

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

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

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18  
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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 (CO<sub>2</sub>) reactivity is an  
25 independent predictor of ischemic stroke and cardiovascular mortality. We sought to test the  
26 hypothesis that cerebrovascular CO<sub>2</sub> reactivity is reduced in South Asians. Middle cerebral  
27 artery blood velocity (MCA V<sub>m</sub>) was measured at rest and during stepwise changes in partial  
28 pressure of end-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) 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% CO<sub>2</sub> (≈21%  
31 Oxygen, Nitrogen balanced). Cerebrovascular CO<sub>2</sub> reactivity was calculated as the change in  
32 MCA V<sub>m</sub> per mmHg change in P<sub>ET</sub>CO<sub>2</sub>. MCA V<sub>m</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> reactivity measures. In  
40 summary, cerebrovascular CO<sub>2</sub> 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; CO<sub>2</sub>, 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<sub>V</sub>, middle cerebral  
55 artery mean blood velocity; N<sub>2</sub>, nitrogen; O<sub>2</sub>, oxygen; P<sub>ET</sub>CO<sub>2</sub>, partial pressure of end-tidal  
56 carbon dioxide; SR<sub>AUC</sub>, 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 CO<sub>2</sub> reactivity) are adequate to assist the maintenance of  
69 central [H<sup>+</sup>]. 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 CO<sub>2</sub> 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 CO<sub>2</sub> (19), and since this time an impaired cerebrovascular  
76 CO<sub>2</sub> 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/CO<sub>2</sub>  
80 homeostasis, nutrient delivery and elimination of potentially toxic metabolites (46). The  
81 mechanism whereby CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> reactivity.

94         The aim of this study was to investigate whether cerebrovascular CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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<sub>2</sub> (hypercapnia),  
166 via a two-way non-rebreathing valve. Specifically, participants received 4 % CO<sub>2</sub> (≈21 % O<sub>2</sub>,  
167 N<sub>2</sub> balanced) for 4-min, followed by 7 % CO<sub>2</sub> (≈21 % O<sub>2</sub>, N<sub>2</sub> 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<sub>ET</sub>CO<sub>2</sub> 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 ( $\Delta$ )  $MCA V_m$  and %  $\Delta$  CVCi versus  $P_{ET}CO_2$  (mmHg)  
199 were calculated. Cerebrovascular  $CO_2$  reactivity was separately expressed as the linear slope  
200 of  $\Delta$   $MCA V_m$  (cm/s) and  $\Delta$  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 %  $\Delta$   $MCA V_m$

203 and %  $\Delta$  CVCi versus  $\Delta$   $P_{ET}CO_2$  (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  $CO_2$   
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  $CO_2$  reactivity*

225 Figure 2 shows the MCA  $V_m$ , CVCi and MAP response to both the hypercapnic and  
226 hypocapnic steps of the cerebrovascular  $CO_2$  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  $CO_2$  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<sub>C</sub>  $P<0.05$ , 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<sub>C</sub> and hypercapnic  
239 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).

242 **DISCUSSION**

243           The major novel finding of the present study is that cerebrovascular CO<sub>2</sub> 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<sub>AUC</sub>), which when accounted for (FMD-to-SR<sub>AUC</sub> ratio), flow-  
248 mediated dilatation was not different between groups. These findings suggest that: 1)  
249 contrary to our hypothesis, cerebrovascular CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> reactivity between young healthy South Asian and Caucasian European  
263 men; neither did we observe between-group differences in MCA V<sub>m</sub> nor CVCi. In a  
264 population-based sample Bathula et al. (4) noted a higher MCA V<sub>m</sub> (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.,  $\Delta P_{ETCO_2}$  of greater than  $\sim 7$ -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.

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346

347 **GRANT AND DISCLOSURES**

348           None.

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479 **TABLES**480 **Table 1.** Participant characteristics.

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

		Caucasian	European	South Asian	Effect Size	P value	
<b>MCA V<sub>m</sub> % (%·mmHg<sup>-1</sup>)</b>	Linear Slope	3.06	[2.77-3.18]	3.26	[2.83-3.45]	0.064	0.221
	R <sup>2</sup>	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 <sup>2</sup>	0.99	[0.97-0.99]	0.98	[0.96-0.99]	0.500	0.221
<b>CVCi % (%·mmHg<sup>-1</sup>)</b>	Linear Slope	2.62	[2.26-2.83]	2.87	[2.66-3.29]	0.234	0.076
	R <sup>2</sup>	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 <sup>2</sup>	0.96	[0.94-0.98]	0.96	[0.93-0.97]	0.123	0.605
<b>Hypercapnic MCA V<sub>m</sub> Slope (cm·s<sup>-1</sup>·mmHg<sup>-1</sup>)</b>	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
<b>Hypocapnic MCA V<sub>m</sub> Slope (cm·s<sup>-1</sup>·mmHg<sup>-1</sup>)</b>	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
<b>Hypercapnic CVCi Slope (cm·s<sup>-1</sup>·mmHg<sup>-2</sup>)</b>	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
<b>Hypocapnic CVCi Slope (cm·s<sup>-1</sup>·mmHg<sup>-2</sup>)</b>	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
<b>Hypercapnic %Δ MCA V<sub>m</sub> /Δ P<sub>ET</sub>CO<sub>2</sub> (%·mmHg<sup>-1</sup>)</b>	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



<b>Hypocapnic %<math>\Delta</math> MCA <math>V_m</math> /<math>\Delta</math> <math>P_{ET}CO_2</math> (%<math>\cdot</math>mmHg<math>^{-1}</math>)</b>	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
<b>Hypercapnic %<math>\Delta</math> CVCi /<math>\Delta</math> <math>P_{ET}CO_2</math> (%<math>\cdot</math>mmHg<math>^{-1}</math>)</b>	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
<b>Hypocapnic %<math>\Delta</math> CVCi /<math>\Delta</math> <math>P_{ET}CO_2</math> (%<math>\cdot</math>mmHg<math>^{-1}</math>)</b>	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.

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

496 mediated dilatation; SR<sub>AUC</sub>, 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 ( $\Delta$ )  
512 (panel A) and  $\Delta$  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.







