03] | 0.072 | 0.986 |

| | | | | | |
|---|---|---|---|---|---|
| **Hypocapnic %Δ MCA $V_m$ /Δ $P_{ET}CO_2$** (%·mmHg$^{-1}$) | BL to -4% | 3.37 (1.69) | 3.35 (1.64) | 0.012 | 0.979 |
| | -4% to -7% | 1.99 (0.67) | 1.87 (0.87) | 0.154 | 0.662 |
| **Hypercapnic %Δ CVCi /Δ $P_{ET}CO_2$** (%·mmHg$^{-1}$) | BL to 4% | 2.36 (1.92) | 3.28 (2.08) | 0.460 | 0.186 |
| | 4% to 7% | 2.92 (1.27) | 2.65 (1.46) | 0.197 | 0.572 |
| **Hypocapnic %Δ CVCi /Δ $P_{ET}CO_2$** (%·mmHg$^{-1}$) | BL to -4% | 3.51 (2.14) | 3.53 (2.06) | 0.009 | 0.969 |
| | -4% to -7% | 1.82 (1.17) | 1.55 (1.33) | 0.215 | 0.529 |

Values are displayed as mean (SD) when normally distributed or median [interquartile range] when non-normally distributed. BL, baseline; $R^2$, coefficient of determination; 4%, first hypercapnic step containing 4% $CO_2$; 7%, second hypercapnic step containing 7% $CO_2$; -4%, first hypocapnic step intended to produce an equal and opposite change in $P_{ET}CO_2$ as observed with 4% $CO_2$; -7%, second hypocapnic step intended to produce an equal and opposite change in $P_{ET}CO_2$ as observed with 7% $CO_2$.

**Table 3.** Flow-mediated dilatation parameters in Caucasian Europeans and South Asians.

| | Caucasian European | South Asian | Effect Size | P value |
|---|---|---|---|---|
| **Baseline diameter** (mm) | 4.13 [3.83-4.37] | 4.30 [4.03-4.51] | 0.194 | 0.285 |
| **Baseline velocity** ($cm.s^{-1}$) | 11.82 [8.65-20.29] | 13.11 [11.28-29.65] | 0.579 | 0.208 |
| **Baseline blood flow** ($ml.min^{-1}$) | 49.32 [37.01-75.30] | 59.31 [42.07-143.41] | 0.632 | 0.196 |
| **Peak diameter** (mm) | 4.47 [4.14-4.68] | 4.55 [4.29-4.79] | 0.011 | 0.666 |
| **Peak blood flow** ($ml.min^{-1}$) | 363.37 (108.93) | 339.88 (128.06) | 0.197 | 0.567 |
| **Time to peak flow** (s) | 12.50 [11.00-14.75] | 11.50 [9.75-13.50] | 0.047 | 0.404 |
| **Absolute FMD** (mm) | 0.31 (0.09) | 0.23 (0.13) | 0.715 | 0.062 |
| **Time to peak diameter** (s) | 68.28 (27.24) | 66.56 (24.72) | 0.066 | 0.849 |
| **$FMD_C$** (%) | 7.39 (2.28) | 5.51 (2.94) | 0.715 | 0.044 |
| **$SR_{AUC}$** ($s^{-1}$) | 19028.11 (8991.70) | 12519.81 (5091.05) | 0.891 | 0.016 |

Values are displayed as mean (SD) when normally distributed or median [interquartile range] when non-normally distributed. FMD, flow-mediated dilatation; $FMD_C$, corrected flow-mediated dilatation; $SR_{AUC}$, shear rate area under the curve.

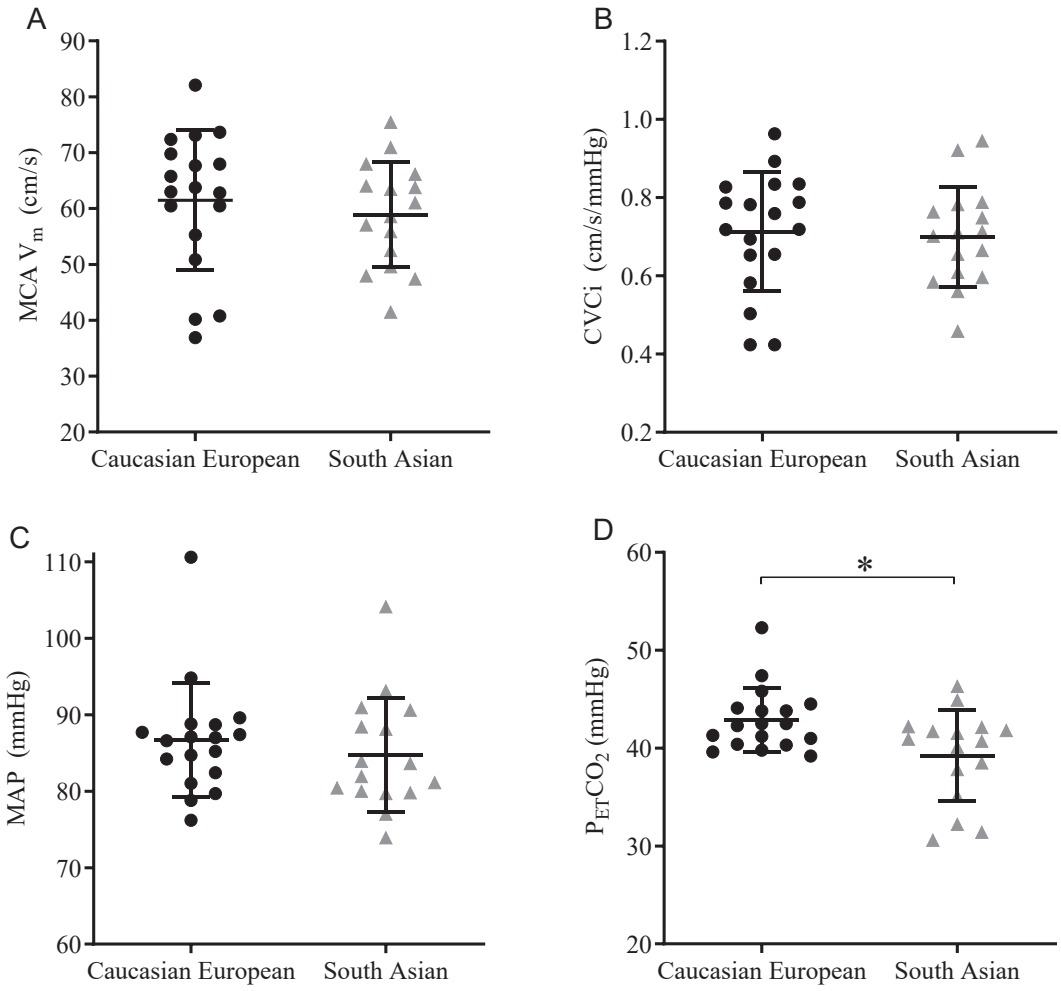
## FIGURE LEGENDS

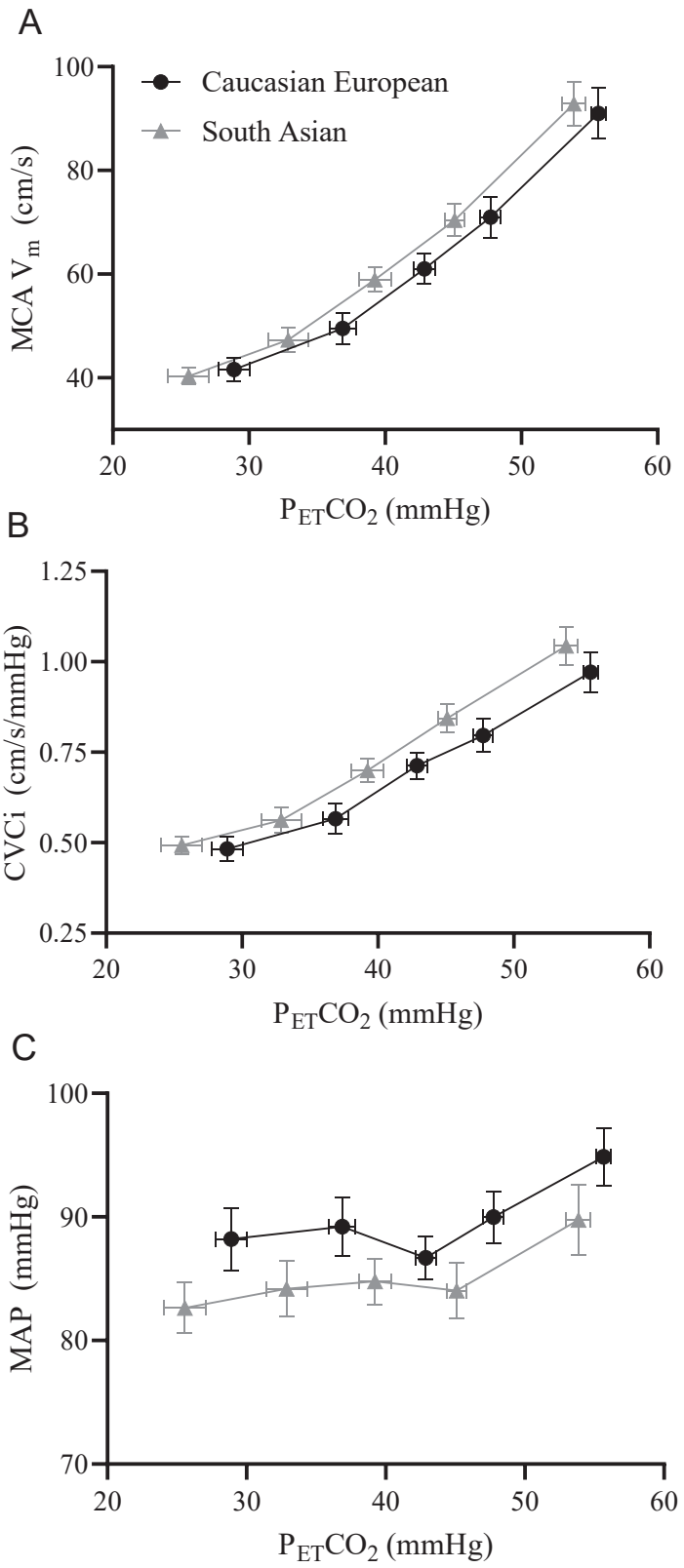
**Figure 1. Baseline MCA $V_m$, CVCi, MAP and $P_{ET}CO_2$ in Caucasian Europeans and South Asians.** MCA $V_m$, middle cerebral artery mean flow velocity; CVCi, cerebrovascular conductance index; MAP, mean arterial pressure; $P_{ET}CO_2$ , partial pressure of end-tidal carbon dioxide. Data expressed as individual values and means with SD. * represents P <0.05.

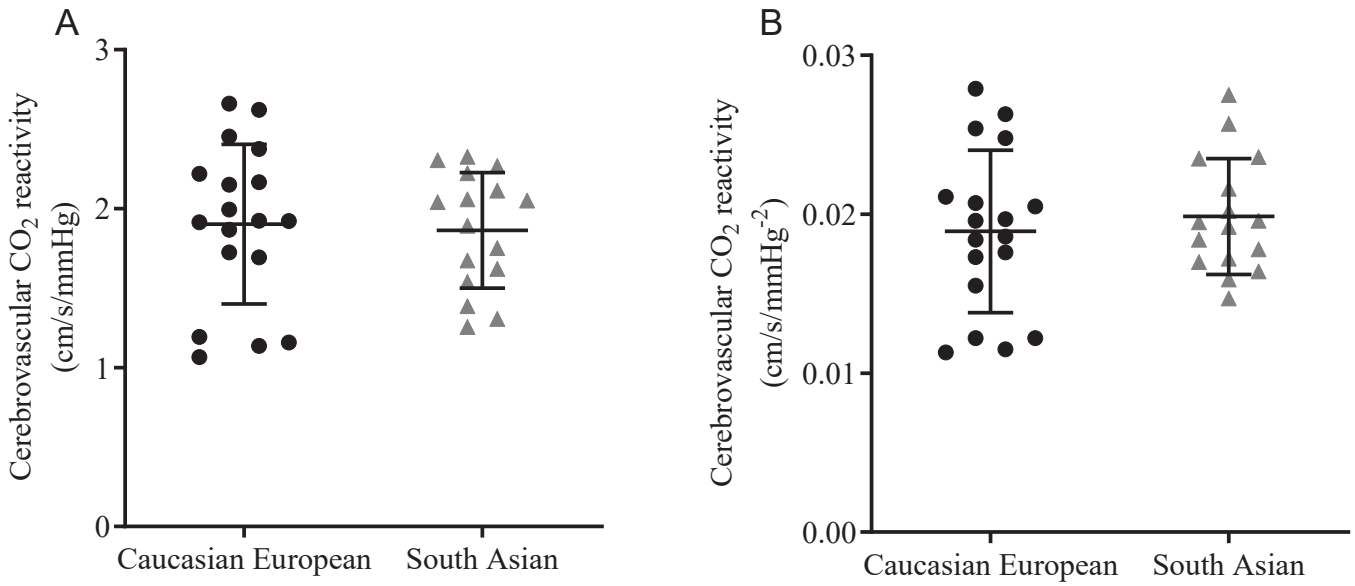
**Figure 2. MCA $V_m$, CVCi and MAP responses to the cerebrovascular $CO_2$ reactivity protocol in Caucasian Europeans and South Asians.** Symbols show mean and standard error of the mean.

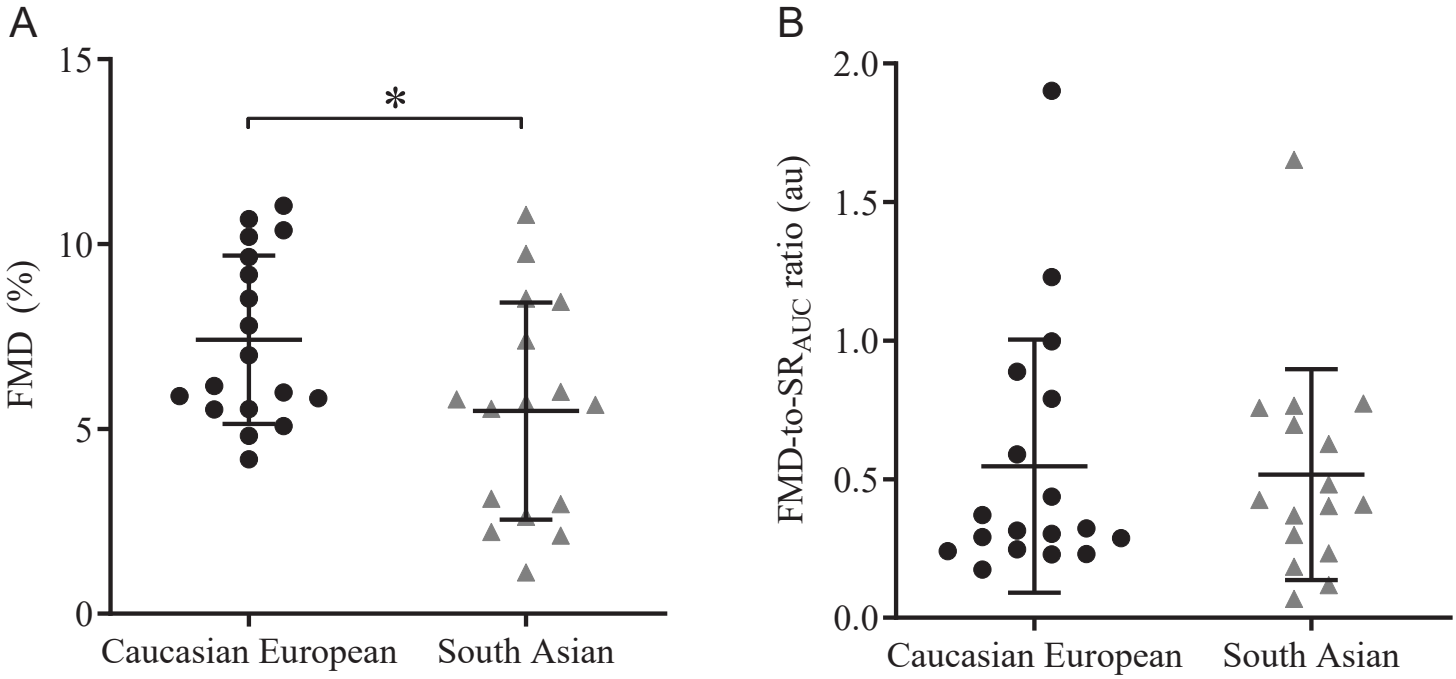
**Figure 3. Cerebrovascular $CO_2$ reactivity in Caucasian Europeans and South Asians.** Cerebrovascular $CO_2$ reactivity is expressed as the slope of MCA $V_m$ change in cm/s (Δ) (panel A) and Δ CVCi (panel B) versus Δ $P_{ET}CO_2$ in mmHg. Horizontal bars show mean and SD.

**Figure 4. Flow-mediated dilatation (FMD) in Caucasian Europeans and South Asians.** FMD is expressed as a percentage change (panel A) and as a ratio between FMD (%) and $SR_{AUC}$ (panel B). Horizontal bars show mean and SD.


A
MCA $V_m$ (cm/s)
90
80
70
60
50
40
30
20
Caucasian European
South Asian
B
CVCi (cm/s/mmHg)
1.2
1.0
0.8
0.6
0.4
0.2
Caucasian European
South Asian
C
MAP (mmHg)
110
100
90
80
70
60
Caucasian European
South Asian
D
$P_{ET}CO_2$ (mmHg)
60
50
40
30
20
*
Caucasian European
South Asian


A
Caucasian European
South Asian
MCA $V_m$ (cm/s)
100
80
60
40
20
30
40
50
60
$P_{ET}CO_2$ (mmHg)
B
CVCi (cm/s/mmHg)
1.25
1.00
0.75
0.50
0.25
20
30
40
50
60
$P_{ET}CO_2$ (mmHg)
C
MAP (mmHg)
100
90
80
70
20
30
40
50
60
$P_{ET}CO_2$ (mmHg)


A
Cerebrovascular $CO_2$ reactivity (cm/s/mmHg)
3
2
1
0
Caucasian European
South Asian
B
Cerebrovascular $CO_2$ reactivity (cm/s/mmHg$^{-2}$)
0.03
0.02
0.01
0.00
Caucasian European
South Asian


A
15
10
5
0
FMD (%)
*
Caucasian European
South Asian
B
2.0
1.5
1.0
0.5
0.0
FMD-to-SR$_{AUC}$ ratio (au)
Caucasian European
South Asian