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DOI:

[10.1152/ajpheart.00641.2019](https://doi.org/10.1152/ajpheart.00641.2019)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Junejo, R, May, S, Alsalahi, S, Alali, M, Ogoh, S & James P, F 2020, 'Cerebrovascular carbon dioxide reactivity and flow mediated dilation in young healthy South Asian and Caucasian European men', *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 318, no. 4, pp. H756-H763.
<https://doi.org/10.1152/ajpheart.00641.2019>

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

3
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14

15
16 **Running Title:** Ethnic differences in cerebrovascular reactivity

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22 **ABSTRACT**

23 South Asians living in the UK have a 1.5-fold greater risk of ischemic stroke than the
24 general population. Impaired cerebrovascular carbon dioxide (CO₂) reactivity is an
25 independent predictor of ischemic stroke and cardiovascular mortality. We sought to test the
26 hypothesis that cerebrovascular CO₂ reactivity is reduced in South Asians. Middle cerebral
27 artery blood velocity (MCA V_m) was measured at rest and during stepwise changes in partial
28 pressure of end-tidal CO₂ (P_{ET}CO₂) in South Asian (n=16) and Caucasian European (n=18)
29 men that were, young (~20 years), healthy and living in the UK. Incremental hypercapnia
30 was delivered via the open circuit steady-state method, with stages of 4% and 7% CO₂ (≈21%
31 Oxygen, Nitrogen balanced). Cerebrovascular CO₂ reactivity was calculated as the change in
32 MCA V_m per mmHg change in P_{ET}CO₂. MCA V_m was not different in South Asian (59 (9)
33 cm/s; mean (standard deviation)) and Caucasian Europeans (61 (12) cm/s; P>0.05). Similarly,
34 cerebrovascular CO₂ reactivity was not different between the groups (South Asian, 2.53
35 (0.76) cm/s/mmHg vs. Caucasian European, 2.61 (0.81) cm/s/mmHg; P>0.05). Brachial
36 artery flow-mediated dilatation was lower in South Asian (5.48 (2.94) %) compared to
37 Caucasian European (7.41 (2.28) %; P<0.05); however when corrected for shear rate, no
38 between group differences in flow-mediated dilatation were observed (P>0.05). Flow-
39 mediated dilatation was not correlated with cerebrovascular CO₂ reactivity measures. In
40 summary, cerebrovascular CO₂ reactivity and flow-mediated dilatation when corrected for
41 shear rate are preserved in young healthy South Asian men living in the UK.

42

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

44 **NEW AND NOTEWORTHY**

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

50

51 **ABBREVIATIONS**

52 BP, blood pressure; CO₂, carbon dioxide; CVCi, cerebrovascular conductance index;
53 ECG, electrocardiograph; FMD, flow-mediated dilation; FMDc, covariate corrected flow-
54 mediated dilation; HR, heart rate; MAP, mean arterial pressure; MCA_V, middle cerebral
55 artery mean blood velocity; N₂, nitrogen; O₂, oxygen; P_{ET}CO₂, partial pressure of end-tidal
56 carbon dioxide; SR_{AUC}, shear rate area under the curve; TCD, transcranial Doppler

57 **INTRODUCTION**

58 South Asian migrants from the Indian sub-continent in the United Kingdom have an
59 ischemic stroke mortality that is ~1.5 times greater than the general population (44), while
60 ischemic stroke onset typically occurs at a younger age in South Asians than ethnically White
61 Caucasian Europeans (20). Although broadly attributable to cultural and socioeconomic
62 factors (12), there is a paucity of information about the underlying physiological mechanisms
63 for such ethnic differences in cerebrovascular health (37). The brain has a high metabolic
64 demand and possesses multiple interactive regulatory mechanisms. The latter ensure that
65 cerebral blood flow remains relatively stable independent of changes in perfusion pressure
66 (cerebral autoregulation), that local perfusion is closely matched to neuronal activation and
67 metabolism (neurovascular coupling), and that cerebrovascular responses to changes in
68 carbon dioxide (cerebrovascular CO₂ reactivity) are adequate to assist the maintenance of
69 central [H⁺]. Bathula et al. (4) observed that cerebral autoregulation is poorer and
70 cerebrovascular resistance is higher in South Asians (of Punjabi Sikh origin) compared to
71 people with “European origins”. However, it remains to be determined whether
72 cerebrovascular CO₂ reactivity is blunted (i.e., diminished cerebral vasodilatory reserve) in
73 South Asians.

74 It has long been established that the cerebral vasculature is highly sensitive to changes
75 in the partial pressure of arterial CO₂ (19), and since this time an impaired cerebrovascular
76 CO₂ reactivity has been established as an independent predictor of ischemic stroke (23) and
77 identified in several cardiovascular, cerebrovascular and neurological disorders (13, 18, 23,
78 41). Cerebrovascular dysfunction may lead to neuronal dysfunction and neurodegeneration
79 since neurons depend on arterial vasodilatation for adequate perfusion to ensure oxygen/CO₂
80 homeostasis, nutrient delivery and elimination of potentially toxic metabolites (46). The
81 mechanism whereby CO₂ modifies cerebral blood vessel tone is complex. Among the various

82 contributory factors, endothelium-derived nitric oxide is considered to be an important local
83 regulator of cerebral blood flow that plays a role in hypercapnia-induced vasodilatation (14,
84 40, 43). Indeed, acute infusion of L-arginine (the substrate for endothelial nitric oxide
85 synthase) restores impairments in cerebrovascular CO₂ reactivity manifest in patients with
86 cardiovascular risk factors (45), while hypercapnia-induced increases in cerebral blood flow
87 are attenuated by inhibition of nitric oxide synthase activity with N-nitro-L-arginine methyl
88 ester (L-NAME) in rats (5). Moreover, individuals or groups in whom impaired peripheral
89 vascular nitric oxide signaling has been identified are reported to demonstrate a reduced
90 cerebrovascular CO₂ reactivity (21). Therefore, the observation that brachial artery flow-
91 mediated dilation, indicative of attenuated endothelium-derived nitric oxide mediated
92 vasodilation, is reduced in South Asians compared to Caucasian Europeans (6, 30) may also
93 point to a reduced cerebrovascular CO₂ reactivity.

94 The aim of this study was to investigate whether cerebrovascular CO₂ reactivity is
95 impaired in young healthy South Asians compared to Caucasian Europeans. Based on prior
96 reports identifying the greater incidence of cerebrovascular events in South Asians and
97 peripheral endothelial dysfunction, we hypothesized that cerebrovascular CO₂ reactivity
98 would be lower in healthy young South Asian adults when compared to age-matched
99 Caucasian Europeans. Brachial artery flow-mediated dilation, a well-established marker of
100 peripheral vascular (endothelial) function, was also determined in accordance with
101 established guidelines (32, 42). Lastly, we assessed whether an association between brachial
102 artery flow-mediated dilation and cerebrovascular CO₂ reactivity existed in the population
103 studied.

104 **METHODS**

105 *Ethical Approval.*

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

111

112 *Participant characteristics.*

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

123

124 *Experimental measures.*

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

129 Finapres Medical Systems BV, Amsterdam, The Netherlands) and corrected with automatic
130 brachial sphygmomanometer readings (Omron 750IT, Milton Keynes, UK). Middle cerebral
131 artery mean blood velocity (MCA V_m) was continuously monitored using transcranial Doppler
132 ultrasonography (Doppler Box X, DWL, Sipplingen, Germany). A 2 MHz probe, mounted on
133 an adjustable headband, was fixed at the temporal window to insonate the right MCA at a
134 depth of 40-65 mm. Participants wore a mouthpiece and nose-clip, and the partial pressure of
135 end-tidal CO_2 ($P_{ET}CO_2$) was provided by a capnograph connected to the mouthpiece by an
136 anesthetic sample line (Gas Analyzer, ADInstruments, Dunedin, New Zealand). Breath-by-
137 breath fluctuations in $P_{ET}CO_2$ were used to calculate respiratory rate. Analogue signals were
138 digitized at 1 kHz (Powerlab, ADInstruments) and recorded using multi-channel data
139 acquisition software (LabChart 7, ADInstruments). Simultaneous recordings of the left
140 brachial artery diameter and flow velocity were obtained with the arm at heart level using
141 Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA).
142 The artery was insonated 10–15 cm proximal to the medial epicondyle at 60°. Duplex
143 imaging was used to obtain a B-mode image of vessel diameter and pulse-wave mode of peak
144 blood velocity using a 4-15 Hz multi-frequency linear-array transducer (Terason uSmart
145 15L4) held in place with an adjustable probe holder. Ultrasound measurements were made in
146 accordance with technical recommendations (32, 42). Recordings were screen captured,
147 stored as video files and offline analysis carried out using automated edge detection and wall
148 tracking software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy) (11).

149

150 ***Experimental Protocol***

151 This cross-sectional study included a screening/familiarization visit prior to the
152 experimental session. Participants were instructed to abstain from food for 2 h, caffeinated
153 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 °C). 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 insonated 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 CO₂ 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 CO₂ (hypercapnia),
166 via a two-way non-rebreathing valve. Specifically, participants received 4 % CO₂ (≈21 % O₂,
167 N₂ balanced) for 4-min, followed by 7 % CO₂ (≈21 % O₂, N₂ 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}CO₂ 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:

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

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) $MCA V_m$ 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 $MCA V_m$

203 and % Δ CVCi versus Δ P_{ET}CO₂ (in mmHg) with the hypercapnic and hypocapnic steps (9,
204 31).

205

206 *Statistical Analysis*

207 Data distribution was assessed by the Shapiro-Wilk test. Normally distributed data
208 were analyzed using two-tailed Students t-test, while non-normally distributed data were
209 analyzed using Mann-Whitney Rank Sum test. The correlation between cerebrovascular CO₂
210 reactivity and flow-mediated dilatation was assessed using Pearson's product moment
211 correlation. Effect size (Cohen's *d*) was calculated as the difference between means of two
212 groups divided by the averaged standard deviation (SD). Statistical analysis was performed
213 using Sigmaplot 13.0 (Systat Software Inc, London, UK). Significance was set at $p < 0.05$.
214 Normally distributed data are presented as mean (SD), unless stated, while non-normally
215 distributed data are presented as median [interquartile range].

216 **RESULTS**

217 *Participant characteristics and baseline haemodynamics*

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

223

224 *Cerebrovascular CO₂ reactivity*

225 Figure 2 shows the MCA V_m , CVCi and MAP response to both the hypercapnic and
226 hypocapnic steps of the cerebrovascular CO₂ reactivity test in the South Asian and Caucasian
227 European groups. As anticipated, hypercapnia produced pronounced increases in MCA V_m
228 and CVCi, while conversely both were reduced with hypocapnia. Of note, no between-group
229 differences were observed in any index of cerebrovascular CO₂ reactivity (Figure 3, Table 2).

230

231 *Brachial artery flow-mediated dilatation*

232 Flow-mediated dilatation was lower in the South Asian than Caucasian European
233 group ($P<0.05$, Figure 4). This between group difference persisted with correction for
234 baseline diameter (FMD_C $P<0.05$, Table 3). Peak reactive hyperemia was not different
235 between groups ($P>0.05$, Table 3). However, SR_{AUC} was lower in South Asians than
236 Caucasian Europeans ($P<0.05$, Table 3) and when brachial artery flow-mediated dilatation
237 was corrected for SR_{AUC} (i.e., FMD-to-SR_{AUC} ratio) the between group difference was no
238 longer evident ($P>0.05$, Figure 4). No significant association between FMD_C and hypercapnic
239 cerebrovascular CO₂ 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).

242 **DISCUSSION**

243 The major novel finding of the present study is that cerebrovascular CO₂ reactivity is
244 not different in young healthy South Asians and Caucasian Europeans. In addition, brachial
245 artery flow-mediated dilatation was lower in South Asians when expressed as a percentage
246 change from baseline. However, during flow-mediated dilation testing South Asians had a
247 lower shear rate response (SR_{AUC}), which when accounted for (FMD-to-SR_{AUC} ratio), flow-
248 mediated dilatation was not different between groups. These findings suggest that: 1)
249 contrary to our hypothesis, cerebrovascular CO₂ reactivity is not lower in healthy young
250 South Asian adults than age-matched Caucasian Europeans, and 2) apparent reductions in
251 brachial artery flow-mediated dilatation in South Asians (6, 30) may be attributable to a
252 reduced ischemic stimulus rather than endothelial dysfunction *per se*.

253 Prior reports have identified a greater incidence of cerebrovascular events in South
254 Asians (20, 44). Given the prognostic significance of impaired cerebrovascular CO₂ reactivity
255 as an independent predictor of ischemic stroke (23) and its association with multiple
256 cardiovascular, cerebrovascular and neurological disorders (13, 18, 23, 41), we anticipated
257 that cerebrovascular CO₂ reactivity would be lower in South Asian adults than age-matched
258 Caucasian Europeans. Moreover, Hurr et al. (15) identified that African Americans (23±4
259 years), a group at higher risk of cardiovascular and cerebrovascular disease, exhibited an
260 attenuated cerebrovascular vasodilatation in response to hypercapnia compared to age-
261 matched Caucasian Americans. Contrary to expectation, we did not observe a difference in
262 cerebrovascular CO₂ reactivity between young healthy South Asian and Caucasian European
263 men; neither did we observe between-group differences in MCA V_m nor CVCi. In a
264 population-based sample Bathula et al. (4) noted a higher MCA V_m (38.0±0.7 vs. 41.4±0.7
265 cm/s) and cerebrovascular resistance (resistivity index), but poorer cerebral autoregulation
266 (low frequency gain, 0.45±0.01 vs. 0.50±0.01 cm/s/mmHg) in South Asians of Punjabi Sikh

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

276 Coronary heart disease risk is elevated in migrant South Asians to the UK (3, 25). Of
277 note, according to the 1991 England and Wales Census data, the relative risk of death from
278 coronary heart disease was 3 in Indian Asian men aged 20-29 years, compared to age-
279 matched Caucasian Europeans (3). The excess coronary heart disease risk in South Asians is
280 not explained by conventional risk factors (e.g., smoking, hypercholesterolemia,
281 hypertension) (24), although an increased prevalence of insulin resistance and diabetes has
282 been implicated (26). Endothelial dysfunction in South Asians (i.e., attenuated brachial artery
283 flow-mediated dilatation and N^G -Monomethyl-L-arginine induced vasoconstriction) is also
284 speculated to contribute to the elevated coronary heart disease risk, and has been identified in
285 both young (30) and older (6) South Asian groups. In the present study when we expressed
286 flow-mediated dilatation simply as the percentage change from baseline in brachial artery
287 diameter, it was reduced in South Asians compared to Caucasian Europeans. This
288 experimental approach and the associated findings are in agreement with previous reports (6,
289 30). It is noteworthy that despite no differences in baseline brachial artery diameter, velocity
290 and blood flow, the SR_{AUC} was attenuated in the South Asian group. Accordingly, when
291 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

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

319 The results of this study should be viewed in the context of the following
320 experimental limitations. Despite the widely acknowledged value of transcranial Doppler in
321 the evaluation of cerebral vascular function, it is an inherent limitation of the method that
322 MCA V_m is only proportional to cerebral blood flow if the cross-sectional area of the MCA
323 remains unchanged. Although, good correlations have been observed between MCA V_m and
324 cerebral blood flow when P_{ETCO_2} is altered (8, 35), there is evidence to suggest MCA
325 diameter increases with robust hypercapnia (i.e., ΔP_{ETCO_2} of greater than ~ 7 -9 mmHg) (1,
326 22, 28). P_{ETCO_2} has been employed as a non-invasive surrogate for the partial pressure of
327 arterial CO_2 (P_aCO_2) in the present study because a strong positive linear correlation between
328 P_{ETCO_2} and P_aCO_2 has been identified (27); however, it is acknowledged that P_{ETCO_2} may
329 underestimate P_aCO_2 at rest (38). We also acknowledge the ongoing debate relating to the
330 relative strengths and weaknesses of approaches developed to determine cerebrovascular CO_2
331 reactivity (10). The method used here has shown a good between-day test-retest reliability
332 (intraclass correlation of 0.938 [95% CI 0.759-0.985] $P < 0.001$; co-efficient of variation for
333 the method error of 6.06%) (18). The extent to which our findings may be more broadly
334 generalized is limited by the inclusion of only healthy young men. We also failed to collect
335 diet and socioeconomic data for the participants and did not objectively assess their activity
336 patterns in a detailed manner. Future studies should consider the important potential
337 interaction between sex, aging, diet, socioeconomic levels, activity patterns and ethnicity in
338 the regulation of peripheral vasculature and cerebrovascular function.

339 In summary, we report for the first time that cerebrovascular CO_2 reactivity is not
340 different in young healthy South Asians and Caucasian Europeans. Furthermore, when the
341 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.

344 **ACKNOWLEDGMENTS**

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

346

347 **GRANT AND DISCLOSURES**

348 None.

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479 **TABLES**480 **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·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·min ⁻¹)	14 [13-15]	15 [13-16]	0.343

481

482 Values are displayed as mean (SD) when normally distributed or median [interquartile range]

483 when non-normally distributed. BMI, body mass index; au, arbitrary units.

484

485 **Table 2.** Cerebrovascular CO₂ 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}CO₂ (%·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 $P_{ET}CO_2$ (%\cdotmmHg$^{-1}$)	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 $P_{ET}CO_2$ (%\cdotmmHg$^{-1}$)	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 $P_{ET}CO_2$ (%\cdotmmHg$^{-1}$)	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

486

487 Values are displayed as mean (SD) when normally distributed or median [interquartile range] when non-normally distributed. BL, baseline; R^2 ,
488 coefficient of determination; 4%, first hypercapnic step containing 4% CO_2 ; 7%, second hypercapnic step containing 7% CO_2 ; -4%, first
489 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
490 to produce an equal and opposite change in $P_{ET}CO_2$ as observed with 7% CO_2 .

491

492 **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

493

494 Values are displayed as mean (SD) when normally distributed or median [interquartile range]

495 when non-normally distributed. FMD, flow-mediated dilatation; FMD_C, corrected flow-

496 mediated dilatation; SR_{AUC}, shear rate area under the curve.

497

498

499 **FIGURE LEGENDS**

500 **Figure 1. Baseline MCA V_m , CVCi, MAP and $P_{ET}CO_2$ in Caucasian Europeans and**
501 **South Asians.** MCA V_m , middle cerebral artery mean flow velocity; CVCi, cerebrovascular
502 conductance index; MAP, mean arterial pressure; $P_{ET}CO_2$, partial pressure of end-tidal
503 carbon dioxide. Data expressed as individual values and means with SD. * represents P
504 <0.05 .

505

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

509

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

514

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







